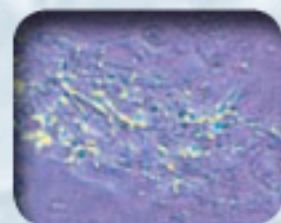




TISSUE DISSOCIATION GUIDE



ABOUT THE COVER and PHOTO CREDITS

Worthington Biochemical Corporation would like to acknowledge and thank the following researchers for providing and kindly allowing reproductions of their images used on the front and back covers and contained within this publication:

Contributors

A citation follows for all the cardiomyocyte images courteously provided by Ronal R. MacGregor, Ph.D., University of Kansas Medical Center, Department of Anatomy & Cell Biology, Kansas City, KS 66160.

A 300g male Sprague-Dawley rat was anesthetized; the heart was removed and perfused at a flow rate of 9 ml per minute with Hank's Balanced Salt Solution (HBSS) for 3 minutes, and then with calcium and magnesium free HBSS (CMFHBSS) containing 2 mM each of taurine, creatine, and carnitine, and 3 mM 1-heptanol (a gap junction blocker) for 9 minutes. The perfusion medium was then changed to Leibovitz' L15 containing the components and amounts as follows:

4 ml sterile water
2 ml 0.15 M MOPS pH 7.4
4 ml PAPL (papain), 50 U/ml in CMFHBSS (5 U/ml final)
133 ul of 30% (300 mg/ml) BSA (bovine serum albumin), culture tested (1 mg/ml final)
29.8 ml L15/PSF/CCT/ITS*
1/2 vial CLS-H (Enzyme vial #2 from the Hepatocyte Isolation System, Worthington)
1 Vial DNase (Enzyme vial #3 from the Hepatocyte Isolation System, Worthington)
1-Heptanol, 18 ul
40 ml

* PSF: Penicillin-Streptomycin-Fungazone; CCT: carnitine, creatine, & taurine; ITS: insulin, transferrin, selenium, 100X (Invitrogen)

Then perfusion was continued for 20 min. All perfusion media were at a temperature of 37 C, warmed by a heat exchanger coil in a water bath. Following the perfusions, the heart was minced, and then placed with 1/2 volume of the final perfusion medium into a 50 mL centrifuge tube, capped, and rotated end-for-end for 1 hour at 6-8 rpm using a rotator from Reliable Scientific Corp. The tube was then removed, shaken briefly, and the dissociated cells and residue were allowed to settle in the tube for 1-2 minutes. The supernatant cells were transferred to a large Petrie dish, allowed to settle at 1 x g, and the supernatant removed and replaced with HBSS. This was repeated once, and the cells were then transferred to a 250 ml round-bottom centrifuge tube and underlaid with about 40 ml of 3% BSA in HBSS. Cells were allowed to settle at 1 x g for 10 min and the supernatant was then removed and replaced with Culture medium which was Leibovitz' L15 supplemented with CCT, PSF, ITS, and made 10% in fetal Bovine serum (Hyclone). Cells were plated in plastic Petri dishes coated with laminin (1 ug/cm², BTI Inc).

After two days of culture, media were changed to contain 10 uM cytosine arabinoside to inhibit cell replication, and culture continued for two days. Media were then changed and culture continued in the absence of cytosine arabinoside. the figures represent cardiomyocytes after culture for a total of 12 days.

The cells were observed using a Nikon Eclipse TE300 microscope attached to a Spot RTKE imaging system by Diagnostics Instruments.

The images representing expression of growth factor receptors and paxillin in rat nerve sheath cells were courteously provided by John Biggerstaff, Ph.D., Research Associate Professor, University of Tennessee, Center for Environmental Biotechnology, 676 Dabney Hall, Knoxville, TN 37996.

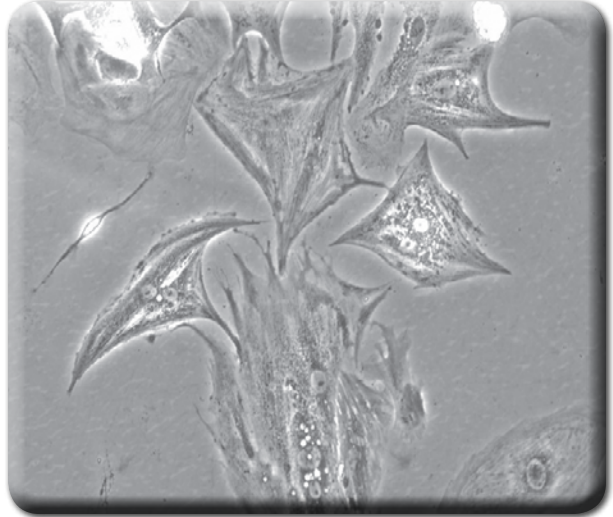
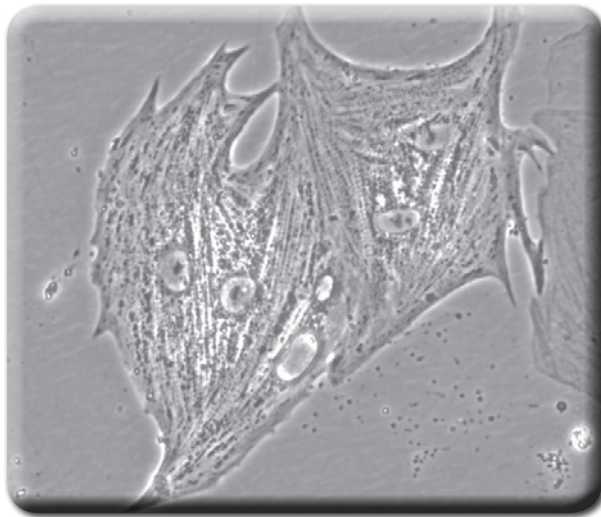
The images of placental cells cultured in a hollow-fiber bioreactor were provided by James Hardy of Hemacell Perfusion Inc. and John Cadwell of FiberCell Systems.

A normal intact human placenta was sourced from a local hospital under conditions approved by the Institutional Review Board (IRB). The umbilical vein was cannulated and a solution of PBS was perfused for a time of one hour. Perfusion was performed utilizing the Machine Pulsatile Perfusion method described elsewhere. Blood forming hematopoietic stem cells and other components were collected from the perfusate. This also reduced the red cell burden. After one hour at room temperature the solution was changed to PBS containing one gram per liter of collagenase (Worthington Type 1, CLS-1).

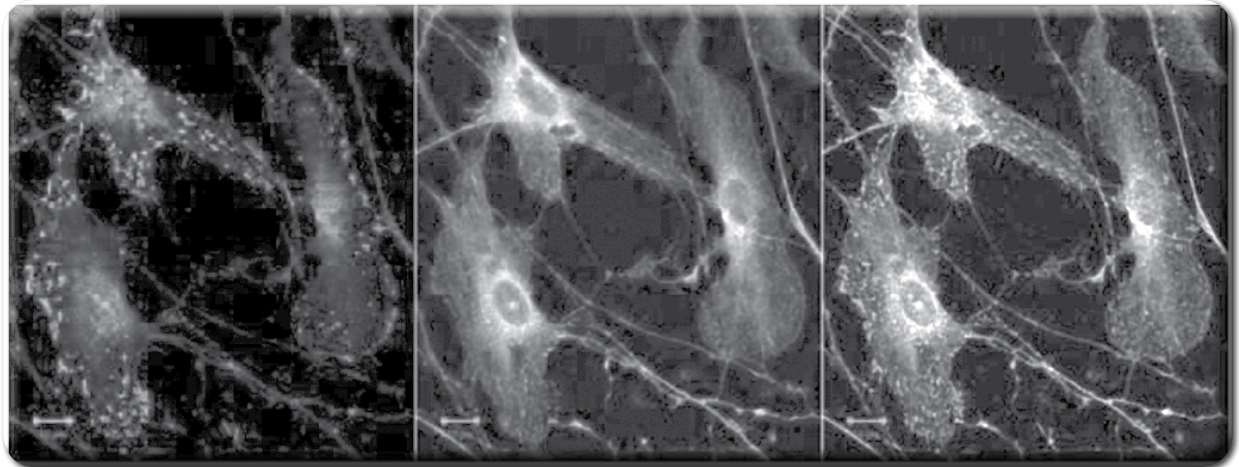
This was perfused for 2 hours at room temperature. The placenta was digitally disaggregated and the cells were collected, washed once in cell culture medium and then the red cells removed using ACK lysing buffer (Quality Biologicals #118-156-101) per manufacturers instructions. Cells were washed once more in cell culture medium. Total number of viable cells was difficult to establish due to the significant amount of cell debris and red cells still present. 20 mls of the cell isolate (out of a total of 40 mls) was seeded into a FiberCell C2008 5kd MWCO polysulfone cartridge and the culture initiated with 100 mls of DMEM/F12 + 10% FBS and 2% pen/strep at a flow rate of 60 mls/minute.

This mixed culture of primary human placental derived cells was maintained for over three months of continuous culture. Every two days harvests of the suspension cells was performed and plated into standard T-75 tissue culture flasks from where the images were taken. The spherical bodies of cells stained intensely for CD105, a mesenchymal stem cell marker.

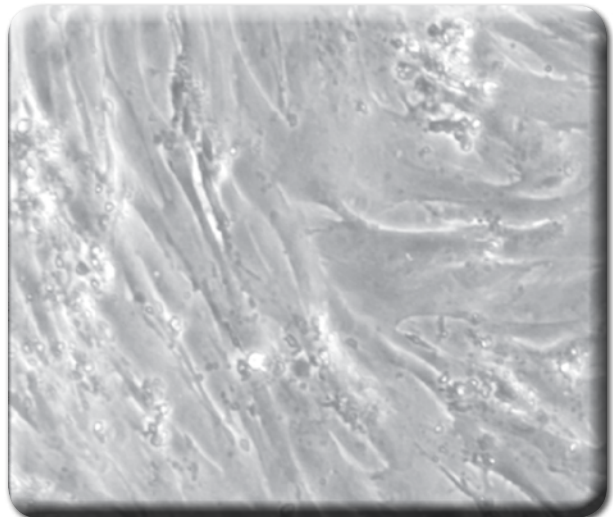
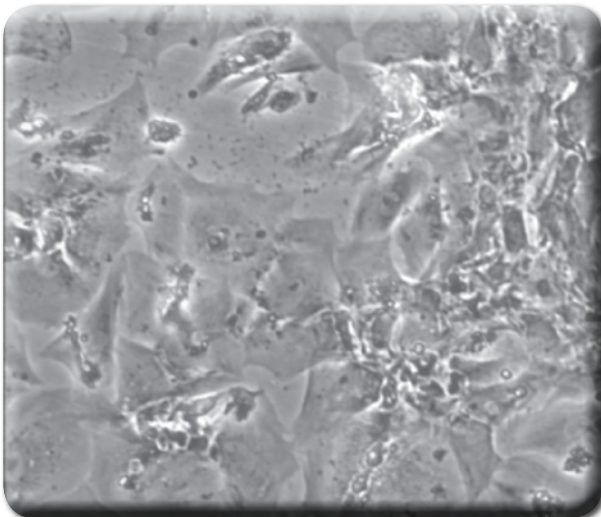
Note: Worthington invites other researchers to submit research-related photos and artwork for our potential use in or on the covers of future corporate publications. Please include complete citations and descriptions and forward submissions to the attention of our Marketing Department (marketing@worthington-biochem.com).



The above images representing rat cardiomyocytes, cultured 12 days, CA treated were provided by Ronal R. MacGregor, Ph.D. University of Kansas Medical Center, Department of Anatomy & Cell Biology, Kansas City, KS 66160.



The above images representing expression of growth factor receptors (center) and paxillin (right) in rat nerve sheath cells were provided by John Biggerstaff, Ph.D. Research Associate Professor, University of Tennessee, Center for Environmental Biotechnology, 676 Dabney Hall, Knoxville, TN 37996.



The above images of placental cells cultured in a hollow-fiber bioreactor were provided by James Hardy of Hemacell Perfusion Inc., and John Cadwell of FiberCell Systems.

Worthington Tissue Dissociation Guide

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FREE Collagenase Sampling Program and Online Lot Selection Tool

Simple as 1, 2, 3... and completely Free!

1. Go to: www.worthington-biochem.com/cls/clsamp.html

Worthington Collagenase Sampling Program

The lot-to-lot variation which is typical of crude enzyme preparations such as Worthington crude collagenase makes it important to pre-test a particular lot of enzyme you are planning to use in your experiment. Many years ago we found that the most practical approach for the researcher is to presample several different lots of collagenase at a time and select the best of the group. As the world's leading manufacturer of collagenase, Worthington is able to offer the greatest number of different lots at any given time and recommend specific lots for an application.

There is no charge for participating in the collagenase sampling program. Under the program, individual researchers are provided with 100 mg samples of up to three different lots of collagenase for evaluation in their own assay systems. A period of 60 days is allowed for your evaluation of these samples. A minimum of 3 grams of each lot will be placed on HOLD, reserved in your name. When you determine which lot performs best for you, simply specify the lot desired when ordering.

To become part of this program, or to discuss any of the Worthington products, just call our **Technical Service group toll-free at 800.445.9603** from anywhere in the United States or Canada or e-mail techservice@worthington-biochem.com

International customers should check our International Distributor listing for a distributor. If you do not have a Worthington Distributor for your country, please contact International Sales or Technical Service.

2. Consider using the interactive lot selection tool...

Collagenase Lot Selection Tool Now Available Online!

Worthington's 'Collagenase Lot Selection Tool' is now available online at our website. This new feature was designed to help researchers select and evaluate current collagenase lots that match previous lots or desired activity profiles. Users may enter target values for collagenase, caseinase, clostripain, and tryptic activities or specify previous lot numbers. Each value can be weighted based on the relative level of importance to the application. After the search for matches is completed, a ranked list of collagenase lots currently available is generated. The selected lots can then be sampled simply by using the built in link to the **Free Collagenase Sampling Program**. As always, Worthington Customer and Technical Service personnel are available via phone at **800.445.9603/732.942.1660** and e-mail to assist with collagenase or any other products.

2. Complete the online Sampling request form...

...samples will be shipped and reserved amount of each lot placed on hold for 60 days pending your evaluation. Completely free and without obligation!

Collagenase Lot Selection Tool

(optional) Look-up assay values for historical lot number:
If you are looking for a lot similar to one you have used in the past, enter it here:
Lot Number:
 (Assay values will be entered below. You may then a matches.)

Find lot matches for specified assay values:

1. Enter amount of collagenase required: mg

2. Select desired collagenase type:

3. Enter target assay values and relative levels of importance:

	u/mg dw	u/mg dw	u/mg dw	u/mg
	caseinase	clostripain	tryptic	
Values:	<input type="text" value="235"/>	<input type="text" value="327"/>	<input type="text" value="2.4"/>	<input type="text" value="0.3"/>
Weights:	<input type="text" value="3"/>	<input type="text" value="3"/>	<input type="text" value="3"/>	<input type="text" value="3"/>

(Report will open in a new window.)

Collagenase Sampling Program Request

Mailing Address:

Email:

Contact Name:

Phone:

Requested Collagenase Type:

Specify up to 3 lot numbers if known:

How much would you like us to reserve for you? grams
(Minimum 3 grams)

Describe tissue to be dissociated:

Introduction

Tissue dissociation/primary cell isolation and cell harvesting are principal applications for enzymes in tissue culture research and cell biology studies. Despite the widespread use of enzymes for these applications over the years, their mechanisms of action in dissociation and harvesting are not well understood. As a result, the choice of one technique over another is often arbitrary and based more on past experience than on an understanding of why the method works and what modifications could lead to even better results.

The goal of a cell isolation procedure is to maximize the yield of functionally viable, dissociated cells. There are many parameters which may affect the outcome of any particular procedure including but not limited to:

1. Type of tissue
2. Species of origin
3. Age of the animal
4. Genetic modification(s) (knockouts, etc.)
5. Dissociation medium used
6. Enzyme(s) used
7. Impurities in any crude enzyme preparation used
8. Concentration(s) of enzyme(s) used
9. Temperature
10. Incubation times

The first four items generally are not a matter of choice. To achieve suitable results the other variable conditions are best defined empirically.

Researchers searching the scientific literature for information on the ideal enzymes and optimal conditions for tissue dissociation are often confronted with conflicting data. Much of the variation stems from the complex and dynamic nature of the extracellular matrix and from the historical use of relatively crude, undefined enzyme preparations for cell isolation applications. Also, the extracellular matrix is composed of a wide variety of proteins, glycoproteins, lipids and glycolipids, all of which can differ in abundance from species to species, tissue to tissue and with developmental age. Commonly used crude enzyme preparations such as Pronase, NF 1:250 and collagenase contain several proteases in variable concentrations, as well as a variety of polysaccharidases, nucleases and lipases.

This guide summarizes our knowledge of how these enzymes accomplish the “routine” operations of tissue dissociation and cell harvesting; describes standard lab procedures; offers a logical experimental approach for establishing a cell isolation protocol; and lists many tissue specific references.

Note: We have not limited the references listed to only those papers using Worthington enzymes. Generally speaking, the tissue dissociation enzymes offered by Worthington can be used interchangeably for most preparations cited.

Cell Isolation Theory

Tissue Types

This section summarizes the general characteristics of extracellular matrices associated with various types of tissue. Coupled with the descriptions of individual enzymes offered in the next section, this information will aid in choosing the enzyme(s) best suited for a particular tissue.

Epithelial Tissue

In the adult, epithelium forms such tissues as the epidermis, the glandular appendages of skin, the outer layer of the cornea, the lining of the alimentary and reproductive tracts, peritoneal and serous cavities, and blood and lymph vessels (where it is usually referred to as “endothelium”). Structures derived from outpouchings from the primitive gut, including portions of the liver, pancreas, pituitary, gastric and intestinal glands, are also composed of epithelial tissue.

Epithelial cells are typically packed so closely together that there is very little intercellular material between them. An extremely tight bond exists between adjacent cells making dissociation of epithelium a difficult process.

On the lateral surfaces of adjacent epithelial cells there are four distinct types of intercellular bonds: the *zonula occludens*, *zonula adherens*, *macula adherens* and *nexus*. The former three are often closely associated to form a junctional complex. In the *zonula occludens*, or “tight junction”, there are multiple sites of actual fusion of the adjacent unit membranes interspersed by short regions of unit membrane separation of approximately 100-150 Å. In a *zonula adherens*, or “intermediate junction”, a fine network of cytoplasmic filaments radiates from the cell membrane into the cytoplasm. The space between unit membranes of adjacent cells is approximately 150-200 Å and is composed of an intercellular amorphous substance of unknown composition. In the *macula adherens*, or “desmosome”, there is a somewhat similar array of intracellular filaments. The adjacent unit membrane space is approximately 150-200 Å and consists of an extracellular protein and glycoprotein ground substance, often with an electron-dense bar visible within it. The integrity of the desmosome requires calcium, and it is broken down by EDTA and calcium-free media. The enzymes collagenase, trypsin and hyaluronidase can also dissociate the desmosome. The *nexus*, or “gap junction”, covers most of the epithelial cell surface. In these areas, the unit membranes appear tightly attached and are separated by only 20Å. The intercellular material consists of an amorphous, darkly-staining substance.

On the basal surface of the epithelium where it overlays connective tissue, there is an extracellular bonding layer or sheet called the basal lamina. The lamina is composed of a network of fine, collagen-like reticular fibers embedded in an amorphous matrix of high and low molecular weight glycoproteins.

Tissue Dissociation Guide

Connective Tissue

Connective tissue develops from mesenchymal cells and forms the dermis of skin, the capsules and stroma of several organs, the sheaths of neural and muscular cells and bundles, mucous and serous membranes, cartilage, bone, tendons, ligaments and adipose tissue.

Connective tissue is composed of cells and extracellular fibers embedded in an amorphous ground substance and is classified as loose or dense, depending upon the relative abundance of the fibers. The cells, which may be either fixed or wandering, include fibroblasts, adipocytes, histiocytes, lymphocytes, monocytes, eosinophils, neutrophils, macrophages, mast cells, and mesenchymal cells.

There are three types of fibers: *collagenous*, *reticular*, and *elastic*, although there is evidence that the former two may simply be different morphological forms of the same basic protein. The proportion of cells, fibers and ground substance varies greatly in different tissues and changes markedly during the course of development.

Collagen fibers are present in varying concentrations in virtually all connective tissues. Measuring 1-10 μm in thickness, they are unbranched and often wavy, and contain repeating transverse bands at regular intervals. Biochemically, native collagen is a major fibrous component of animal extracellular connective tissue; skin, tendon, blood vessels, bone, etc. In brief, collagen consists of fibrils composed of laterally aggregated polarized tropocollagen molecules (M.W. 300,000). Each rod-like tropocollagen unit consists of three helical polypeptide α -chains wound around a single axis. The strands have repetitive glycine residues at every third position and an abundance of proline and hydroxyproline. The amino acid sequence is characteristic of the tissue of origin. Tropocollagen units combine uniformly in a lateral arrangement reflecting charged and uncharged amino acids along the molecule, thus creating an axially repeating periodicity. Fibroblasts and possibly other mesenchymal cells synthesize the tropocollagen subunits and release them into the extracellular matrix where they undergo enzymatic processing and aggregation into native collagen fibers. Interchain cross-linking of hydroxyprolyl residues stabilizes the collagen complex and makes it more insoluble and resistant to hydrolytic attack by most proteases. The abundance of collagen fibers and the degree of cross-linking tend to increase with advancing age, making cell isolation more difficult.

Reticular fibers form a delicate branching network in loose connective tissue. They exhibit a regular, repeating subunit structure similar to collagen and may be a morphological variant of the typical collagen fibers described above. Reticular fibers tend to be more prevalent in tissues of younger animals.

Elastic fibers are less abundant than the collagen varieties. They are similar to reticular fibers in that they form branching networks in connective tissues. Individual fibers are usually less than 1 μm thick and exhibit no transverse periodicity. The fibers contain longitudinally-arranged bundles of microfibrils embedded in an

amorphous substance called elastin. Like collagen, elastin contains high concentrations of glycine and proline, but in contrast has a high content of valine and two unusual amino acids, desmosine and isodesmosine. Fibroblasts and possibly other mesenchymal cells synthesize the elastin precursor, tropoelastin, and release it into the extracellular matrix where enzymes convert the lysine residues into the desmosines. Polymerization of elastin occurs during interchain cross-linking of the latter. In this state, elastin is very stable and also highly resistant to hydrolytic attack by most proteases.

The viscous extracellular ground substance in which connective tissue cells and fibers are embedded is a complex mixture of various glycoproteins, the most common being hyaluronic acid, chondroitin sulfate A, B, and C and keratin sulfate. Each of these glycoproteins is an unbranching polymer of two different alternating monosaccharides attached to a protein moiety. Hyaluronic acid, for example, contains acetyl glucosamine and glucuronate monomers and about 2% protein, while the chondroitin sulfates contain acetyl galactosamine and glucuronate or iduronate monomers and more than 15% protein. The relative abundance of these glycoproteins varies with the origin of the connective tissue.

Dissociating Enzymes

While many enzyme systems have been investigated by researchers performing primary cell isolations, the enzymes discussed here have been found satisfactory for a wide variety of tissues from many different species of various ages.

Collagenase

Bacterial collagenase is a crude complex containing a collagenase more accurately referred to as clostridiopeptidase A which is a protease with a specificity for the X-Gly bond in the sequence Pro-X-Gly-Pro, where X is most frequently a neutral amino acid. Such sequences are often found in collagen, but only rarely in other proteins. While many proteases can hydrolyze single-stranded, denatured collagen polypeptides, clostridiopeptidase A is unique among proteases in its ability to attack and degrade the triple-helical native collagen fibrils commonly found in connective tissue.

True collagenase may cleave simultaneously across all three chains or attack at a single strand. Mammalian collagenases split collagen in its native triple-helical conformation at a specific site yielding fragments, TC A and TC B, representing 3/4 and 1/4 lengths of the tropocollagen molecule. After fragmentation the pieces tend to uncoil into random polypeptides and are more susceptible to attack by other proteases.

Bacterial collagenases are usually extracted from host invasive strains. These enzymes differ from mammalian collagenases in that they attack many sites along the helix. Collagenases from *Clostridium histolyticum*, first prepared by Mandl, *et al.*, have been most thoroughly studied. Commercially available collagenase has been limited primarily to that from *Cl. histolyticum*; although, other sources have recently become available. Clostridial collagenase also degrades the helical regions in native collagen preferentially

Tissue Dissociation Guide

at the X-Gly bond in the sequence Pro-X-Gly-Pro where X is most frequently a neutral amino acid. This bond in synthetic peptide substrates may also be split.

Purified clostridiopeptidase A alone is usually inefficient in dissociating tissues due to incomplete hydrolysis of all collagenous polypeptides and its limited activity against the high concentrations of non-collagen proteins and other macromolecules found in the extracellular matrix. The collagenase most commonly used for tissue dissociation is a crude preparation containing clostridiopeptidase A in addition to a number of other proteases, polysaccharidases and lipases. Crude collagenase is well suited for tissue dissociation since it contains the enzyme required to attack native collagen and reticular fibers in addition to the enzymes which hydrolyze the other proteins, polysaccharides and lipids in the extracellular matrix of connective and epithelial tissues.

The first commercially available collagenase was offered by Worthington in 1959. At that time we offered one type of crude enzyme which we tested only for collagenase activity. Eventually, with the cooperation of many in the research community, four basic profiles were identified:

Type 1 containing average amounts of assayed activities (collagenase, caseinase, clostripain, and tryptic activities). It is generally recommended for epithelial, liver, lung, fat, and adrenal tissue cell preparations.

Type 2 containing greater proteolytic activities, especially clostripain activities. It is generally used for heart, bone, muscle, thyroid and cartilage.

Type 3 selected because of low proteolytic activity. It is usually used for mammary cells.

Type 4 selected because of low tryptic activity. It is commonly used for islets and other applications where receptor integrity is crucial.

Introduced in 2007, Animal Origin Free collagenase (code CLSAFA) is derived from cultures grown in medium completely devoid of animal based components and designed for bioprocessing applications where introduction of potential animal derived pathogens must be prevented. Levels of secondary proteases are similar to Types 1 and 2.

Correlations between type and effectiveness with different tissues have been good, but not perfect, due in part to variable parameters of use. Nevertheless most researchers consider the tissue-typing of crude collagenase lots to be a valuable service. A detailed description of the Worthington collagenase assay can be found in the Worthington Enzyme Manual or at: www.worthington-biochem.com.

If you find one of the types of collagenases suitable for your cell isolation procedure, you may want to try Worthington's Collagenase Sampling Program. This cost-free program lets researchers pre-sample different lots of collagenase and evaluate them in their specific applications to achieve the best combination of cell yield and viability. (See page 150 of this guide for further information.)

Trypsin

Trypsin is a pancreatic serine protease with a specificity for peptide bonds involving the carboxyl group of the basic amino acids, arginine and lysine. Trypsin is one of the most highly specific proteases known, although it also exhibits some esterase and amidase activity.

Purified trypsin alone is usually ineffective for tissue dissociation since it shows little selectivity for extracellular proteins. Combinations of purified trypsin and other enzymes such as elastase and/or collagenase have proven effective for dissociation.

"Trypsin" is also the name commercial suppliers have given to pancreatin, a crude mixture of proteases, polysaccharidases, nucleases and lipases extracted from porcine pancreas. NF 1:250, a commonly used "trypsin" preparation, has the potency to bring about the proteolytic digestion of 250 times its weight of casein under assay conditions specified by the National Formulary. It is important to realize that this assay procedure is not specific for trypsin, although pancreatin does contain this enzyme. Nomenclature notwithstanding, crude "trypsins" like NF 1:250 and 1:300 are widely used for dissociating tissues, perhaps because the tryptic and contaminating proteolytic and polysaccharidase activities do bring about a preferential attack of the extracellular matrix. It appears, however, that crude trypsin and crude collagenase dissociate tissues by different mechanisms, and difficulties are often encountered when using NF 1:250 preparations -- the most common being incomplete solubility, lot-to-lot variability, cell toxicity, and cell surface protein/receptor damage.

In tissue culture laboratories, researchers use purified trypsin to release cells into suspension from monolayers growing on the interior surfaces of culture vessels. Most cells originating from normal tissues and not highly adapted to artificial culture conditions grow in monolayers, i.e., a layer of cells one cell thick adhering to the interior surface of the culture vessel. Because such cells are more like cells in normal tissues, many tissue culture researchers are studying cells that grow in monolayer culture.

Monolayer cultures are commonly grown in glass or polystyrene roller bottles, culture flasks, or Petri dishes. Plastic vessels used in tissue culture work are specially treated to ensure good adherence of cells to the vessel walls. For a detailed discussion of cell harvesting, see page 6 of this guide.

Some of the most frequently used grades of purified trypsin for cell isolation procedures are the Worthington product Codes: TL, TRL, TRLS, and TRTVMF. These products are suitable for cell harvesting as well as tissue dissociation.

Elastase

Pancreatic elastase is a serine protease with a specificity for peptide bonds adjacent to neutral amino acids. It also exhibits esterase and amidase activity. While elastase will hydrolyze a wide variety of protein substrates, it is unique among proteases in its ability to hydrolyze native elastin, a substrate not attacked by trypsin, chymotrypsin or pepsin. It is produced in the pancreas as an

Tissue Dissociation Guide

inactive zymogen, proelastase, and activated in the duodenum by trypsin. Elastase is also found in blood components and bacteria.

Because elastin is found in highest concentrations in the elastic fibers of connective tissues, elastase is frequently used to dissociate tissues which contain extensive intercellular fiber networks. For this purpose, it is usually used with other enzymes such as collagenase, trypsin, and chymotrypsin. Elastase is the enzyme of choice for the isolation of Type II cells from the lung.

Hyaluronidase

Hyaluronidase is a polysaccharidase with a specificity for endo-N-acetylhexosaminic bonds between 2-acetoamido-2-deoxy-beta-D-glucose and D-glucuronate. These bonds are common in hyaluronic acid and chondroitin sulfate A and C. Because these substances are found in high concentrations in the ground substance of virtually all connective tissues, hyaluronidase is often used for the dissociation of tissues, usually in combination with a crude protease such as collagenase.

Papain

Papain is a sulfhydryl protease from *Carica papaya* latex. Papain has wide specificity and it will degrade most protein substrates more extensively than the pancreatic proteases. It also exhibits esterase activity.

With some tissues papain has proven less damaging and more effective than other proteases. Huettner and Baughman (*J. Neuroscience*, 6, 3044 (1986)) describe a method using papain to obtain high yields of viable, morphologically intact cortical neurons from postnatal rats which is the basis of the Worthington Papain Dissociation System described on page 14.

Chymotrypsin

Chymotrypsin is a protease which preferentially catalyzes the hydrolysis of peptide bonds involving the aromatic amino acids tyrosine, phenylalanine, and tryptophan. In addition it acts upon the peptide bonds of leucyl, methionyl, asparagenyl and glutamyl residues, and the amides and esters of susceptible amino acids.

Chymotrypsin is used to a limited extent in tissue dissociation, usually in combination with trypsin and elastase.

Deoxyribonuclease I

Often as a result of cell damage, deoxyribonucleic acid leaks into the dissociation medium increasing viscosity and causing handling and recovery problems. Purified deoxyribonuclease (DNase) is sometimes included in cell isolation procedures to digest the nucleic acids without damaging the intact cells.

Neutral Protease (Dispase)

Neutral Protease (Dispase) is a bacterial enzyme produced by *Bacillus polymyxa* that hydrolyses N-terminal peptide bonds of non-polar amino acid residues and is classified as an aminopeptidase. Its mild proteolytic action makes the enzyme especially useful for the isolation of primary and secondary (subcultivation) cell culture since it maintains cell membrane integrity.

Neutral Protease (Dispase) is also frequently used as a secondary enzyme in conjunction with collagenase and/or other proteases in many primary cell isolation and tissue dissociation applications. Neutral Protease (Dispase) dissociates fibroblast-like cells more efficiently than epithelial-like cells so it has also been used for differential isolation and culture applications. Other advantages are its non-mammalian (bacterial) source and its ability to be inhibited by EDTA.

Trypsin Inhibitor (soybean)

The trypsin inhibitor from soybean inactivates trypsin on an equimolar basis; however it exhibits no effects on the esterolytic, proteolytic or elastolytic activities of porcine elastase. Cell isolation procedures occasionally call for a trypsin inhibitor, usually the inhibitor from soybean (Worthington Code: SIC).

Trypsin Inhibitor (ovomuroid)

The trypsin inhibitor from ovomucoid (egg white) also inhibits papain activity. With papain's increased use in neural and endocrine cell isolation applications, ovomucoid trypsin inhibitor is more widely used as an efficient way to stop these digestions. It is also a component of Worthington's Papain (Neural) Dissociation System, supplied combined with BSA to create a single step density gradient for papain inhibition and cell recovery.

Animal Origin Free (AOF) Enzymes

General interest in Animal Origin Free (AOF) tissue dissociation enzymes has dramatically increase to avoid potential contamination with mammalian agents such as prions and viruses. Worthington produces several AOF collagenases, proteases and nucleases for those requiring AOF enzymes, please check online or our current catalog for these products.

Note: Application specific cell isolation systems have been developed by Worthington to eliminate the need for experimenting with various enzyme combinations and use testing several lots of collagenase. Descriptions for these systems begin on page 10 of this guide.

Cell Isolation Techniques

Methods and Materials

Working With Enzymes

- All of the enzymes Worthington offers for tissue dissociation applications are available as lyophilized powders for convenience, versatility, and stability. As such they may be stored at 2 – 8°C, and they can be shipped without special handling. While lyophilization makes shipping and storing the enzymes easier, special care is required when opening any of the vials.
- Lyophilized proteins tend to be very hygroscopic so they should not be opened in humid areas. Be sure that any vial has

Tissue Dissociation Guide

been brought to room temperature before opening. Ideally, the vials should be taken from the refrigerator at least a half hour before opening, and they should be left in a dessicator. Before opening any of the vials, be sure it is not at all cool to the touch. All of the cell isolation enzymes cited in this section can be repeatedly warmed to room temperature and then returned to the refrigerator as long as these precautions are followed.

- Once diluted with media or buffer, proteolytic enzymes may undergo autolysis. Dissolve enzymes immediately before use.
- Special care must be taken with the deoxyribonuclease. This product is very prone to shear denaturation. Mix gently.
- Reconstituted enzymes should not be stored at 2–8°C. If necessary they can be aliquoted and frozen at –20°C. Avoid repeated freeze-thaw cycles.
- All enzymes, upon reconstitution, can be sterile filtered through a 0.22µm pore size membrane.
- Generally most of the enzymes used in cell isolation procedures (except trypsin) can be directly dissolved in a balanced salt solution or buffer of choice. Stock solutions of trypsin should be made initially by reconstituting the enzyme in 0.001N HCl. This solution can be diluted into the digestion medium or buffer immediately prior to use.

Basic Primary Cell Isolation Protocol

(Refer to references for application specific parameters)

- For non-perfusion, mince or cut the isolated piece of tissue into 2–4 millimeter pieces with sterile scissors or scalpel.
- Add the tissue pieces to the appropriate buffer or balanced salt solution on ice and wash 2–3 times.
- Add appropriate amount of enzyme(s) and incubate at optimum temperature (usually 37°C) for appropriate time, mixing intermittently.
- Gently disperse the cells by pipeting (trituration).
- Filter the cell suspension through fine mesh.
- Allow the cells to settle and decant excess liquid containing enzymes. Wash and repeat 2–3 times.
- Resuspend cells in appropriate medium or buffer.
- Quantitate cell yield and viability.
- Seed cells for culture, if required.

Perfusion procedures require special equipment and techniques for recirculating the buffers, media and enzymes. Please refer to referenced texts for additional information and guidance.

Balanced Salt Solutions

The compilation of standard balanced salt solutions with their references found in the following table can be helpful in selecting an appropriate dissociation solution.

Standard Solution Table

Composition of Selected Balanced Salt Solutions^{a,b}

	Ringer ^c	Tyrode ^{d,e}	Gey ^f	Earle ^g	Puck ^h	Hanks ⁱ	Dulbecco (PBS) ^{j,k}
NaCl	9.00	8.00	7.00	6.80	8.00	8.00	8.00
KCl	0.42	0.20	0.37	0.40	0.40	0.40	0.20
CaCl ₂	0.25	0.20	0.17	0.20	0.012	0.14	0.40
MgCl ₂ •6H ₂ O		0.10	0.21			0.10	0.10
MgSO ₄ •7H ₂ O			0.07	0.10	0.154	0.10	
Na ₂ HPO ₄ •1 ₂ H ₂ O			3.00		0.39	0.12	2.31
NaH ₂ PO ₄ •H ₂ O		0.05		0.125			
KH ₂ PO ₄			0.03		0.15	0.06	0.20
NaHCO ₃		1.00	2.27	2.20		0.35	
Glucose		1.00	1.00	1.00	1.10	1.00	
Phenol Red				0.05	0.005	0.02	
Atmosphere	air	air	95%air/ 5%CO ₂	95%air/ 5%CO ₂	air	air	air

a Amounts are given as grams per liter of solution

b In some instances the values given represent calculations from data presented by the authors to account for the use of hydrated or anhydrous salts

c S. Ringer, *J. Physiol. (London)* 18, 425 (1895)

d M.V. Tyrode, *Arch. Int. Pharmacodyn. Ther.*, 20, 2025 (1910)

e R.C. Parker, "Methods of Tissue Culture", 3rd ed., p. 57, Harper, New York, 1961

f G.O. Gey and M.K. Hey, *Am J. Cancer*, 27, 55 (1936)

g W.R. Earle, *J. Natl. Cancer Inst*, 4, 165 (1943)

h T.T. Puck, S.J. Cieciora, and A. Robinson, *J. Exp. Med.* 108, 945 (1958)

i J.H. Hanks and R.E. Wallace, *Proc. Soc. Exp. Biol. Med.*, 71, 196 (1949)

j PBS, phosphate-buffered saline

k R. Dulbecco and M. Vogt, *J. Exp. Med.*, 99, 167 (1954)

Equilibration with 95% O₂:5% CO₂

In many cell isolation procedures it is important to the survival of the tissue during dissociation that the incubation medium be both well oxygenated and buffered at physiological pH. Both requirements are satisfied when the medium is equilibrated with 95%O₂:5%CO₂. Several balanced salt solutions contain the pH sensitive indicator dye, phenol red. When it is red or purple in color, the medium is too alkaline. This sometimes occurs when the tissue is placed in the dissociation enzyme solution. Reequilibration with O₂:CO₂ is usually necessary prior to incubation.

Gas should not be bubbled directly into any solution containing protein. This can result in frothing and denaturation of the protein with loss of biological activity. Gas can be sterilized by passage through a 0.22 micron membrane filter or through a sterile fiber plug such as the cotton plug in a sterile Pasteur or volumetric pipette. While mixing the solution, pass O₂:CO₂ continuously through the space above the liquid until color indicates pH 7.2–7.4. The balanced salt solution is often pre-gassed but should be equilibrated with sterile O₂:CO₂ each time the bottle is opened.

Buffered balanced salt solutions will usually maintain constant pH regardless of the degree of oxygenation/carbonation and as a result can be easier to work with. Certain cell types may be

Tissue Dissociation Guide

sensitive to particular buffer salts. The reference tables can be useful in selecting an appropriate balanced salt solution, buffer, or dissociation media for a specific application.

Trituration

(Cell dispersion through mild pumping action)

This can be a crucial procedure. It serves to break up the tissue fragments following incubation in the dissociation mix. If done too vigorously, cells will be destroyed lowering viability; too weakly and tissue fragments will be left intact lowering yield. Gentle trituration, using a 10ml pipette, constitutes filling and emptying the barrel at a rate of about 3.0ml per sec. You can best determine a suitable rate for your tissue through trial and error. Avoid bubbling the cell suspension.

Enzymatic Cell Harvesting

Most non-malignant cells growing *in vitro* move about and divide until they form a monolayer one cell thick completely covering the surfaces of the culture vessel. Movement and proliferation normally cease when confluence is reached. Harvesting cells for study, processing or subculture requires dissociation and detachment of the monolayer. Limited treatment of the cell layer with the enzyme trypsin is the method most frequently applied.

It was formerly thought that trypsin preparations simply hydrolyzed a proteinaceous adhesive bonding substance responsible for the tenacious attachment of cells to their substratum with the resultant detachment of the cells from the culture vessel. It is now felt that the mechanism of action of trypsin in cell harvesting is more complex. This section summarizes recent information on this subject.

Cell Adhesion and Harvesting

During interphase, fibroblast-like cells in culture are spread out on the substratum in a characteristic, spindle-shaped configuration. There are differences of opinion as to whether the actual areas of cell adhesion are distributed over most of the undersurface of the cell or are localized in relatively narrow patches near the cell margins, principally in the vicinity of ruffling activity. In either case, these areas of adhesion appear to be composed of clusters of attachment points, each about 1 μm in diameter. The individual attachment points are apparently the distal portions of a cell cytoskeleton structure bound to the substratum.

Within minutes after subjecting cultured cells to cold temperatures, chelating agents or trypsin solutions, they change shape drastically by rounding up and blebbing. Electron micrographs show many long retraction fibers with a diameter of 0.25 – 0.5 μm running from the surface of the rounded cell body to enlarged, terminal bulb attachment points previously located on the flattened cell's undersurface.

The cells remain attached to the substratum until the fibers are broken, either mechanically by tapping or shaking the culture

vessel, or chemically by the continued action of chelators and/or trypsin. (Cold temperatures alone are sufficient for rounding up but not for detachment. These conditions also greatly diminish the entry of trypsin into the cell.) Soon after cell detachment from the surface of the culture vessel, and subculture into new vessels containing trypsin-free medium, cytoplasm flows into the broken retraction fibers and refills them. Within an hour the rounded cells begin to take on their characteristic shape.

Trypsin for Cell Harvesting

In 1916, Rous and Jones used “the trypsin powders of Merck, Brubler and Kahlbaum” to digest the plasma clots in which living cells were growing in order to obtain a cell suspension for subculturing. Vogelaar and Erlichman in 1934 were the next researchers to utilize the digestive enzymes in a crude trypsin preparation to liquify the coagulated plasma in which human fibroblasts were growing prior to subculturing. Techniques using trypsin similar to those used today were introduced by Scherer, Syverton and Gey in 1953 to harvest the then newly cultivated HeLa cell strain for subculturing and biochemical analysis. These workers tested both recrystallized trypsin and NF 1:250 trypsin for cell harvesting and found that the purified trypsin was more potent and less toxic to cells. Nevertheless the NF 1:250 preparation was employed for routine harvesting simply because it was less expensive.

Relatively crude pancreatic preparations like NF 1:250 trypsin are still used today for cell harvesting in spite of the fact that they exhibit considerable lot-to-lot variability and contain extraneous substances and other enzymatic activities. Impurities in crude trypsin can cause unnecessary damage to cells and a reduction of cloning efficiency. Use of higher purity crystalline trypsin can eliminate many of these difficulties.

None of the contaminants present in the NF 1:250 materials appears to be essential for cell harvesting activity since purified trypsin is very effective for monolayer dissociation, and since crude NF 1:250 trypsin plus soybean trypsin inhibitor is ineffective.

McKeehan and Ham report markedly improved viability and multiplication potential to single cells in low serum medium when harvesting with crystalline trypsin at reduced temperatures, i.e., at 4°C.

Cell Release Procedure

In order to transfer or pass cells in monolayer culture from one culture vessel to another it is necessary to release cells from the monolayer into suspension so that they can be easily handled by pipetting and diluting.

Releasing cells from the monolayer is almost always accomplished with purified trypsin by a procedure known as trypsinization. A usual trypsinization procedure follows.

Trypsinization Procedure

1. Remove culture medium from cells.
2. Add sterile trypsin solution (in BSS-balanced salt solution, normally calcium-free Hanks).
3. Allow trypsin solution to act on monolayer for several minutes at room temperature or 37°C. (or longer at 2-8°C.)
4. Remove trypsin solution gently, so as not to disturb cells.
5. Add BSS or media (often with serum or trypsin inhibitor to inactivate residual trypsin) and agitate vessel to disrupt monolayer and suspend cells.

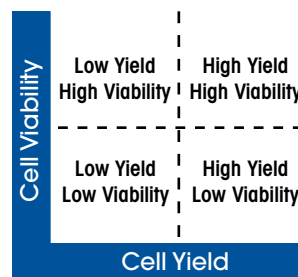
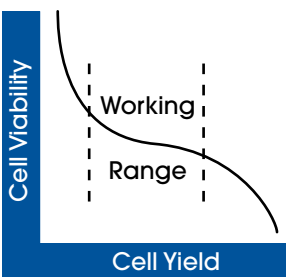
Note: Some researchers have found that procedures using crystalline trypsin can provide increased viability in cells after they are released. Viability is usually determined by measuring cloning efficiency, i.e., the ability of a single cell to attach to the wall of a culture vessel and divide to produce a colony of cells which is visible to the naked eye after staining.

Optimization Techniques

General Guidelines

Although optimization of a cell isolation procedure for a particular cell type is dependent upon the adequate recovery of cells having various required characteristics, some guidelines can be established. The information in this guide regarding cell isolation and the enzymes used, when combined with logic and suitable experimental design, should lead to the development of a satisfactory cell isolation method. (See Freshney, 1987 for a detailed discussion) The complex relationship between cell yield and viability can be represented by the simplified illustrations shown below. In general there is an area of optimized recovery balanced between yield and viability; working near the middle of this range will reduce variability in the results of the cell isolation procedure. Understanding this relationship and how it can vary with a particular cell type and application, can make the optimization process easier.

For troubleshooting purposes various possible results, along with suggested corrective actions are listed below. Keep in mind that there are no clear lines between the quadrants but rather converging zones with variable areas of overlap.



Low Yield/Low Viability: *Over/under dissociation, cellular damage.* Change to less digestive type enzyme and/or decrease working concentration. (e.g. from trypsin to collagenase/ from Type 2 collagenase to Type 1).

Low Yield/High Viability: *Under dissociation.* Increase enzyme concentration and/or incubation time and monitor both yield and viability response.

If yield remains poor, evaluate a more digestive type enzyme and/or the addition of secondary enzyme(s).

High Yield/Low Viability: *Good dissociation, cellular damage.* Enzyme overly digestive and/or at too high a working concentration. Reduce concentration and/or incubation time and monitor yield and viability response.

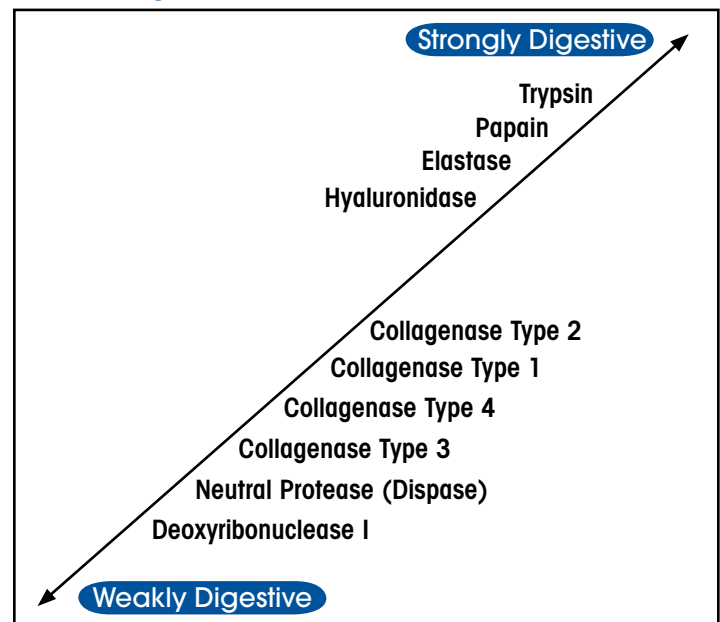
Try diluting the proteolytic action by adding bovine serum albumin (BSA) (0.1 = 0.5% w/v) or soybean trypsin inhibitor (0.01 - 0.1% w/v) to the dissociation.

Try using less proteolytic enzyme although yield may be affected and should be monitored.

High Yield/High Viability: *The place to be!* Consider evaluating the effect of dissociation parameters to learn their limitations for future reference.

A scale showing the relative digestive power of the enzymes commonly used follows for reference. Refer to this scale when troubleshooting a dissociation and planning isolation strategy.

Enzyme Digestion Scale



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Optimization Strategy

Review the reference tables starting on page 22 for the particular tissue and cell type of interest, and then apply this information to the practical application of tissue dissociation. An example of a basic optimization strategy follows:

Based upon the enzyme(s) cited, working concentrations and the buffer or media system used, set up proposed preliminary dissociation conditions similar to the closest available reference(s) listed in the tables.

Note: If a majority of the most similar referenced procedures cite the use of more than one enzyme, optimize the concentration of the primary enzyme (the one at the highest relative concentration) before adding the secondary enzyme(s). For example, if the two most similar references cite collagenase 0.1% with DNase 0.01% and collagenase 0.075% with hyaluronidase 0.025%, optimize the collagenase concentration empirically before evaluating the effects of either the hyaluronidase or the deoxyribonuclease. After optimizing the primary enzyme's concentration and incubation conditions evaluate any secondary enzyme(s).

1. Initially vary the concentration of the primary enzyme approximately 50% relative to the referenced procedure(s). The above example of collagenase concentrations 0.1% and 0.075% suggests an evaluation of enzyme concentrations between 0.025% and 0.15%. The concentration increments should be evenly distributed to cover this entire range. As a result incremental concentrations of 0.025%, 0.05%, 0.075%, 0.10%, 0.125% and 0.15% would be indicated. To simplify the initial screening the middle of the range can be selected and, after evaluation of yield and viability results, a decision can be made regarding the need for further studies. In this case initial collagenase concentrations evaluated may be 0.05%, 0.075%, 0.10% and 0.125%.

Note: Historically, most tissue dissociation and cell isolation protocols have cited the enzyme concentration used in terms of weight per unit volume (w/v). More recently, however, some researchers have begun to use the enzymes on an activity basis, that is, units per milliliter (u/ml). Use either method but consider the advantages and disadvantages of each:

1. The traditional weight per unit volume method most likely resulted from the use of cruder, partially purified mixtures of enzymes and is used independently of any specific or contaminating activities which may be present. With some of these crude preparations the lot-to-lot variation can be significant resulting in up to a two-fold difference in the amount of enzymatic activity added on a weight basis.
2. Adding by activity can result in a possible two-fold difference in the amount of weight added to a dissociation; however, normalizes the potency used based upon the primary activity for each lot.

Both methods ignore the relative contaminant activity levels. Upon establishing a basic method, consider pre-sampling different lots of enzyme(s) to evaluate these factors and to select a lot of enzyme which has minimal effect upon the critical parameters of a specific application.

Important: For accurate evaluation of a particular procedure's performance, cell yield and viability should be quantitated and compared. After optimizing basic dissociation and isolation conditions, the specific application parameters such as metabolic function(s) or receptor binding capability should also be evaluated. Based upon these results the method may be judged suitable for use or re-optimized for higher retention of native cellular characteristics.

Cell Quantitation

It is important to quantitate the results of each dissociation step in order to effectively evaluate each procedure. The use of a cell counting chamber (hemocytometer) for yield quantitation and the use of trypan blue for viability quantitation are recommended. The use of a hemocytometer for cell yield quantitation is outlined; however, newcomers to this procedure can refer to more detailed discussions (see Freshney, *Culture of Animal Cells*, page 227).

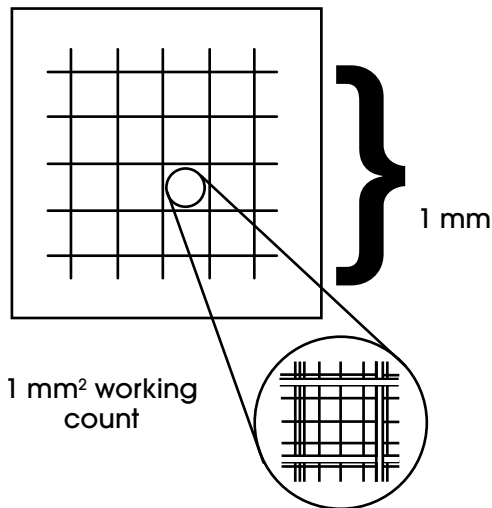
Required Supplies:

Improved Neubauer Hemocytometer
Cell Compatible Media or BSS
Pasteur Pipet or Micropipettor
Microscope (10X)
Counter

Procedure:

1. Carefully clean the counting chamber surface and the coverslip of the hemocytometer with 70% isopropanol and allow to air dry. Be careful not to scratch these surfaces.
2. Wet the sides of the coverslip with reagent grade water and align the coverslip over the counting chamber.
3. Take a **well mixed** 20-50 μ l aliquot of the dissociated cell suspension using either a Pasteur pipet or a micropipettor only drawing the cells into the tip. **Immediately** transfer the cell suspension to the counting chamber by placing the tip of the pipet at the edge of the chamber and allowing the chamber to fill completely via capillary action. Do not over- or underfill the chamber.
4. Repeat this procedure using another aliquot sample for the second chamber on the opposite side of the hemocytometer.
5. Place the hemocytometer on the microscope stage and, using the 10X objective, focus on the counting chamber grid lines. Adjust the contrast as needed to clearly see both the grid and the dispersed cells.

6. Adjust the field area by slowly moving the slide to obtain a central grid bounded by three lines on all sides (see figure below). Count the total number of cells present in this 1 mm² area including those cells which are on the top and left borders and excluding those on the right and bottom borders.



7. For accuracy count at least 100-500 cells. Depending upon yield and density more or fewer areas may be counted.
8. Repeat the count for the second chamber. If no second chamber exists, the slide should be cleaned and the process repeated.

Calculation:

$$C = \bar{N} \times 10^4$$

where C = cells per milliliter
 \bar{N} = average of cells counted
 10^4 = volume conversion factor for 1 mm²

$$\text{Total Yield} = C \times V$$

where V = total volume of cells (ml)

Example:

$$\text{Count}_1 = 182 \text{ cells/mm}^2$$

$$\text{Count}_2 = 175 \text{ cells/mm}^2$$

$$\text{Volume of Cells} = 55 \text{ ml}$$

$$\begin{aligned} \text{Average cells counted} &= \frac{\text{Count}_1 + \text{Count}_2}{2} \\ &= \frac{182 + 175}{2} \\ &= 178.5 \end{aligned}$$

$$C = 178.5 \times 10^4 = 1,785,000 \text{ cells/ml}$$

$$\text{Total yield} = C \times V = 1,785,000 \times 55 = 98,175,000 \text{ cells}$$

Note: For best results the cell density should be at least 10⁵ cells per milliliter. Common errors occur by improper mixing of the cell suspension prior to sampling and/or by allowing the cells to settle in the pipet prior to loading the hemocytometer counting chamber. Avoid the counting of multiple cell aggregates; the presence of aggregates indicates incomplete dissociation which may require further optimization of the isolation parameters. A single cell suspension provides the best results.

Measure of Viability

One of the simplest methods to approximate cell viability is the dye exclusion technique. This method utilizes an indicator dye to demonstrate cell membrane damage. Cells which absorb the dye become stained and are considered non-viable. Dyes such as trypan blue, erythrosin, and nigrosin are commonly used with trypan blue being the most common in preliminary cell isolation procedures.

This procedure can be performed along with the cell counting procedure but cell density may require adjustment in order to obtain approximately 10⁶ cells per milliliter.

Procedure

1. Mix 1 drop of trypan blue with one drop of the cell suspension and allow 1 - 2 minutes for absorption
2. Prepare hemocytometer and load chambers as described in "Cell Quantitation".
3. Count both the total number of cells and the number of stained (dark) cells.

Calculation

$$\text{Percent Viability} =$$

$$\frac{\text{Total Cells Counted} - \text{Stained Cells}}{\text{Total Cells Counted}} \times 100$$

Example

$$\text{Total Cells} / 1 \text{ mm}^2 = 182$$

$$\text{Stained Cells} = 24$$

$$\begin{aligned} \% \text{ Viability} &= \frac{182 - 24}{182} \times 100 \\ &= \frac{158}{182} \times 100 \\ &= 86.8\% \text{ Viability} \end{aligned}$$

Note: Dye exclusion viability procedures tend to give high estimates of cell viability when compared to cell attachment or metabolic assays, but for optimization of cell isolation procedures trypan blue does provide a rapid estimate of dissociation performance in conjunction with yield quantitation.

Tissue Dissociation Guide

Use-Tested Cell Isolation Systems

Worthington currently offers Cell Isolation Systems which are kits containing enzymes and other required reagents for performing tissue dissociation without having to purchase individual packages of one or more enzymes and pre-testing various lots of some enzymes. Some are designed for working with specific tissues, and one kit is a general purpose procedure development system. In all cases the enzymes which are included in the kits are regular Worthington products which can be purchased independently as needed.

Cell Isolation Optimizing System (CIT)

The Cell Isolation Optimizing System is a complete method development kit containing an assortment of enzymes most frequently used in tissue dissociation and cell isolation procedures. The kit includes instructions, references and strategies for the handling, use and optimization of enzymatic cell isolation methods to achieve maximum yield of viable cells. The system is designed to offer versatility in developing a method of obtaining cells from many different tissue types and sources in a cost-efficient manner.

The “System” contains all of the enzymes produced by Worthington commonly referenced in tissue dissociation and cell isolation procedures along with the **Cell Isolation Guide** detailing the various enzymes, tissue culture techniques, and protocol optimization guidelines similar to those outlined in this guide. In addition the guide lists hundreds of cell and tissue specific isolation references for getting started in enzymatic cell isolation.

CIT Kit Contents

Enzyme	Code*	Qty/Vial
Collagenase Type 1	CLS1	500 mg dw
Collagenase Type 2	CLS2	500 mg dw
Collagenase Type 3	CLS3	500 mg dw
Collagenase Type 4	CLS4	500 mg dw
Trypsin	TRL	500 mg dw
Hyaluronidase	HSE	50,000 Units
Elastase	ESL	100 mg P
Neutral Protease (Dispase®)	NPRO	10 mg dw
Papain	PAPL	100 mg P
Deoxyribonuclease I	DP	25 mg dw
Trypsin Inhibitor	SIC	100 mg dw

dw = dry weight

P = protein

*The code which appears in the table for each of the enzymes corresponds to the codes found in our regular catalog.

It is intended to serve both as a development tool for the experienced researcher and as an educational aid for students of cell biology.

Hepatocyte Isolation System

Introduction

Most traditional methods published for isolating hepatocytes use crude and partially purified enzyme preparations including various types of collagenase and other proteases. More recently the use of better characterized preparations of collagenase such as Worthington Types 1 and 4 (CLS-1, 4) have provided better results. All crude collagenase preparations can contain lot-variable contaminating proteases, esterases and other enzymes requiring researchers to pre-screen several lots of enzyme and/or continually modify isolation parameters and protocols.

The Worthington Hepatocyte Isolation System has been developed to provide researchers with a reliable, convenient, and consistent hepatocyte cell isolation system. By using the pre-optimized combination of enzymes contained in this kit, it is possible to minimize the lot-to-lot variation and improve the quality of the isolated hepatocytes. In addition, Worthington use-tests each lot by isolating hepatocytes from adult rat to assure performance, reliability, and consistent yield of viable cells.

The method is based on that described by Berry, M.N., modified by Seglen, P.O. (Methods in Cell Biology, vol XIII, David M. Prescott ed., Academic Press, 1976; Chapter 4, “Preparation of Isolated Rat Liver Cells”, pp 29-83), and further optimized in conjunction with several researchers.

The Hepatocyte Isolation System has also been adapted for the isolation of hepatocytes from mice. Please contact Worthington’s Technical Service for additional mouse application information.

Description and Package Contents

The package contains sufficient materials for five separate adult rat liver perfusions. For larger or smaller tissue applications, prepare proportionate volumes of reagents at each step and combine them in the same ratio as described in the protocol.

Vial #1: 10X CMF-HBSS Concentrate, 1 bottle, 500ml
Sterile calcium- and magnesium-free Hank’s Balanced Salt Solution (CMF-HBSS). The solution is used for washing and perfusing the liver prior to the addition of the dissociating enzyme solution.

Vial #2: Collagenase-Elastase Enzyme Vial, 5 Vials
Worthington collagenase (Code: CLS-1) and elastase (Code: ESL), filtered through 0.22µm pore size membrane, and lyophilized. Before use, reconstitute with the L-15/MOPS solution and swirl gently to dissolve contents as directed in the following procedure. Store unconstituted vials at 2–8°C.

Vial #3: 1,000 Units DNase I each, 5 Vials
Worthington DNase I (Code: D), filtered through 0.22µm pore size membrane, and lyophilized. Before use, reconstitute with L-15/MOPS solution and swirl gently to dissolve contents as directed in the following procedure. Store unconstituted vials at 2–8°C.

Vial #4: 0.15M MOPS, pH 7.5, 1 bottle, 75ml
0.15M MOPS, pH 7.5 buffer concentrate, used to buffer the reconstituted Leibovitz L-15 media.

Tissue Dissociation Guide

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Tel: 800.445.9603 Fax: 800.368.3108 • 732.942.9270
www.worthington-biochem.com • www.tissuedissociation.com

Vial #5: 7.5% Sodium Bicarbonate (NaHCO₃), 1 bottle, 100ml
7.5% Sodium bicarbonate concentrate, used to buffer the diluted CMF-HBSS.

Pouch, containing Leibovitz L-15 Media Powder: 1 x 1L
Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing approximately 800ml of cell culture grade water. Rinse pouch 2 - 3 times with an additional 100ml water. Bring total volume to 1000ml and filter through a 0.22 micron pore size membrane.

Required for Perfusion Isolation but not Included:

- Equipment and tools for animal anesthesia and surgery
 - A perfusion apparatus with a bubble trap suitable for liver perfusion at 10-30ml/min, 37°C. The tubing to be inserted into the portal vein is thin-walled with an inner diameter of 0.35-0.45mm
- Note: Measure the dead volume of the perfusion circuit*
- A low-speed centrifuge suitable for sedimentation of hepatocytes
 - Labware for cell sedimentation, and culture or incubation including sterile 150 X 25mm culture plates
 - A means to count or estimate the yield of cells
 - A means to sterile-filter solutions, if desired
 - Cell culture media and supplies, if needed
 - Sterile cell culture grade water
 - Concentrated antibiotics: penicillin, streptomycin, Fungazone, etc. for culture, if needed.
 - Surgical thread, silk, size 000
 - Heparin (optional)

For Cell Quantitation and Viability Assessment:

- Improved Neubauer hemocytometer
- Counter
- Pasteur pipet or micropipettor
- Microscope (10X), preferably inverted phase-contrast
- Standard 10ml serological pipets

Note: The following procedure presumes previous experience in liver digestion and cell isolation. For those not experienced, refer to the publication by Seglen referenced above, or to Alpini *et al.* entitled "Recent Advances in the Isolation of Liver Cells" published in *Hepatology* (1994) 20:494-514. Perfusion of the liver while still in the peritoneal cavity is described in "Isolated Hepatocytes Preparation, Properties and Application", by Berry, M.N., Edwards, A.M. and Barritt, GJ; RH Burdon and PH Van Knippenberg, eds., Elsevier, Amsterdam, New York, Oxford, Chapter 2.

Procedure For Cell Isolation

I. Preliminary Steps for Digestion of 1 Liver

The volumes specified in the following protocol are suitable for perfusion volumes of approximately 80-100ml. Proportional adjustments may be necessary for different perfusion systems.

Note: Sterile techniques, glassware and plasticware should be used. The use of a sterile hood is also recommended to avoid culture contamination.

Prepare:

• **Vial #1, 10X CMF-HBSS:** Dilute 100ml of the 10X CMF-HBSS with 850ml of sterile water and add 4.7ml of 7.5% Sodium Bicarbonate (Vial #5, NaHCO₃) in a sterile 1L bottle. Adjust pH if necessary to 7.4. Bring (QS) to a total volume of 1L with sterile water. If sterile water is not available, mix ingredients and sterile (0.22u) filter. Makes a total of 5L.

• **Leibovitz L-15 Media, 1 x 1L:** Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing 800ml of cell culture grade water. Rinse pouch 2 - 3 times with an additional 100ml water. Bring total volume to 1000ml and filter through a 0.22 micron pore size membrane.

• **Enzyme Buffer Solution:** Combine 13.3ml of MOPS concentrate with 10ml sterile water and 76.7ml of L-15 in a sterile 100ml bottle. Transfer sufficient L-15/MOPS into one each of Vial #2 and into one Vial #3 to dissolve the contents, mix gently to completely dissolve and transfer the enzymes back to the 100ml bottle. The collagenase, elastase and DNase concentrations will be approximately 225U/ml, 0.3U/ml and 10U/ml, respectively.

• Flush the sterile perfusion apparatus with CMF-HBSS, eliminating all air from the system except that in a bubble trap.

• Place the 150 x 25mm or equivalent Petri dish close to the perfusion apparatus to receive the perfused liver.

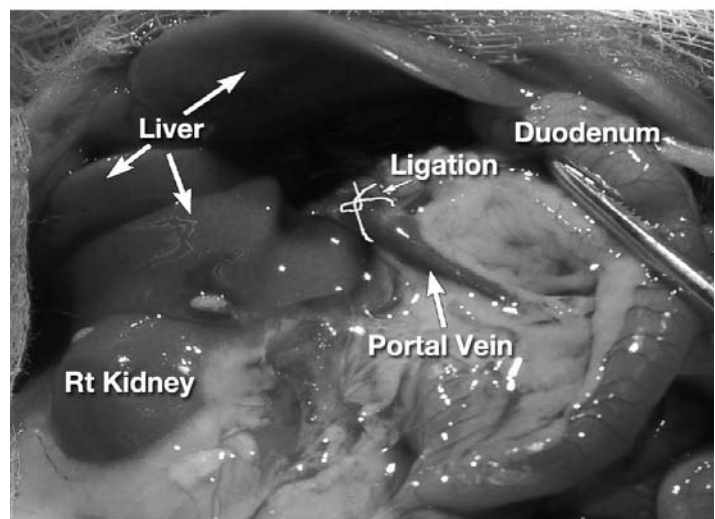
II. Perfusion and Digestion of Adult Rat Liver

The following steps should be performed in a laminar flow hood or safety cabinet. In particular, the digested liver should be processed under sterile conditions unless acute incubations will terminate the procedures.

1. Pretreatment of the rat with heparin is helpful. Inject i.p. about 20 minutes before perfusion, or into a vein (Seglen suggests the ilio-lumbar vein) after opening the abdomen. Use from 100-200U/100g body weight.

2. Anesthetize a rat, 200-400g weight, and position it for dissection. Install sufficient padding under the rat to hold the blood and initial perfusate. Place the rat on its back, tape down the legs, sterilize the abdomen with an iodine solution or 70% ethanol, and open the abdomen to expose the liver. Move the intestines to the left side of the abdomen (to the right as you look down with the rat's head away from you) exposing the hepatic portal vein.

3. Using a pair of fine, curved forceps, place a segment of 000 surgical thread underneath and around the portal vein just above (toward the head) the intersection of the portal vein and the final mesenteric vein close to the liver. Tie a loose half-square or equivalent knot around the vein. Locate the vena cava so it can be opened for drainage just before the portal vein (vena porta) is cannulated.



Tissue Dissociation Guide

Neonatal Cardiomyocyte Isolation System (NCIS)

4. Turn on the perfusion pump containing plain CMF-HBSS with a flow rate 10-15ml/min so that the tubing or cannula can be inserted into the portal vein. The bath temperature is adjusted so that the perfusate temperature is 37°C. Cut a nick in the vena cava near the right kidney to lower the blood pressure, and then with fine surgical scissors cut a nick in the portal vein (partially through) about 5mm below (towards the tail) the knotted thread. Insert the tubing into the portal vein towards the liver and only several millimeters past the loose knot. The liver should clear of blood. Tie the surgical threads tightly around the portal vein and tubing. Cut the vena cava through and increase the perfusate flow rate to 20-25ml/min.

Note: Establishment of an effective perfusion that flushes the entire vasculature is essential to the success of the digestion.

5. Remove the liver from the animal with great care; do not rush. Place the liver onto a mesh stage in such a manner that it can be perfused in a recirculating fashion. The initial CMF-HBSS perfusate, however, goes to waste.

6. After 7-10 min of CMF-HBSS perfusion, switch to perfusion with the Enzyme Buffer Solution (L-15 digestion medium containing the enzymes). Start recirculation after one system-dead-volume of the remaining CMF-HBSS has gone to waste.

7. Perfuse the liver with the digestion mixture until it swells fully (but not prematurely) and the liver is fully digested, about 20-30 minutes.

Note: Halt the perfusion immediately by stopping the pump and removing the liver if the portal vein breaks or if the surface of the liver shows signs of disintegration when touched with forceps or a blunt object.

8. At the end of the perfusion, stop the pump, gently place the liver in the 150ml or equivalent culture dish and remove the perfusion tube. Transfer the culture dish to a sterile hood if not already in one, and add approximately 150ml of fresh CMF-HBSS to the dish.

9. In the culture dish, gently pull off the lobular capsule membranes with forceps or dog comb (recommended by Seglen), and rake out the cells. Remove the large central tree of connective and vascular tissue, and any undigested tissue or connective tissue.

10. Gently agitate the dish to disperse the cells. Place the dish at an angle by propping one side on the lid. Allow clumps or connective tissue to settle for a minute or so, then remove the dispersed cells from the top of the buffer at the deepest part of the plate, i.e. close to the lower edge, and transfer the cell suspension to 50ml sterile tubes.

11. Centrifuge for three minutes at low speed (just rapidly enough for loose cell pellets, e.g. 100 x g) at room temperature.

12. Add more CMF-HBSS to the culture dish and repeat the process to increase the yield of cells. Repeat as long as clean cells can be removed.

13. As soon as cells are sedimented, add fresh CMF-HBSS, suspend the cells by inverting the capped tubes, and re-centrifuge as above. Repeat process once more to remove traces of the digestive enzymes from the cells. Discard the supernatant(s) and transfer cells to culture medium or buffered medium in a second 100mm or 150mm culture dish. The yield of cells from a good digestion of a liver of a 300gm rat is approximately 4-5ml of packed volume after gentle sedimentation in a centrifuge.

The Worthington Neonatal Cardiomyocyte Isolation System has been introduced to provide researchers with a reliable, convenient, and consistent cell isolation system. By utilizing purified rather than crude enzyme preparations, it has been possible to minimize the lot-to-lot variation. In addition, Worthington use-tests the kits by isolating cardiomyocytes from neonatal rat hearts to assure performance, reliability, and consistent yield of viable cells.

The kit has been formulated in conjunction with Dr. Ronal MacGregor. The method is based on that described by Toraason, *et al.* (1988) in which the minced tissue is incubated overnight with trypsin in the cold. As pointed out by Toraason, this step reduces the hands on time required to harvest cells compared to the time involved in sequential incubations in warm trypsin or collagenase.

The package contains sufficient materials for five separate tissue dissociations, each containing up to twelve hearts. For larger or smaller tissue samples prepare proportionate volumes of reagents at each step and combine them in the same ratio as described in the protocol.

NCIS Kit Contents

Vial #1: 1 bottle, 500 ml

Sterile calcium- and magnesium-free Hank's Balanced Salt Solution (CMF HBSS), pH 7.4. The solution is used for reconstituting the contents of Vials #2 and #3 in addition to serving as the medium for the dissociation.

Vial #2: 5 vials, 1000 µg each

Worthington Trypsin (Code: TRLS), 3X crystallized, dialyzed against 1 mM HCl, filtered through 0.22 µm pore size membrane, and lyophilized. Before use, reconstitute with 2 ml CMF HBSS (Vial #1) and swirl gently to dissolve contents. Store at 2-8°C.

Vial #3: 5 vials, 2000 µg each

Worthington Soybean Trypsin Inhibitor (Code: SIC), a 0.22 µm pore size membrane filtered, lyophilized powder. Before use, reconstitute with 1 ml CMF HBSS (Vial #1) and swirl gently to dissolve contents. Store at 2-8°C.

Vial #4: 5 vials, 1500 Units each

Worthington Purified Collagenase (Code: CLSPA), a 0.22 µm pore size membrane filtered, lyophilized powder which has been chromatographically purified. It contains less than 50 caseinase units per milligram and is composed of two separable but very similar collagenases. Before use, reconstitute with 5 ml Leibovitz L-15 Media (prepared as described below) and swirl gently to dissolve contents. Store at 2-8°C.

Pouch containing Leibovitz L-15 Media Powder: 1x1 L

Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing 800 ml of cell culture grade water. Rinse pouch 2 - 3 times with additional 100 ml. Bring total volume to 1 liter and filter through a 0.22 micron filter.

The kit also includes 5 Cell Strainers (Falcon), and a card correlating phenol red color with pH for checking the pH of balanced salt solution and culture medium.

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NCIS Procedure

Day 1: Perform the following in the afternoon

Prepare:

- **Reagent #1**, CMF HBSS: 50-60 ml from Vial #1, *ice cold*.
- **Reagent #2**, Trypsin: reconstitute one of Vial #2 with 2 ml Reagent #1, *ice cold*.
- One sterile 50 ml centrifuge tube, *in ice*.
- 10 cm Petri dish, sterile, *on ice*.

1. Transfer 30–40 ml of Reagent #1 to the centrifuge tube.
2. Anesthetize each rat pup, sterilize the abdomen with an antiseptic solution, and surgically remove the beating heart; immediately place the heart in the centrifuge tube to chill and rinse. Repeat for remaining rat pups. Swirl the tube to rinse hearts, then pour off most of the liquid. Rinse the hearts with 10 ml of Reagent #1, pour off the liquid as before, then transfer the hearts to the Petri dish. Mince the tissue with small scissors or a razor blade to less than 1 mm³ pieces keeping tissue at 0°C.
3. Add Reagent #1 to Petri dish to a final volume of approximately 9 ml.
4. Transfer 1 ml of the contents of the trypsin vial (Vial #2) into the Petri dish and mix completely by swirling. Final trypsin concentration is 50 µg/ml.
5. Place the lid on the petri dish and immediately place in refrigerator overnight (16–20 hours) at 2–8°C.

Note: If animals are 4 days old or older, increase the trypsin concentration up to a maximum of 100 µg/ml.

Day 2: Begin the following in the morning:

Prepare:

- **Reagent #1**, CMF HBSS: 30 ml. *ice cold*.
- **Reagent #3**, Trypsin Inhibitor: reconstitute one of Vial #3 with 1 ml Reagent #1. Room temperature.
- **Reagent #4** Collagenase: reconstitute one of Vial #4 with 5 ml prepared Leibovitz L-15. Room temperature.
- Enough culture medium containing calcium and magnesium for digestion, centrifugations, and plating in cultureware. (approximately 100 ml for 10 hearts). Room temperature.
- Wide-mouth 10 ml serological pipet, sterile (opening about 3 mm diameter)
- Standard 10 ml plastic serological pipet

6. Remove Petri dish from refrigerator and bring to sterile hood on ice. Transfer tissue and buffer to 50 ml centrifuge tube on ice using wide-mouth pipet.
7. Transfer contents of Vial #3 into tube and mix.
8. Oxygenate tissue for 30 seconds to 1 minute if O₂ is available by passing oxygen over the surface of the liquid.
9. Warm tissue and buffer to 30–37°C in water bath, maintaining sterility (i.e. cap if needed). DO NOT add calcium-containing medium until tissue fragments are warm.
10. Slowly transfer the contents of Vial #4 into tube and mix. Cap tube tightly.
11. Place tube in/on slowly rotating (tumbling) or shaking instrument (2–4 rpm) at 37°C and incubate for 30 to 45 minutes.

All subsequent steps at room temperature.

12. Remove tube from incubator and return to sterile hood. With standard 10 ml plastic serological pipet, triturate about 10 times to release cells. (Trituration is discussed in the following inset.) Pipet as gently as possible consistent with successful tissue dispersion.

13. Rinse a Cell Strainer with 1 ml of the L-15 culture medium. Allow tissue residue to settle 3–4 minutes, then (with same pipet) filter the supernatant through the Cell Strainer into a fresh 50 ml centrifuge tube.
14. Add 5 ml additional L-15 culture medium to tissue residue, repeat trituration step. Allow tissue residue to settle as before, then filter cells through the same Cell Strainer. Rinse mesh gently with 2 ml culture medium, oxygenate cells 1 minute, then allow filtered cells to remain undisturbed for about 20 minutes at room temperature. This allows complete digestion of the partially degraded collagen. Cells can be held up to 1 hour at this point.
15. Swirl cells gently; if no clumps have formed and appearance is uniform, sediment cells at 50–100 x g for 5 minutes (enough to settle the myocytes and some but not all red cells.) Suspend cells in additional portions of L-15 culture medium and repeat sedimentation as desired. If no sedimentation is desired, cells can be plated directly from the initial filtrate. Serum is generally required for plating cells in cultureware.
16. Suspend final cell pellet in suitable culture medium. Pipet gently to disperse. No clumps or connective tissue strands should be visible. Count the cells using a hemocytometer or other method, adjust cell concentration and add serum as desired, then dispense to tissue cultureware. (Some brands of uncoated cultureware do not encourage high plating efficiencies. Use Falcon or equivalent for best results.) Routine cell yields are 2–3 x 10⁶ cardiomyocytes per heart digested. Good (fairly heavy) seeding levels of cells should be obtained at 125,000 cardiomyocytes per cm² of culture wells or flasks. Adhesion may be improved by collagen or fibronectin coating of the plastic Cell Quantitation and Estimation of Viability are discussed in the following sections.
17. Place each plate or flask in a 37°C incubator as soon as it is plated. Do not touch or otherwise disturb the cells for at least 24 hours.

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Papain Dissociation System (PDS)

The Worthington Papain Dissociation System is a set of reagents intended for use in the neural cell isolation method of Huettner and Baughman. The materials are designed for convenience and simplicity and are useful to the occasional user as well as the more experienced and frequent user. Each lot is use tested for performance in tissue dissociation and provides freshly prepared enzyme solutions for each dissociation.

The reagents are stable at ambient temperatures for the periods of time expected in normal shipping procedures, but the package should be refrigerated upon arrival and can be stored at 2-8°C for up to 4 months before use.

PDS Kit Contents

The package contains sufficient materials for dissociation of five separate tissue aliquots of up to 0.3 - 0.4 cm³ each. For larger tissue samples prepare proportionately larger volumes of reagents at each step and combine them in the same ratio as described in the protocol.

Vial #1: 1 bottle, 250 ml

Sterile Earle's Balanced Salt Solution (EBSS) with bicarbonate and phenol red. Aliquots of this vial are used to reconstitute other vials and to prepare dilute inhibitor solution. Refrigerate between uses and equilibrate with sterile O₂:CO₂ before each use.

Vial #2: 5 vials, 100 Units each

Papain containing L-cysteine and EDTA. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with five mls of EBSS (vial 1) yields a solution at 20 units of papain per ml in one millimolar L-cysteine with 0.5 millimolar EDTA. Brief incubation is needed to insure full solubility and activity.

Vial #3: 5 vials, 1000 Units each

Deoxyribonuclease I (DNase). This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 0.5 ml of EBSS (vial #1) yields a solution at 2000 units of deoxyribonuclease per ml. Avoid vigorous mixing.

Vial #4: 1 vial, 320 mg

Ovomucoid protease inhibitor with bovine serum albumin. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 32 mls of EBSS (vial #1) yields a solution at an effective concentration of 10 mgs of ovomucoid inhibitor and 10 mgs of albumin per ml. The inner rubber stopper can be discarded after reconstitution. Aliquots of this vial are used for each dissociation. Refrigerate between uses and equilibrate with sterile O₂:CO₂ before each use. Stable after reconstitution when stored at 4°C.

Also included is a card correlating color with pH for use as a guide in O₂:CO₂ equilibration.

PDS Procedure

Sterile procedures should be used throughout.

1. Add 32 mls of EBSS (vial 1) to the albumin ovomucoid inhibitor mixture (vial 4) and allow the contents to dissolve while preparing the other components. Mix before using and equilibrate with O₂:CO₂. Reconstitute for the first use, then store and reuse.
2. Add 5 mls of EBSS (vial 1) to a papain vial (vial #2). Place vial #2 in a 37°C water bath for ten minutes or until the papain is completely dissolved and the solution appears clear. If solution appears alkaline (red or purple) equilibrate the solution with 95% O₂:5%CO₂. The solution should be used promptly but can be held at room temperature during the dissection. A separate papain vial

is provided for each dissociation. (If desired the papain can be transferred to a centrifuge tube or other container before proceeding.)

3. Add 500 μ ls of EBSS to a DNase vial (vial #3). Mix gently -- DNase is sensitive to shear denaturation. Add 250 μ ls of this solution to the vial containing the papain. This preparation contains a final concentration of approximately 20 units/ml papain and 0.005% DNase. Save the balance of the DNase vial to use in step #7. A separate DNase vial is provided for each dissociation.
4. Place tissue in the papain solution. Tissue should be slightly minced or cut into small pieces (this can be done separately or on the side of the tube containing the papain). Displace air in vial with sterile O₂:CO₂. Do not bubble gas through the solution. Immediately cap vial.
5. Incubate the vial containing the tissue at 37°C with constant agitation (a rocker platform is ideal) for 30 min to 1 1/2 hrs. The amount of time must be determined empirically; however, embryonic tissue generally requires less time than postnatal tissue.
6. Triturate the mixture with 10 ml pipette. Allow any pieces of undissociated tissue remaining after trituration to settle to the bottom of the tube. Vigorous trituration of neuronal tissue results in a high yield of cells, most of which are spherical and devoid of processes. Gentle trituration results in more undissociated tissue fragments and a lower yield of cells although many of these now retain their proximal processes.
7. Carefully remove the cloudy cell suspension, place in sterile screwcapped tube and centrifuge at 300g for 5 minutes at room temperature. Be careful to avoid including any pieces of undissociated tissue during this time -- prepare medium to resuspend the pelleted cells.

Mix 2.7 mls EBSS (vial #1) with 300 μ ls reconstituted albumin-ovomucoid inhibitor solution (vial #4) in a sterile tube. Add 150 μ ls of DNase solution (vial #3) saved at step #3.
8. Discard supernatant and immediately resuspend cell pellet in DNase dilute albumin-inhibitor solution.
9. Prepare discontinuous density gradient. Add 5.0 ml of albumin-inhibitor solution (vial #4) to centrifuge tube, carefully layer cell suspension on top, then centrifuge at 70g for 6 minutes at room temperature. The interface between the two layers of the gradient should be clearly visible although minimal mixing at this boundary does not affect the result. Dissociated cells pellet at the bottom of the tube, membrane fragments remain at the interface.
10. Discard the supernatant and immediately resuspend the pelleted cells in medium for cell culture or for flow cytometric analysis.

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Tissue/Cell Culture Glossary

Adventitious: Developing from unusual points of origin, such as shoots or root tissues from callus or embryos from sources other than zygotes. This term can also be used to describe agents which contaminate cell cultures:

Anchorage-dependent cells or cultures: Cells, or cultures derived from them, which will grow, survive, or maintain function only when attached to a surface such as glass or plastic. The use of this term does not imply that the cells are normal or that they are or are not neoplastically transformed.

Aneuploid: The situation which exists when the nucleus of a cell does not contain an exact multiple of the haploid number of chromosomes; one or more chromosomes being present in greater or lesser number than the rest. The chromosomes may or may not show rearrangements.

Asepsis: Without infection or contaminating microorganisms.

Aseptic technique: Procedures used to prevent the introduction of fungi, bacteria, viruses, mycoplasma or other microorganisms into cell, tissue and organ culture. Although these procedures are used to prevent microbial contamination of cultures, they also prevent cross contamination of cell cultures as well. These procedures may or may not exclude the introduction of infectious molecules.

Attachment efficiency: The percentage of cells plated (seeded, inoculated) which attach to the surface of the culture vessel within a specified period of time. The conditions under which such a determination is made should always be stated.

Autocrine cell: In animals, a cell which produces hormones, growth factors or other signaling substances for which it also expresses the corresponding receptors. (See also Endocrine and Paracrine.)

Axenic culture: A culture without foreign or undesired life forms. An axenic culture may include the purposeful cocultivation of different types of cells, tissues or organisms.

Callus: An unorganized, proliferative mass of differentiated plant cells; a wound response.

Cell culture: Term used to denote the maintenance or cultivation of cells *in vitro* including the culture of single cells. In cell cultures, the cells are no longer organized into tissues.

Cell generation time: The interval between consecutive divisions of a cell. This interval can best be determined, at present, with the aid of cinephotomicrography. This term is not synonymous with "population doubling time".

Cell hybridization: The fusion of two or more dissimilar cells leading to the formation of a synkaryon.

Cell line: A cell line arises from a primary culture at the time of the first successful subculture. The term "cell line" implies that cultures from it consist of lineages of cells originally present in the primary culture. The terms finite or continuous are used as prefixes if the status of the culture is known. If not, the term line will suffice. The term "continuous line" replaces the term "established line". In any published description of a culture, one must make every attempt to publish the characterization or history of the culture. If such has already been published, a reference to the original publication must be made. In obtaining a culture, as originally named and described, must be maintained and any deviations in cultivation from the original must be reported in any publication.

Cell strain: A cell strain is derived either from a primary culture or a cell line by the selection or cloning of cells having specific properties or markers. In describing a cell strain, its specific features must be defined. The terms finite or continuous are to be used as prefixes if the status of the culture is known. If not, the term strain will suffice. In any published description of a cell strain, one must make every attempt to publish the characterization or history of the strain. If such

has already been published, a reference to the original publication must be made. In obtaining a culture from another laboratory, the proper designation of the culture, as originally named and described, must be maintained and any deviations in cultivation from the original must be reported in any publication.

Chemically defined medium: A nutritive solution for culturing cells in which each component is specifiable and ideally, is of known chemical structure.

Clonal propagation: Asexual reproduction of plants that are considered to be genetically uniform and originated from a single individual or explant.

Clone: In animal cell culture terminology a population of cells derived from a single cell by mitoses. A clone is not necessarily homogeneous and, therefore, the terms clone and cloned do not indicate homogeneity in a cell population, genetic or otherwise. In plant culture terminology, the term may refer to a culture derived as above or it may refer to a group of plants propagated only by vegetative and asexual means, all members of which have been derived by repeated propagation from a single individual.

Cloning efficiency: The percentage of cells plated (seeded, inoculated) that form a clone. One must be certain that the colonies formed arose from single cells in order to properly use this term. (See Colony forming efficiency)

Colony forming efficiency: The percentage of cells plated (seeded, inoculated) that form a colony.

Complementation: The ability of two different genetic defects to compensate for one another.

Contact inhibition of locomotion: A phenomenon characterizing certain cells in which two cells meet, locomotory activity diminishes, and the forward motion of one cell over the surface of the other is stopped.

Continuous cell culture: A culture which is apparently capable of an unlimited number of population doublings; often referred to as an immortal cell culture. Such cells may or may not express the characteristics of *in vitro* neoplastic or malignant transformation. (See also Immortalization)

Crisis: A stage of the *in vitro* transformation of cells. It is characterized by reduced proliferation of the culture, abnormal mitotic figures, detachment of cells from the culture substrate, and the formation of multinucleated or giant cells. During this massive cultural degeneration, a small number of colonies usually, but not always, survive and give rise to a culture with an apparent unlimited *in vitro* lifespan. This process was first described in human cells following infection with an oncogenic virus (SV40). See also Cell line, *In vitro* transformation and *In vitro* senescence.

Cryopreservation: Ultra-low temperature storage of cells, tissues, embryos or seeds. This storage is usually carried out using temperatures below -100°C.

Cumulative population doublings: See Population doubling level.

Cybrid: The viable cell resulting from the fusion of a cytoplasm with a whole cell, thus creating a cytoplasmic hybrid.

Cytoplasm: The intact cytoplasm remaining following the enucleation of a cell.

Cytoplasmic hybrid: Synonymous with "cybrid"

Cytoplasmic inheritance: Inheritance attributable to extranuclear genes; for example genes in cytoplasmic organelles such as mitochondria or chloroplasts, or in plasmids, etc.

Density-dependent inhibition of growth: Mitotic inhibition correlated with increased cell density.

Differentiated: Cells that maintain, in culture, all or much of the specialized structure and function typical of the cell type *in vivo*.

Diploid: The state of the cell in which all chromosomes, except sex chromosomes, are two in number and are structurally identical with those of the species from which the culture was derived. Where

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there is a Commission Report available, the experimenter should adhere to the convention for reporting the karyotype of the donor. Commission Reports have been published for mouse¹, human², and rat³. In defining a diploid culture, one should present a graph depicting the chromosome number distribution leading to the modal number determination along with representative karyotypes.

¹ Committee on Standardized Genetic Nomenclature of Mice. Standard karyotype of the mouse, *Mus musculus*. *J. Hered.* 63, 69-72 (1972)

² Paris Conference (1971), Supplement (1975). Standardization in Human cytogenetics. *Birth Defects: Original Article Series, XI*, 9, 1975. The National Foundation, New York (Reprinted in *Cytogenet. Cell Genet.*, 15, 201-238, 1975.

³ Committee for a Standardized Karyotype of *Rattus norvegicus*. Standard karyotype of the Norway rat, *Rattus norvegicus*., *Cytogenet. Cell Genet.* 12, 199-205, 1973

Electroporation: Creation, by means of an electrical current, of transient pores in the plasmalemma usually for the purpose of introducing exogenous material, especially DNA, form the medium.

Embryo culture: *In vitro* development or maintenance of isolated mature or immature embryos.

Embryogenesis: The process of embryo initiation and development.

Endocrine cell: In animals, a cell which produces hormones, growth factors or other signaling substances for which target cells, expressing the corresponding receptors, are located at a distance. (See also Autocrine and Paracrine)

Epigenetic event: Any change in a phenotype which does not result from an alteration in DNA sequence. This change may be stable and heritable and includes alteration in DNA methylation, transcriptional activation, translational control and posttranslational modifications

Epigenetic variation: Phenotypic variability which has a nongenetic basis.

Epithelial-like: Resembling or characteristic of, having the form or appearance of epithelial cells. In order to define a cell as an epithelial cell, it must possess characteristics typical of epithelial cells. Often one can be certain of the histologic origin and/or function of the cells placed into culture and, under these conditions, one can be reasonably confident in designating the cells as epithelial. It is incumbent upon the individual reporting on such cells to use as many parameters as possible in assigning this term to a culture. Until such time as a rigorous definition is possible, it would be most correct to use the term "epithelial-like".

Euploid: The situation which exists when the nucleus of a cell contains exact multiples of the haploid number of chromosomes.

Explant: Tissue taken from its original site and transferred to an artificial medium for growth or maintenance.

Explant culture: The maintenance or growth of an explant in culture.

Feeder layer: A layer of cells (usually lethally irradiated for animal cell culture) upon which are cultured a fastidious cell type. (See also Nurse culture)

Fibroblast-like: Resembling or characteristic of, having the form or appearance of fibroblast cells. In order to define a cell as a fibroblast cell, it must possess characteristics typical of fibroblast cells. Often one can be certain of the histologic origin and/or function of the cells placed into culture and, under these conditions, one can be reasonably confident in designating the cells as fibroblast. It is incumbent upon the individual reporting on such cells to use as many parameters as

Finite cell culture: A culture which is capable of only a limited number of population doubling after which the culture ceases proliferation. (See *In vitro* senescence)

possible in assigning this term to a culture. Until such time as a rigorous definition is possible, it would be most correct to use the term "fibroblast-like."

Friability: A term indicating the tendency for plant cells to separate from one another.

Gametoclonal variation: Variation in phenotype, either genetic or epigenetic in origin, expressed by gametoclones.

Gametocclone: Plants regenerated from cell cultures derived from meiospores, gametes or gametophytes.

Habituation: The acquired ability of a population of cells to grow and divide independently of exogenously supplied growth regulators.

Heterokaryon: A cell possessing two or more genetically different nuclei in a common cytoplasm, usually derived as a result of cell-to-cell fusion.

Heteroploid: The term given to a cell culture when the cells comprising the culture possess nuclei containing chromosome numbers other than the diploid number. This is a term used only to describe a culture and is not used to describe individual cells. Thus, a heteroploid culture would be one which contains aneuploid cells.

Histiotypic: The *in vitro* resemblance of cells in culture to a tissue in form or function or both. For example, a suspension of fibroblast-like cells may secrete a glycosaminoglycan-collagen matrix and the result is a structure resembling fibrous connective tissue, which is, therefore, histiotypic. This term is not meant to be used along with the word "culture". Thus, a tissue culture system demonstrating form and function typical of cells *in vivo* would be said to be histiotypic.

Homokaryon: A cell possessing two or more genetically identical nuclei in a common cytoplasm, derived as a result of cell-to-cell fusion.

Hybrid cell: The term used to describe the mononucleate cell which results from the fusion of two different cells, leading to a formation of a synkaryon.

Hybridoma: The cell which results from the fusion of an antibody producing tumor cell (myeloma) and an antigenically-stimulated normal plasma cell. Such cells are constructed because they produce a single antibody directed against the antigen epitope which stimulated the plasma cell. This antibody is referred to as a monoclonal antibody.

Immortalization: The attainment by a finite cell culture, whether by perturbation or intrinsically, of the attributes of a continuous cell line. An immortalized cell is not necessarily one which is neoplastically or malignantly transformed.

Immortal cell culture: See Continuous cell culture.

Induction: Initiation of a structure, organ or process *in vitro*.

***In vitro* neoplastic transformation:** The acquisition, by cultured cells, of the property to form neoplasms, benign or malignant, when inoculated into animals. Many transformed cell populations which arise *in vitro* intrinsically or through deliberate manipulation by the investigator, produce only benign tumors which show no local invasion or metastasis following animal inoculation. If there is supporting evidence, the term "*in vitro* malignant neoplastic transformation" or "*in vitro* malignant transformation" can be used to indicate that an injected cell line does, indeed, invade or metastasize.

***In vitro* propagation:** Propagation of plants in a controlled, artificial environment, using plastic or glass culture vessels, aseptic techniques and a defined growing medium.

***In vitro* senescence:** In vertebrate cell cultures, the property attributable to finite cell cultures; namely, their inability to grow beyond a finite number of population doublings. Neither invertebrate nor plant cell cultures exhibit this property.

***In vitro* transformation:** A heritable change, occurring in cells in culture, either intrinsically or from treatment with chemical carcinogens, oncogenic viruses, irradiation, transfection with oncogenes, etc. and leading to the acquisition of altered morphological, antigenic, neoplastic, proliferative or other properties. This expression is distinguished from "*in vitro* neoplastic transformation" in that the

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alterations occurring in the cell population may not always include the ability of the cells to produce tumors in appropriate hosts. The type of transformation should always be specified in any description.

Juvenile: A phase in the sexual cycle of a plant characterized by differences in a appearance form the adult and which lacks the ability to respond to flower-inducing stimuli,

Karyoplast: A cell nucleus, obtained from the cell by enucleation, surrounded by a narrow rim of cytoplasm and a plasma membrane.

Line: See Cell line.

Liposome: A closed lipid vesicle surrounding an aqueous interior; may be used to encapsulate exogenous materials for ultimate delivery of these into cells by fusion with the cell.

Meristem culture: *In vitro* culture of a generally shiny, dome-like structure measuring less than 0.1 mm in length when excised, most often excised from the shoot apex.

Microcell: A cell fragment, containing one to a few chromosomes, which is formed by the enucleation or disruption of a micronucleated cell.

Micronucleated cell: A cell which has been mitotically arrested and in which small groups of chromosomes function as foci for the reassembly of the nuclear membrane thus forming micronuclei the maximum of which could be equal to the total number of chromosomes.

Micropropagation: *In vitro* clonal propagation of plants from shoot tips or nodal explants, usually with an accelerated proliferation of shoots during subcultures.

Morphogenesis: (a) The evolution of a structure from an undifferentiated to a differentiated state. (b) The process of growth and development of differentiated structures.

Mutant: A phenotypic variant resulting from a changed or new gene.

Nurse culture: In the culture of plant cells, the growth of a cell or cells on a contiguous culture of different origin which in turn is in contact with the tissue culture medium. The cultured cell or tissue may be separated from the feeder layer by a porous matrix such as filter paper or membranous filters. (See also Feeder layer)

Organ culture: The maintenance or growth of organ primordia or the whole or parts of an organ *in vitro* in a way that may allow differentiation and preservation of the architecture and/or function.

Organized: Arranged into definite structures.

Organogenesis: The evolution, from dissociated cells, of a structure which shows natural organ form or function or both.

Organotypic: Resembling an organ *in vivo* in three dimensional form or function or both. For example, a rudimentary organ in culture may differentiate in an organotypic manner, or a population of dispersed cells may become rearranged into an organotypic structure and may also function in an organotypic manner. This term is not meant to be used along with the word "culture" but is meant to be used as a descriptive term.

Paracrine: In animals, a cell which produces hormones, growth factors or other signaling substances for which the target cells, expressing the corresponding receptors, are located in its vicinity, or in a group adjacent to it. (See also Autocrine and Endocrine)

Passage: The transfer or transplantation of cell, with or without dilution, from one culture vessel to another. It is understood that any time cells are transferred from one vessel to another, a certain portion of the cells may be lost and, therefore, dilution of cells, whether deliberate or not, may occur. This term is synonymous with the term "subculture".

Passage number: The number of times the cells in the culture have been subcultured or passaged. In descriptions of this process, the ration or dilution of the cells should be stated so that the relative cultural age can be ascertained.

Pathogen free: Free from specific organisms based on specific tests for the designated organisms.

Plant tissue culture: The growth or maintenance of plant cells, tissues, organs or whole plants *in vitro*.

Plating efficiency: This is a term which originally encompasses the terms "Attachment ("Seeding") efficiency", Cloning efficiency", and "colony forming efficiency" and which is now better described by using one or more of them in its place as the term "plating" is not sufficiently descriptive of what is taking place. (See Attachment, Cloning, Colony forming efficiency)

Population density: The number of cells per unit area or volume of a culture vessel. Also the number of cells per unit volume of medium in a suspension culture.

Population doubling level: The total number of population doubling of a cell line or strain since its initiation *in vitro*. A formula to use for the calculation of "population doublings" in a single passage is:

$$\text{Number of population doublings} = \log_{10}(N/N_0) \times 3.33$$

where: N=number of cells in the growth vessel at the end of a period of growth. N_0 =number of cells plated in the growth vessel. It is best to use the number of viable cells or number of attached cells for this determination. Population doubling level is synonymous with "cumulative population doublings."

Population doubling time: The interval, calculated during the logarithmic phase of growth in which, for example, 1.0×10^6 cells increase to 2.0×10^6 cells. This term is not synonymous with "cumulative population doublings".

Primary culture: A culture started from cells, tissues or organs taken directly from organisms. A primary culture may be regarded as such until it is successfully subcultured for the first time. It then becomes a "cell line".

Protoplast: A cell from which the entire cell wall has been removed. This term is used to describe such plant, bacterial or fungal cells. (See Spheroplast for comparison.)

Protoplast fusion: Technique in which protoplasts are fused into a single cell.

Pseudodiploid: This describes the condition where the number of chromosomes in a cell is diploid but, as a result of chromosomal rearrangements, the karyotype is abnormal and linkage relationships may be disrupted.

Recon: The viable cell reconstructed by the fusion of a karyoplast with a cytoplast.

Reconstituted cell: Synonymous with "Recon".

Reculture: The process by which a cell monolayer or a plant explant is transferred, without subdivision, into fresh medium. (See also Passage)

Regeneration: In plant cultures, a morphogenetic response to a stimulus that results in the production of organs, embryos or whole plants.

Saturation density: The maximum cell number attainable, under specified culture conditions, in a culture vessel. This term is usually expressed as the number of cells per square centimeter in a monolayer culture or the number of cells per cubic centimeter in a suspension culture.

Seeding efficiency: (See Attachment efficiency)

Senescence: (See *In vitro* senescence)

Shoot apical meristem: Undifferentiated tissue, located within the shoot tip, generally appearing as a shiny dome-like structure distal to the youngest leaf primordium and measuring less than 0.1 mm in length when excised.

Shoot tip (apex) culture: A structure consisting of the shoot apical meristem plus one to several primordial leaves, usually measuring from 0.1-1.0 mm in length; in instances where more mature leaves are included, the structure can measure up to several centimeters in length.

Somaclonal variation: Phenotypic variation, either genetic or epigenetic in origin, displayed among somaclones.

Tissue Dissociation Guide

Somaclone: Plants derived from any form of cell culture involving the use of somatic plant cells.

Somatic cell hybrid: The cell or plant resulting from the fusion of animal cells or plant protoplasts respectively, derived from somatic cells which differ genetically.

Somatic cell genetics: The study of genetic phenomena of somatic cells. The cells under study are most often cells grown in culture.

Somatic cell hybridization: The *in vitro* fusion of animal cells or plant protoplasts derived from somatic cells which differ genetically.

Somatic embryogenesis: In plant culture, the process of embryo initiation and development from vegetative or nongametic cells.

Spheroplast: A cell from which most of the cell wall has been removed. (See Protoplasts for comparison)

Stage I: A step in *in vitro* propagation characterized by the establishment of an aseptic tissue culture of a plant.

Stage II: A step in *in vitro* plant propagation characterized by the rapid numerical increase of organs other structures

Stage III: A step in *in vitro* plant propagation characterized by preparation of propagules for successful transfer to soil, a process involving rooting of shoot cuttings, hardening of plants and initiating the change from the heterotrophic to the autotrophic state.

Stage IV: A step in *in vitro* plant propagation characterized by the establishment in soil of a tissue culture derived plant, either after undergoing a Stage III pretransplant treatment or, in certain species, after the direct transfer of plants from Stage II into soil.

Sterile: (a) Without Life. (b) Inability of an organism to produce functional gametes.

Strain: See Cell strain.

Subculture: See Passage. With plant cultures, this is the process by which the tissue or explant is first subdivided, then transferred into fresh culture medium.

Substrain: A substrain can be derived from a strain by isolation a single cell or groups of cells having properties or markers not shared by all cells of the parent strain.

Surface or substrate dependent cells or cultures: See Anchorage dependent cells.

Suspension culture: A type of culture in which cells, or aggregates of cells, multiply while suspended in liquid medium.

Synkaryon: A hybrid cell which results from the fusion of the nuclei it carries.

Tissue culture: The maintenance or growth of tissues, *in vitro*, in a way that may allow differentiation and preservation of their architecture and/or function.

Totipotency: A cell characteristic in which the potential for forming all the cell types in the adult organism is retained.

Transfection: The transfer, for the purpose of genomic integration, of naked, foreign DNA into cells in culture. The traditional microbiological usage of this term implied that the DNA being transferred was derived from a virus. The definition as stated here is that which is in use to describe the general transfer of DNA irrespective of its source. (See also Transformation)

Transformation: In plant cell culture, the introduction and stable genomic integration of foreign DNA into a plant cell by any means, resulting in a genetic modification. This definition is the traditional microbiological definition. For animal cell culture, see *In vitro* transformation, *In vitro* neoplastic transformation and Transfection.

Type I callus: A type of adventive embryogenesis found with gramineous monocots, which has been induced on an explant where the somatic embryos are arrested at the coleptilar or scutellar stage of embryogeny. The embryos are often fused together especially at the coleorhizal end of the embryo axis. This tissue can be subcultured and maintain this morphology.

Type II callus: A type of adventive embryogenesis found with gramineous monocots, which has been induced on an explant where the somatic embryos are arrested at the globular stage of embryogeny. The globular embryos often arise individually from a common base. The tissue can be subcultured and maintain this morphology.

Variant: A culture exhibiting a stable phenotypic change whether genetic or epigenetic in origin.

Vegetative propagation: Reproduction of plants using a nonsexual process involving the culture of plant parts such as stem and leaf cuttings.

Undifferentiated: With plant cells, existing in a state of cell development characterized by isodiametric cell shape, very little or no vacuole, and a large nucleus, and exemplified by cells comprising an apical meristem or embryo. With animal cells, this is the state wherein the cell in culture lacks the specialized structure and/or function of the cell type *in vivo*.

Virus-free: Free from specified viruses based on tests designed to detect the presence of the organisms in question.

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Stem Cell Glossary

Adult stem cell: See somatic stem cell.

Astrocyte: A type of supporting (glial) cell found in the nervous system.

Blastocoel: The fluid-filled cavity inside the blastocyst, an early, preimplantation stage of the developing embryo.

Blastocyst: A preimplantation embryo of about 150 cells produced by cell division following fertilization. The blastocyst is a sphere made up of an outer layer of cells (the trophoblast), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass).

Bone marrow stromal cells: A population of cells found in bone marrow that are different from blood cells.

Bone marrow stromal stem cells (skeletal stem cells): A multipotent subset of bone marrow stromal cells able to form bone, cartilage, stromal cells that support blood formation, fat, and fibrous tissue.

Cell-based therapies: Treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cells or tissues.

Cell culture: Growth of cells *in vitro* in an artificial medium for research or medical treatment.

Cell division: Method by which a single cell divides to create two cells. There are two main types of cell division depending on what happens to the chromosomes: mitosis and meiosis.

Chromosome: A structure consisting of DNA and regulatory proteins found in the nucleus of the cell. The DNA in the nucleus is usually divided up among several chromosomes. The number of chromosomes in the nucleus varies depending on the species of the organism. Humans have 46 chromosomes.

Clone: (v) To generate identical copies of a region of a DNA molecule or to generate genetically identical copies of a cell, or organism; (n) The identical molecule, cell, or organism that results from the cloning process.

1. In reference to DNA: To clone a gene, one finds the region where the gene resides on the DNA and copies that section of the DNA using laboratory techniques.

2. In reference to cells grown in a tissue culture dish: a clone is a line of cells that is genetically identical to the originating cell. This cloned line is produced by cell division (mitosis) of the original cell.

3. In reference to organisms: Many natural clones are produced by plants and (mostly invertebrate) animals. The term clone may also be used to refer to an animal produced by somatic cell nuclear transfer (SCNT) or parthenogenesis.

Cloning: See Clone.

Cord blood stem cells: See Umbilical cord blood stem cells.

Culture medium: The liquid that covers cells in a culture dish and contains nutrients to nourish and support the cells. Culture medium may also include growth factors added to produce desired changes in the cells.

Differentiation: The process whereby an unspecialized embryonic cell acquires the features of a specialized cell such as a heart, liver, or muscle cell. Differentiation is controlled by the interaction of a cell's genes with the physical and chemical conditions outside the cell, usually through signaling pathways involving proteins embedded in the cell surface.

Directed differentiation: The manipulation of stem cell culture conditions to induce differentiation into a particular cell type.

DNA: Deoxyribonucleic acid, a chemical found primarily in the nucleus of cells. DNA carries the instructions or blueprint for making all the structures and materials the body needs to function. DNA consists of both genes and non-gene DNA in between the genes.

Ectoderm: The outermost germ layer of cells derived from the inner cell mass of the blastocyst; gives rise to the nervous system, sensory organs, skin, and related structures.

Embryo: In humans, the developing organism from the time of fertilization until the end of the eighth week of gestation, when it is called a fetus.

Embryoid bodies: Rounded collections of cells that arise when embryonic stem cells are cultured in suspension. Embryoid bodies contain cell types derived from all 3 germ layers.

Embryonic germ cells: Pluripotent stem cells that are derived from early germ cells (those that would become sperm and eggs). Embryonic germ cells (EG cells) are thought to have properties similar to embryonic stem cells.

Embryonic stem cells: Primitive (undifferentiated) cells that are derived from preimplantation-stage embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.

Embryonic stem cell line: Embryonic stem cells, which have been cultured under in vitro conditions that allow proliferation without differentiation for months to years.

Endoderm: The innermost layer of the cells derived from the inner cell mass of the blastocyst; it gives rise to lungs, other respiratory structures, and digestive organs, or generally "the gut."

Enucleated: Having had its nucleus removed.

Epigenetic: Having to do with the process by which regulatory proteins can turn genes on or off in a way that can be passed on during cell division.

Feeder layer: Cells used in co-culture to maintain pluripotent stem cells. For human embryonic stem cell culture, typical feeder layers include mouse embryonic fibroblasts (MEFs) or human embryonic fibroblasts that have been treated to prevent them from dividing.

Fertilization: The joining of the male gamete (sperm) and the female gamete (egg).

Fetus: In humans, the developing human from approximately eight weeks after conception until the time of its birth.

Gamete: An egg (in the female) or sperm (in the male) cell. See also Somatic cell.

Gastrulation: The process in which cells proliferate and migrate within the embryo to transform the inner cell mass of the blastocyst stage into an embryo containing all three primary germ layers.

Gene: A functional unit of heredity that is a segment of DNA found on chromosomes in the nucleus of a cell. Genes direct the formation of an enzyme or other protein.

Germ layers: After the blastocyst stage of embryonic development, the inner cell mass of the blastocyst goes through gastrulation, a period when the inner cell mass becomes organized into three distinct cell layers, called germ layers. The three layers are the ectoderm, the mesoderm, and the endoderm.

Hematopoietic stem cell: A stem cell that gives rise to all red and white blood cells and platelets.

Human embryonic stem cell (hESC): A type of pluripotent stem cells derived from early stage human embryos, up to and including the blastocyst stage, that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.

Induced pluripotent stem cell (iPSC): A type of pluripotent stem cell, similar to an embryonic stem cell, formed by the introduction of certain embryonic genes into a somatic cell.

In vitro: Latin for "in glass"; in a laboratory dish or test tube; an artificial environment.

In vitro fertilization: A technique that unites the egg and sperm in a laboratory instead of inside the female body.

Inner cell mass (ICM): The cluster of cells inside the blastocyst. These cells give rise to the embryo and ultimately the fetus. The ICM may be used to generate embryonic stem cells.

Long-term self-renewal: The ability of stem cells to replicate themselves by dividing into the same non-specialized cell type over long periods (many months to years) depending on the specific type of stem cell.

Mesenchymal stem cells: A term that is currently used to define non-blood adult stem cells from a variety of tissues, although it is not clear that mesenchymal stem cells from different tissues are the same.

Meiosis: The type of cell division a diploid germ cell undergoes to produce gametes (sperm or eggs) that will carry half the normal chromosome number. This is to ensure that when fertilization occurs, the fertilized egg will carry the normal number of chromosomes rather than causing aneuploidy (an abnormal number of chromosomes).

Mesoderm: Middle layer of a group of cells derived from the inner cell mass of the blastocyst; it gives rise to bone, muscle, connective tissue, kidneys, and related structures.

Microenvironment: The molecules and compounds such as nutrients and growth factors in the fluid surrounding a cell in an organism or in the laboratory, which play an important role in determining the characteristics of the cell.

Mitosis: The type of cell division that allows a population of cells to increase its numbers or to maintain its numbers. The number of chromosomes remains the same in this type of cell division.

Multipotent: Having the ability to develop into more than one cell type of the body. See also pluripotent and totipotent.

Neural stem cell: A stem cell found in adult neural tissue that can give rise to neurons and glial (supporting) cells. Examples of glial cells include astrocytes and oligodendrocytes.

Neurons: Nerve cells, the principal functional units of the nervous system. A neuron consists of a cell body and its processes—an axon and one or more dendrites. Neurons transmit information to other neurons or cells by releasing neurotransmitters at synapses.

Oligodendrocyte: A supporting cell that provides insulation to nerve cells by forming a myelin sheath (a fatty layer) around axons.

Parthenogenesis: The artificial activation of an egg in the absence of a sperm; the egg begins to divide as if it has been fertilized.

Tissue Dissociation Guide

Passage: In cell culture, the process in which cells are disassociated, washed, and seeded into new culture vessels after a round of cell growth and proliferation. The number of passages a line of cultured cells has gone through is an indication of its age and expected stability.

Pluripotent: The state of a single cell that is capable of differentiating into all tissues of an organism, but not alone capable of sustaining full organismal development.

Scientists demonstrate pluripotency by providing evidence of stable developmental potential, even after prolonged culture, to form derivatives of all three embryonic teratoma after injection into an immunosuppressed mouse.

Polar Body: A polar body is a structure produced when an early egg cell, or oogonium, undergoes meiosis. In the first meiosis, the oogonium divides its chromosomes evenly between the two cells but divides its cytoplasm unequally. One cell retains most of the cytoplasm, while the other gets almost none, leaving it very small. This smaller cell is called the first polar body. The first polar body usually degenerates. The ovum, or larger cell, then divides again, producing a second polar body with half the amount of chromosomes but almost no cytoplasm. The second polar body splits off and remains adjacent to the large cell, or oocyte, until it (the second polar body) degenerates. Only one large functional oocyte, or egg, is produced at the end of meiosis.

Preimplantation: With regard to an embryo, preimplantation means that the embryo has not yet implanted in the wall of the uterus. Human embryonic stem cells are derived from preimplantation-stage embryos fertilized outside a woman's body (in vitro).

Proliferation: Expansion of the number of cells by the continuous division of single cells into two identical daughter cells.

Regenerative medicine: A field of medicine devoted to treatments in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cell populations or tissues. (See also cell-based therapies).

Reproductive cloning: The process of using somatic cell nuclear transfer (SCNT) to produce a normal, full grown organism (e.g., animal) genetically identical to the organism (animal) that donated the somatic cell nucleus. In mammals, this would require implanting the resulting embryo in a uterus where it would undergo normal development to become a live independent being. The first mammal to be created by reproductive cloning was Dolly the sheep, born at the Roslin Institute in Scotland in 1996. See also Somatic cell nuclear transfer (SCNT).

Signals: Internal and external factors that control changes in cell structure and function. They can be chemical or physical in nature.

Somatic cell: Any body cell other than gametes (egg or sperm); sometimes referred to as "adult" cells. See also Gamete.

Somatic cell nuclear transfer (SCNT): A technique that combines an enucleated egg and the nucleus of a somatic cell to make an embryo. SCNT can be used for therapeutic or reproductive purposes, but the initial stage that combines an enucleated egg and a somatic cell nucleus is the same. See also therapeutic cloning and reproductive cloning.

Somatic (adult) stem cells: A relatively rare undifferentiated cell found in many organs and differentiated tissues with a limited capacity for both self renewal (in the laboratory) and differentiation. Such cells vary in their differentiation capacity, but it is usually limited to cell types in the organ of origin. This is an active area of investigation.

Stem cells: Cells with the ability to divide for indefinite periods in culture and to give rise to specialized cells.

Stromal cells: Connective tissue cells found in virtually every organ. In bone marrow, stromal cells support blood formation.

Subculturing: Transferring cultured cells, with or without dilution, from one culture vessel to another.

Surface markers: Proteins on the outside surface of a cell that are unique to certain cell types and that can be visualized using antibodies or other detection methods.

Telomere: The end of a chromosome, associated with a characteristic DNA sequence that is replicated in a special way. A telomere counteracts the tendency of the chromosome to shorten with each round of replication.

Teratoma: A multi-layered benign tumor that grows from pluripotent cells injected into mice with a dysfunctional immune system. Scientists test whether they have established a human embryonic stem cell (hESC) line by injecting putative stem cells into such mice and verifying that the resulting teratomas contain cells derived from all three embryonic germ layers.

Tetraploid complementation assay: An assay that can be used to test a stem cell's potency. Scientists studying mouse chimeras (mixing cells of two different animals) noted that fusing two 8-cell embryos produces cells with 4 sets of chromosomes (tetraploid cells) that are biased toward developing into extra-embryonic tissues such as the placenta. The tetraploid cells do not generate the embryo itself; the embryo proper develops from injected diploid stem cells. This tendency has been exploited to test the potency of a stem cell. Scientists begin with a tetraploid embryo. Next, they inject the stem cells to be tested. If the injected cells are pluripotent, then an embryo develops. If no embryo develops, or if the resultant embryo cannot survive until birth, the scientists conclude that the cells were not truly pluripotent.

Therapeutic cloning: The process of using somatic cell nuclear transfer (SCNT) to produce cells that exactly match a patient. By combining a patient's somatic cell nucleus and an enucleated egg, a scientist may harvest embryonic stem cells from the resulting embryo that can be used to generate tissues that match a patient's body. This means the tissues created are unlikely to be rejected by the patient's immune system. See also Somatic cell nuclear transfer (SCNT).

Totipotent: Having the ability to give rise to all the cell types of the body plus all of the cell types that make up the extraembryonic tissues such as the placenta. (See also Pluripotent and Multipotent).

Transdifferentiation: The process by which stem cells from one tissue differentiate into cells of another tissue.

Trophoblast: The outer layer of the preimplantation embryo in mice. It contains trophoblast cells.

Trophoblast: The outer cell layer of the blastocyst. It is responsible for implantation and develops into the extraembryonic tissues, including the placenta, and controls the exchange of oxygen and metabolites between mother and embryo.

Umbilical cord blood stem cells: Stem cells collected from the umbilical cord at birth that can produce all of the blood cells in the body (hematopoietic). Cord blood is currently used to treat patients who have undergone chemotherapy to destroy their bone marrow due to cancer or other blood-related disorders.

Undifferentiated: A cell that has not yet developed into a specialized cell type.

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Note: The following abbreviations appear throughout the Tissue Tables:

BALB	Bagg Albine (obtained from H.J Bagg in 1923)
BSA	Bovine Serum Albumin
BSS	Balanced Salt Solution
CF	Calcium Free
CLSPA	Worthington Purified Collagenase
CMF	Calcium Magnesium Free
DMEM	Dulbecco's Modified Eagle Medium
EBSS	Earle's Balanced Salt Solution
FBS	Fetal Bovine Serum
HBSS	Hank's Balanced Salt Solution
HIS	Hepatocyte Isolation System
L-15	Liebowitz L-15 Medium
MEM	Minimum Essential Medium
NCIS	Worthington Neonatal Cardiomyocyte Isolation System
PBS	Phosphate Buffered Saline
PDS	Worthington Papain Dissociation System
RPMI	Roswell Park Memorial Institute (Moore, <i>et al</i> , <i>Tissue Culture Association Manual</i> , 3, 503-508, 1976)
SD	Sprague-Dawley
SW	Swiss Webster

Tissue Dissociation Guide

Adipose/Fat			
Species		Cell(s)	Enzyme(s)
Bovine	Bovine	Adipocytes	Collagenase Type 1: 40 u/ml
Canine	Canine	Renal adipose derived cells	Collagenase Type 1: 0.3%
	Canine, 20-25 kg	Adipose stem cells	Collagenase: see reference
Equine	Equine	Adipose derived stem cells	Collagenase Type 1: 0.1%
	Equine	Adipose derived stem cells	Collagenase Type 1: 0.1%
Fish	Fish, Atlantic salmon	Preadipocytes	Collagenase Type 1: 0.1%
Gerbil	Gerbil of unknown age (also rat, hamster, rabbit, lamb, guinea-pig)	Brown fat	Collagenase Type 1: 0.10%
Guinea-Pig	Guinea-pig, adult (also rat, hamster, gerbil, rabbit, lamb)	Brown fat	Collagenase Type 1: 0.10%
Hamster	Hamster, adult (also rat, gerbil, rabbit, lamb, guinea-pig)	Brown fat	Collagenase Type 1: 0.10%
Human	Human, male 40-60 years	Adipose derived stem cells	Collagenase: 0.25% Deoxyribonuclease I: 0.002%
	Human	Renal adipose derived cells	Collagenase Type 1: 0.3%
	Human	Adipocytes	Collagenase Type 1: 0.1%
	Human	Adipose derived stromal cells	Collagenase Type 1: see reference
	Human	Adult stem cells	Collagenase Type 1: 0.1%
	Human	Adipocytes	Collagenase Type 1: 0.2%
	Human	Adipose derived stromal cells	Collagenase Type 1: 0.15%
	Human	Mesenchymal stem	Collagenase Type 1: 0.1%
	Human	Adipocytes, stromal vascular	Collagenase: 0.2%
	Human	Multipotent adipose derived stem	Collagenase: 0.2%
	Human, adult, obese	Adipocytes	Collagenase Type 2: 0.1%

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Adipose/Fat	
Medium	Reference
Krebs-Ringer bicarbonate	Yang YT, Baldwin RL: Preparation and metabolism of isolated cells from bovine adipose tissue, <i>J Dairy Sci</i> 56, 350-65, 1973 (10340)
DMEM	Basu, J., Genheimer, C., Sangha, N., Quinlan, S., Guthrie, K., Kelley, R., Ilagan, R., Jain, D., Bertram, T. and Ludlow, J.: Organ Specific Regenerative Markers in Peri-Organ Adipose: Kidney., <i>Lipids Health Dis</i> Vol. 10, 171, 2011 (10665)
Media-199	Fischer, L., McIlhenny, S., Tulenko, T., Golesorkhi, N., Zhang, P., Larson, R., Lombardi, J., Shapiro, I. and DiMuzio, P.: Endothelial Differentiation of Adipose-Derived Stem Cells: Effects of Endothelial Cell Growth Supplement and Shear Force., <i>J Surg Res</i> 152, 157, 2009 (10599)
PBS	Vidal, M., Robinson, S., Lopez, M., Paulsen, D., Borkhsenius, O., Johnson, J., Moore, R. and Gimble, J.: Comparison of Chondrogenic Potential in Equine Mesenchymal Stromal Cells Derived from Adipose Tissue and Bone Marrow., <i>Vet Surg</i> Vol. 37, 713, 2008 (10561)
PBS	Vidal, M., Kilroy, G., Lopez, M., Johnson, J., Moore, R. and Gimble, J.: Characterization of Equine Adipose Tissue-Derived Stromal Cells: Adipogenic and Osteogenic Capacity and Comparison with Bone Marrow-Derived Mesenchymal Stromal Cells., <i>Vet Surg</i> Vol. 36, 613, 2007 (10533)
HBSS	Todorovic, M., Vegusdal, A., Gjoen, T., Sundvold, H., Torstensen, B., Kjaer, M. and Ruyter, B.: Changes in Fatty Acids Metabolism During Differentiation of Atlantic Salmon Preadipocytes; Effects of n-3 and n-9 Fatty Acids., <i>Biochim Biophys Acta</i> 1781, 326, 2008 (10597)
Bicarbonate buffer	Nedergaard, J. and Lindberg, O.: The Brown Fat Cell, <i>Int Rev Cytol</i> 74, 187, 1982 (544)
Bicarbonate buffer	Nedergaard, J. and Lindberg, O.: The Brown Fat Cell, <i>Int Rev Cytol</i> 74, 187, 1982 (544)
Bicarbonate buffer	Nedergaard, J. and Lindberg, O.: The Brown Fat Cell, <i>Int Rev Cytol</i> 74, 187, 1982 (544)
PBS	Blasi, A., Martino, C., Balducci, L., Saldarelli, M., Soleti, A., Navone, S., Canzi, L., Cristini, S., Invernici, G., Parati, E. and Alessandri, G.: Dermal Fibroblasts Display Similar Phenotypic and Differentiation Capacity to Fat-Derived Mesenchymal Stem Cells, but Differ in Anti-Inflammatory and Angiogenic Potential, <i>Vasc Cell</i> Vol. 3, 5, 2011 (10486)
DMEM	Basu, J., Genheimer, C., Sangha, N., Quinlan, S., Guthrie, K., Kelley, R., Ilagan, R., Jain, D., Bertram, T. and Ludlow, J.: Organ Specific Regenerative Markers in Peri-Organ Adipose: Kidney., <i>Lipids Health Dis</i> Vol. 10, 171, 2011 (10665)
KRB	Peters, R., Wolf, M., van den Broek, M., Nuvolone, M., Dannenmann, S., Stieger, B., Rapold, R., Konrad, D., Rubin, A., Bertino, J., Aguzzi, A., Heikenwalder, M. and Knuth, A.: Efficient Generation of Multipotent Mesenchymal Stem Cells from Umbilical Cord Blood in Stroma-Free Liquid Culture., <i>PLoS ONE</i> 5, e15689, 2010 (10669)
DMEM	Traktuev, D., Merfeld-Clauss, S., Li, J., Kolonin, M., Arap, W., Pasqualini, R., Johnstone, B., and March, K.: A Population of Multipotent CD34-Positive Adipose Stromal Cells Share Pericyte and Mesenchymal Surface Markers, Reside in a Periendothelial Location, and Stabilize Endothelial Networks, <i>Circ Res</i> 102, 77, 2008 (10350)
PBS	Devireddy, R., Thirumala, S. and Gimble, J.: Cellular Response of Adipose Derived Passage-4 Adult Stem Cells to Freezing Stress., <i>J Biomech Eng</i> 127, 1081, 2005 (10600)
HBSS	Bujalska Iwona J, Durrani Omar M, Abbott Joseph, Onyimba Claire U, Khosla Pamela, Moosavi Areeb H, Reuser Tristan T Q, Stewart Paul M, Tomlinson Jeremy W, Walker Elizabeth A, Rauz Saaeha: Characterisation of 11beta-hydroxysteroid dehydrogenase 1 in human orbital adipose tissue: a comparison with subcutaneous and omental fat, <i>J Endocrinol</i> 192, 279-88, 2007 (10222)
DMEM	Schaffler A, Buchler C: Concise review: adipose tissue-derived stromal cells--basic and clinical implications for novel cell-based therapies, <i>Stem Cells</i> 25, 818-27, 2007 (10308)
HBSS	Jeon Eun Su, Song Hae Young, Kim Mi Ra, Moon Hyun Jung, Bae Yong Chan, Jung Jin Sup, Kim Jae Ho: Sphingosylphosphorylcholine induces proliferation of human adipose tissue-derived mesenchymal stem cells via activation of JNK, <i>J Lipid Res</i> 47, 653-64, 2006 (10328)
HBSS	Boquest Andrew C, Shahdadfar Aboulghassem, Fronsdaal Katrine, Sigurjonsson Olafur, Tunheim Siv H, Collas Philippe, Brinchmann Jan E: Isolation and transcription profiling of purified uncultured human stromal stem cells: alteration of gene expression after in vitro cell culture, <i>Mol Biol Cell</i> 16, 1131-41, 2005 (10312)
DMEM	Rodriguez Anne-Marie, Pisani Didier, Dechesne Claude A, Turc-Carel Claude, Kurzenne Jean-Yves, Wdziekonski Brigitte, Villageois Albert, Bagnis Claude, Breittmayer Jean-Philippe, Groux Herve, Ailhaud Gerard, Dani Christian: Transplantation of a multipotent cell population from human adipose tissue induces dystrophin expression in the immunocompetent mdx mouse, <i>J Exp Med</i> 201, 1397-405, 2005 (10326)
DMEM/F12	Seboek, D., Linscheid, P., Zulewski, H., Langer, I., Christ-Crain, M., Keller, U., and Muller, B.: Somatostatin Is Expressed and Secreted by Human Adipose Tissue Upon Infection and Inflammation, <i>J Clin Endocrinol Metab</i> 89(10), 4833, 2004 (9794)

Tissue Dissociation Guide

Species

Adipose/Fat (con't)

Cell(s)

Enzyme(s)

	Human, adult, female	Preadipocytes	Collagenase Type 1: 0.2%
	Human, adult	Adipocytes	Collagenase Type 1: 0.13%
	Human	Stromal vascular, adipocytes	Collagenase: 0.2%
	Human	Stromal vascular, adipocytes, stem	Collagenase: 300 u/ml
	Human, adult, female, non-obese	Adipocytes	Collagenase Type 2: 0.05%
	Human	Adipocytes	Collagenase Type 1: see reference
	Human, adult, male and female	Adipocytes	Collagenase Type 1: 0.2%
	Human	Processed lipoaspirate cells	Collagenase Type 1: 0.075%
	Human	Adipocytes	Collagenase Type 1: 0.2%
	Human, adult, male and female	Adipocytes	Collagenase Type 1: 0.1%
	Human, adult, male and female	Adipocytes	Collagenase Type 1: 0.1%
	Human, non-diabetic, male	Adipocytes	Collagenase Type 1: 0.05%
Mouse	Mouse	Stem and progenitor	Collagenase Type 2: 0.2%
	Mouse, 3 week	Adipocytes	Collagenase Type 1: 0.2%
	Mouse	White adipocytes	Collagenase Type 2: 0.1%
	Mouse, C57Bl/6J	Adipocytes	Collagenase Type 1: 0.15%
	Mouse, 3-6 day and 2-3 month	Adipose-derived stromal cells	Collagenase Type 2: 0.075%
	Mouse	Stromal vascular, adipocytes	Collagenase: 0.2%
	Mouse, C57Bl/6J and FVB, male, 8-9 week	Adipocytes	Collagenase:
	Mouse, both sexes	Adipocytes	Collagenase: 0.05%
	Mouse, B6D2F1, F1 hybrids, New Zealand black female & New Zealand white male	Vascular endothelial	Collagenase: 0.2%
	Porcine	Porcine, female, <1 year	Adipose mesenchymal stem
Porcine, crossbred, male 1-4 day		Adipocytes	Collagenase Type 1: 0.2%

Tissue Dissociation Guide

Medium	Reference	Adipose/Fat (con't)
DMEM/F-12	Quickler, M., Sinha, B., Tomlinson, J., Bujalska, I., Stewart, P., Arlt, W.: Androgen generation in adipose tissue in women with simple obesity--a site-specific role for 17beta-hydroxysteroid dehydrogenase type 5, <i>J Endocrinol</i> 183, 331, 2004 (9795)	
(see reference)	Fain JN, Madan AK, Hiler ML, Cheema P, and Bahouth SW.: Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans, <i>Endocrinology</i> 145, 2273, 2004 (10064)	
DMEM/F12	Planat-Benard Valerie, Silvestre Jean-Sebastien, Cousin Beatrice, Andre Mireille, Nibbelink Maryse, Tamarat Radia, Clergue Michel, Manneville Carole, Saillan-Barreau Corinne, Duriez Micheline, Tedgui Alain, Levy Bernard, Penicaud Luc, Casteilla Louis: Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives, <i>Circulation</i> 109, 656-63, 2004 (10298)	
PBS	Miranville A, Heeschen C, Sengenès C, Curat CA, Busse R, Bouloumie A: Improvement of postnatal neovascularization by human adipose tissue-derived stem cells, <i>Circulation</i> 110, 349-55, 2004 (10300)	
DMEM	Gesta, S., Lolmede, K., Daviaud, D., Berlan, M., Bouloumie, A., Lafontan, M., Valet, P., and Saulnier-Blache, J.: Culture of human adipose tissue explants leads to profound alteration of adipocyte gene expression, <i>Horm Metab Res</i> 35, 158, 2003 (1306)	
Saline	Patwardhan, R., Tubbs, R., Leonard, R., Kelly, D., Killingsworth, C., Rollins, D., Smith, W., Ideker, R., and Oakes, W.: Discernment of Adipose versus Nervous Tissue: A Novel Adjunct Solution in Lipomyelomeningocele Surgery, <i>Ped Neurosurg</i> 36, 314, 2002 (1308)	
DMEM/F-12	McTernan, P., Anderson, L., Anwar, A., Eggo, M., Crocker, J., Barnett, A., Stewart, P., and Kumar, S.: Glucocorticoid Regulation of P450 Aromatase Activity in Human Adipose Tissue: Gender and Site Differences, <i>J Clin Endocrinol Metab</i> 87, 1327, 2002 (9792)	
PBS	Zuk Patricia A, Zhu Min, Ashjian Peter, De Ugarte Daniel A, Huang Jerry I, Mizuno Hiroshi, Alfonso Zeni C, Fraser JohnK, Benhaim Prosper, Hedrick Marc H: Human adipose tissue is a source of multipotent stem cells, <i>Mol Biol Cell</i> 13, 4279-95, 2002 (10333)	
HBSS	McTernan PG, Anwar A, Eggo MC, Barnett AH, Stewart PM, Kumar S: Gender differences in the regulation of P450 aromatase expression and activity in human adipose tissue, <i>Int J Obes Relat Metab Disord</i> 24, 875-81, 2000 (10330)	
DMEM/Ham's F-12	Gottschling-Zelle, H., Birgel, M., Scriba, D., Blum, W., and Hauner, H.: Depot-specific Release of Leptin from Subcutaneous and Omental Adipocytes in Suspension Culture: Effect of Tumor Necrosis Factor-alpha and Transforming Growth Factor-beta1, <i>Eur J Endocrinol</i> 141 (4), 436, 1999 (1309)	
DMEM/ F-12	Zhang, H., Kumar, S., Barnett, A., and Eggo, M.: Intrinsic Site-Specific Differences in the Expression of Leptin in Human Adipocytes and Its Autocrine Effects on Glucose Uptake, <i>J Clin Endocrinol Metab</i> 84, 2550, 1999 (9789)	
Kreb's Ringer bicarbonate buffer	Anderson, O., Gliemann, J., and Gammeltoft: Receptor Binding and Biological Effect of Insulin in Human Adipocytes, <i>Diabetologia</i> 13, 589, 1977 (674)	
HBSS	Han, J., Koh, Y., Moon, H., Ryoo, H., Cho, C., Kim, I. and Koh, G.: Adipose Tissue is an Extramedullary Reservoir for Functional Hematopoietic Stem and Progenitor Cells., <i>Blood</i> 115, 957, 2010 (10494)	
HBSS	De Matteis, R., Zingaretti, M., Murano, I., Vitali, A., Frontini, A., Giannulis, I., Barbatelli, G., Marcucci, F., Bordinchia, M., Sarzani, R., Raviola, E. and Cinti, S.: In Vivo Physiological Transdifferentiation of Adult Adipose Cells., <i>Stem Cells</i> 27, 2761, 2009 (10552)	
DMEM	Wong, K., Szeto, F, Zhang, W., Ye, H., Kong, J., Zhang, Z., Sun, X. and Li, Y: Involvement of the Vitamin D Receptor in Energy Metabolism: Regulation of Uncoupling Proteins., <i>Am J Physiol/Endo</i> 296, 820, 2009 (10572)	
DMEM/F12	Aoyagi T, Shimba S, Tezuka M: Characteristics of Circadian Gene Expressions in Mice White Adipose Tissue and 3T3-L1 Adipocytes, <i>J Health Sci</i> 51, 21, 2005 (10028)	
PBS	Cowan Catherine M, Shi Yun-Ying, Aalami Oliver O, Chou Yu-Fen, Mari Carina, Thomas Romy, Quarto Natalina, Contag Christopher H, Wu Benjamin, Longaker Michael T: Adipose-derived adult stromal cells heal critical-size mouse calvarial defects, <i>Nat Biotechnol</i> 22, 560-7, 2004 (10140)	
DMEM/F12	Planat-Benard Valerie, Silvestre Jean-Sebastien, Cousin Beatrice, Andre Mireille, Nibbelink Maryse, Tamarat Radia, Clergue Michel, Manneville Carole, Saillan-Barreau Corinne, Duriez Micheline, Tedgui Alain, Levy Bernard, Penicaud Luc, Casteilla Louis: Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives, <i>Circulation</i> 109, 656-63, 2004 (10298)	
DMEM	Ruan, H., Zarnowski, MJ., Cushman, S., and Lodish, H.: Standardized Isolation of Primary Adipose Cells from Mouse Epididymal Fat Pads Induces Inflammatory Mediators and Down-regulates Adipocyte Genes, <i>J Biol Chem</i> 278, 47585, 2003 (9793)	
Krebs-Ringer Phosphate HEPES (KRPH)	Nadler, S., Stoehr, J., Rabaglia, M., Schueler, K., Birnbaum, M., and Attie, A.: Normal Akt/PKB with Reduced PI3K Activation in Insulin-resistant Mice, <i>Am J Physiol/Endo</i> 281, E1249, 2001 (1310)	
PBS	Lauder, T., Gegen, N., Knedler, A., and Harbeck, R.: The Isolation and Characterization of Enriched Microvascular Endothelial Cells From Mouse Adipose Tissue, <i>J Immunol Methods</i> 102, 45, 1987 (882)	
DMEM	Williams, K., Picou, A., Kish, S., Giraldo, A., Godke, R. and Bondioli, K: Isolation and Characterization of Porcine Adipose Tissue-Derived Adult Stem Cells., <i>Cells Tissues Organs</i> 188, 251, 2008 (10370)	
DMEM/F12	Ramsay, T.G.: Porcine Leptin Inhibits Lipogenesis in Porcine Adipocytes, <i>J Anim Sci</i> 81, 3008, 2003 (9797)	

Tissue Dissociation Guide

Species

Adipose/Fat (con't)

Cell(s)

Enzyme(s)

Species	Adipose/Fat (con't)	Cell(s)	Enzyme(s)
Rat	Porcine, 8-9 week	Adipose	Collagenase Type 1: 300 u/ml
	Porcine, neonatal	Adipocytes	Collagenase Type 1: 0.3%
	Porcine, crossbred, 1-3 day	Adipose, Stromal-vascular	Collagenase Type 1: 0.2%
	Rat, Lewis, male	Renal adipose derived cells	Collagenase Type 1: 0.3%
	Rat, SD, neonatal	Brown adipocytes	Collagenase Type 4: 0.1% Neutral Protease: 0.1% Trypsin: 0.05%
	Rat, Wistar, 4 week	Adipocytes	Collagenase: 0.2%
	Rat, SD	Adipocytes	Collagenase Type 1: 0.2%
	Rat, SD, male, 4-7 weeks	Brown adipocytes	Deoxyribonuclease I: 0.5%
	Rat	Adipocytes	Collagenase Type 2: 0.2%
	Rat, SD, male, 130-160 g	Adipose Epididymal fat pads	Collagenase: 0.3%
	Rat, 3 day	Preadipocytes	Collagenase Type 3: 0.10%
	Rat, SD, various weights and ages	Brown adipocytes Interscapular & cervical depots	Collagenase: 0.2% Soybean Trypsin Inhibitor: 0.3%
	Rat, CD, male, 150-200 g	Adipocytes Epididymal fat pads	Collagenase: 0.1%
	Rat, Wistar, albino, male, 100-140 g	Adipocytes Epididymal-fat pads	Collagenase: 0.3%
	Rat, Fischer, 344, male, 9 - 13 week	White fat	Collagenase: 0.3%
	Rat (CFE), albino, female	Brown fat	Collagenase Type 1: 0.10%
Rat, SD, male, 160-210 g	Fat	Collagenase: 0.3%	

Adrenal

Species

Cell(s)

Enzyme(s)

Species	Adrenal	Cell(s)	Enzyme(s)
Bovine	Bovine	Chromaffin cells	Collagenase: 0.1% Deoxyribonuclease I: 30 u/ml
	Bovine, 6 month	Chromaffin	Collagenase Type 1: 0.125%
	Bovine	Chromaffin	Collagenase: 0.2%
	Bovine	Chromaffin	Deoxyribonuclease I: 30 u/mg
	Bovine (also rat)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%
	Bovine	Chromaffin	Collagenase Type 1: 0.25 %
	Bovine	Chromaffin	Collagenase: 0.05%
	Bovine	Chromaffin	Collagenase: 0.15%
	Bovine	Chromaffin	Collagenase: 0.1%

Medium	Reference	Adipose/Fat (con't)
HEPES	Ding, S., McNeel, R., and Mersmann, H.: Expression of Porcine Adipocyte Transcripts: Tissue Distribution and Differentiation <i>In Vitro</i> and <i>In Vivo</i> , <i>Comp Biochem Physiol B</i> 123, 307, 1999 (1144)	
Krebs-Ringer bicarbonate albumin	Wang Y, Fried SK, Petersen RN, Schoknecht PA: Somatotropin regulates adipose tissue metabolism in neonatal swine, <i>J Nutr</i> 129, 139-45, 1999 (10339)	
DMEM/F12	Suryawan, A., Swanson, L., and Hu, C.: Insulin and Hydrocortisone, But Not Triiodothyronine, Are Required for the Differentiation of Pig Preadipocytes in Primary Culture, <i>J Anim Sci</i> 75, 105, 1997 (9790)	
DMEM	Basu, J., Genheimer, C., Sangha, N., Quinlan, S., Guthrie, K., Kelley, R., Ilagan, R., Jain, D., Bertram, T. and Ludlow, J.: Organ Specific Regenerative Markers in Peri-Organ Adipose: Kidney., <i>Lipids Health Dis</i> Vol. 10, 171, 2011 (10665)	
PBS	Liu, Z., Wang, H., Zhang, Y., Zhou, J., Lin, Q., Wang, Y., Duan, C., Wu, K. and Wang, C.: Efficient Isolation of Cardiac Stem Cells from Brown Adipose., <i>J Biomed Biotechnol</i> Vol. 2010, 104296, 2010 (10598)	
Ham's F12	Aoki, S., Toda, S., Sakemi, T., and Sugihara, H.: Coculture of Endothelial Cells and Mature Adipocytes Actively Promotes Immature Preadipocyte Development <i>In Vitro</i> , <i>Cell Struct Funct</i> 28, 55, 2003 (9791)	
KRHB	Mora, S., Yang, C., Ryder, J., Boeglin, D and Pessin, J: The MEF2A and MEF2D Isoforms are Differentially Regulated in Muscle and Adipose Tissue during States of Insulin Deficiency, <i>Endocrinology</i> 142, 1999, 2001 (9796)	
DMEM	Omatsu-Kanbe, M., and Matsuura, H.: Inhibition of Store-operated Ca ²⁺ Entry by Extracellular ATP in Rat Brown Adipocytes, <i>J Physiol</i> 521 (3), 601, 1999 (1307)	
DMEM /F-12	Serrero, G: Primary Culture in Defined Medium of Adipocyte Precursors, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 11B:6.1, 1995 (1285)	
Kreb's-Ringer bicarbonate buffer modified	Charron, M.J. and Kahn, B.B.: Divergent Molecular Mechanisms for Insulin-Resistant Glucose Transport in Muscle and Adipose Cells <i>In Vivo</i> , <i>J Biol Chem</i> 265, 7994, 1990 (571)	
Parker Medium 199	Gaben-Cogneville, A., Poussin, B., Chamblier, M., Forgue-Fafitte, M., and Rosselin, G.: Development of Insulin and Epidermal Growth Factor Receptors During the Differentiation of Rat Preadipocytes in Primary Culture, <i>Biochim Biophys Acta</i> 968, 231, 1988 (336)	
Krebs Ringer bicarbonate buffer	Woodward, Julie A. and Saggerson, E.: Effect of Adenosine Deaminase, N6-Phenylisopropyladenosine and Hypothyroidism on the Responsiveness of Rat Brown Adipocytes to Noradrenaline, <i>Biochem J</i> 238, 395, 1986 (311)	
Krebs Ringer bicarbonate buffer	Pessin, J.E., Gitomer, W., Oka, Y., Oppenheimer, C.L., and Czech, M.P.: B-Adrenergic Regulation of Insulin and Epidermal Growth Factor Receptors in Rat Adipocytes, <i>J Biol Chem</i> 258, 7386, 1983 (558)	
Kreb's Ringer	Green, A. and Newsholme, E.: Sensitivity of Glucose Uptake and Lipolysis of White Adipocytes of the Rat to Insulin and Effects of Some Metabolites, <i>Biochem J</i> 180, 365, 1979 (310)	
Kreb's Ringer bicarbonate buffer	Stiles, J.W., Francendese, A.A. and Masoro, E.J.: Influence of Age on Size and Number of Fat Cells in the Epididymal Depot, <i>Am J Physiol</i> 229, 1561, 1975 (285)	
Bicarbonate buffer	Fain, J., Reed, N., and Saperstein, R.: Isolation and Metabolism of Brown Fat Cells, <i>J Biol Chem</i> 242, 1887, 1967 (549)	
Albumin-bicarbonate buffer	Rodbell, M.: Metabolism of Isolated Fat Cells. I. Effects of Hormones on Glucose Metabolism and Lipolysis, <i>J Biol Chem</i> 239 (2), 375, 1964 (548)	

Adrenal

Medium	Reference	
DMEM	Hahn, S., Chen, Y., Vinson, C. and Eiden, L.: A Calcium-Initiated Signaling Pathway Propagated Through Calcineurin and cAMP Response Element-Binding Protein Activates Proenkephalin Gene Transcription after Depolarization., <i>Mol Pharmacol</i> 64, 1503, 2003 (10565)	
Locke's solution	Moustafa T, Girod S, Tortosa F, Li R, Sol JC, Rodriguez F, Bastide R, Lazorthes Y, Sallerin B: Viability and functionality of bovine chromaffin cells encapsulated into alginate-PLL microcapsules with a liquefied inner core, <i>Cell Transplant</i> 15, 121-33, 2006 (10341)	
Locke's solution	Ortega, J., Sagen, J., and Pappas, G.: Short-term Immunosuppression Enhances Long-term Survival of Bovine Chromaffin Cell Xenografts in Rat CNS, <i>Cell Transplant</i> 1, 33, 1992 (359)	
HEPES	Zhu, J., Li, W., Toews, M., and Hexum, T.: Neuropeptide Y Inhibits Forskolin-Stimulated Adenylate Cyclase in Bovine Adrenal Chromaffin Cells via a Pertussis Toxin-Sensitive Process, <i>J Pharmacol Exp Ther</i> 263 (3), 1479, 1992 (1232)	
25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, <i>In Vitro Cell Dev Biol</i> 26, 335, 1990 (438)	
DMEM	Dahmer, M., Hart, P., and Perlman, R.: Studies on the Effect of Insulin-Like Growth Factor-I on Catecholamine Secretion from Chromaffin Cells, <i>J Neurochem</i> 54 (3), 931, 1990 (1231)	
Locke's solution, CMF	Aunis, D., Rotllan, P., and Miras-Portugal, M.: Incorporation of Adenosine into Nucleotides of Chromaffin Cells in Culture, <i>Neurochem Int</i> 7, 89, 1985 (644)	
Kreb's, CMF	Almazan, G., Aunis, D., Garcia, A., Montiel, C., Nicolas, G., and Sanchez-Garcia, P.: Effects Of CLS on the Release of Noradrenaline From Chromaffin Cells, <i>Br J Biomed Sci</i> 81, 599, 1984 (343)	
(see reference)	Pollard, H., Pazoles, C., Creutz, C., Scott, J., Zinder, O., and Hotchkiss, A.: An Osmotic Mechanism For Exocytosis From Dissociated Chromaffin Cells, <i>J Biol Chem</i> 259, 1114, 1984 (559)	

Tissue Dissociation Guide

	Bovine	Chromaffin	Collagenase Type 1: 0.05%
	Bovine	Chromaffin	Collagenase Type 1: 0.025%
	Bovine	Medulla	Hyaluronidase: 0.2%
	Bovine	Medulla	Collagenase: 0.2%
	Bovine	Chromaffin	Deoxyribonuclease I: 15 µg/ml
	Bovine	Medulla	Protease: 0.2%
	Bovine	Chromaffin	Collagenase: 0.05%
	Bovine, adult (also rat, Hanover-Wistar, young; guinea-pig, newborn)	Chromaffin	Collagenase: 0.5%
	Bovine	Chromaffin	Collagenase: 0.25%
	Bovine	Medulla	Collagenase: 0.05%
	Bovine, adult	Medulla	Collagenase Type 1: 0.5%
	Bovine	Chromaffin	Collagenase: 0.05%
	Bovine	Medullary	Collagenase Type 2: 0.2%
	Bovine	Foreskin	Collagenase: 0.5%
	Bovine	Medulla	Collagenase Type 1: 0.05%
	Bovine	Chromaffin	Hyaluronidase: 0.2%
Guinea-Pig	Guinea-pig, 500-700 g	Chromaffin Medulla	Collagenase:
	Guinea-pig (also rat, Hanover-Wistar, young; newborn; cattle)	Chromaffin	Collagenase: 0.5%
Hamster	Guinea-pig	Adrenal Chromaffin	Collagenase: 0.05%-0.20%
	Hamster (<i>Mesocricetus auratus</i>) 100-150 g	Adrenal Chromaffin	Hyaluronidase: 0.20%
Human	Human	Chromaffin cells	Collagenase: 0.2%
	Human	Adrenocortical	Collagenase Type 1: 0.2% Deoxyribonuclease I: 0.01%
	Human, adult	Chromaffin	Trypsin: 0.25%
	Human, adult and child	Foreskin	Collagenase: 0.5%
Mouse	Mouse, embryonic	Chromaffin cells	Papain: 20-25 u/ml
Ovine	Ovine, adult and fetal	Adrenocortical	Collagenase Type 1: 0.4%
	Ovine, fetal	Adrenocortical	Collagenase Type 1: 0.4%

Medium	Reference
CF Kreb's	Cena, V., Garcia, A., Montiel, C., and Sanchez-Garcia, P.: Uptake of [³ H]-nicotine and [³ H]-noradrenaline by Cultured Chromaffin Cells, <i>Br J Pharmacol</i> 81, 119, 1984 (342)
HBSS, modified	Waymire, J., Bennett, W., Boehme, R., Hankins, L., Gilmer-Waymire, K., and Haycock, J.: Bovine Adrenal Chromaffin Cells: High-Yield Purification and Viability in Suspension Culture, <i>J Neurosci Methods</i> 7, 329, 1983 (608)
Saline w/BSA 0.5%	Knight, D. and Baker, P.: Stimulus-Secretion Coupling in Isolated Bovine Adrenal Medullary Cells, <i>Q J Exp Physiol</i> 68, 123, 1983 (715)
Krebs-Ringer bicarbonate buffer, CMF	Greenberg, A. and Zinder, O.: alpha- and beta-Receptor Control of Catecholamine Secretion from Isolated Adrenal Medulla Cells, <i>Cell Tissue Res</i> 226, 655, 1982 (356)
Medium A (see reference)	Wilson, S.P., and Viveros, O.H.: Primary Culture of Adrenal Medullary Chromaffin Cells in a Chemically Defined Medium, <i>Exp Cell Res</i> 133, 159, 1981 (392)
Saline	Baker, P., and Knight, D.: Calcium Control of Exocytosis and Endocytosis in Bovine Adrenal Medullary Cells, <i>Phil Trans R Soc Lond</i> 296, 83, 1981 (1158)
Locke's solution, CMF	Trifaro, J.M., and Lee, R.W.: Morphological Characteristics and Stimulus-Secretion Coupling in Bovine Adrenal Chromaffin Cell Cultures, <i>Neuroscience</i> 5, 1533, 1980 (647)
HBSS	Unsicker, K., Rieffert, B., and Ziegler, W.: Effects of Cell Culture Conditions, Nerve Growth Factor, Dexamethasone, and Cyclic AMP on Adrenal Chromaffin Cells <i>In Vitro</i> , <i>Adv Biochem Psychopharmacol</i> 255, 51, 1980 (713)
F-12 medium	Kumakura, K., Karoum, F., Guidotti, A., and Costa, E.: Modulation of Nicotinic Receptors by Opiate Receptor Agonists in Cultured Adrenal Chromaffin Cells, <i>Nature</i> 283, 489, 1980 (714)
Locke's solution, CF	Kilpatrick, D., Ledbetter, F., Carson, K., Kirshner, A., Slepets, R., and Kirshner, N.: Stability of Bovine Adrenal Medulla Cells in Culture, <i>J Neurochem</i> 35 (3), 679, 1980 (1157)
HBSS	Unsicker, K., and Griesser, G.: Establishment, Characterization and Fibre Outgrowth of Isolated Bovine Adrenal Medullary Cells in Long-Term Cultures, <i>Neuroscience</i> 5, 1445, 1980 (1160)
DMEM	Aunis, D., Guerold, B., Bader, M-F., and Cieselski-Treska, J.: Immunocytochemical and Biochemical Demonstration of Contractile Proteins in Chromaffin Cells, <i>Neuroscience</i> 5, 2261, 1980 (1161)
HEPES	Hersey, R., and DiStefano, V.: Control of Phenylethanolamine N-Methyltransferase by Glucocorticoids in Cultured Bovine Adrenal Medullary Cells, <i>J Pharmacol Exp Ther</i> 209 (1), 147, 1979 (1159)
Dulbecco's MEM w/10% calf serum	Folkman, J., Haudenschild, C. C., and Zetter, B. R.: Long-term Culture of Capillary Endothelial Cells, <i>Proc Natl Acad Sci U S A</i> 76, 5217, 1979 (653)
Kreb's, CF	Fenwick, E., Fajdiga, P., Howe, N., and Livett, B.: Functional and Morphological Characterization of Isolated Bovine Adrenal Medullary Cell, <i>J Cell Biol</i> 76, 12, 1978 (591)
HEPES, CF	Brooks, J.C.: The Isolated Bovine Adrenomedullary Chromaffin Cell: A Model of Neuronal Excitation-Secretion, <i>Endocrinology</i> 101, 1369, 1977 (373)
BSS (see reference)	Role, L.W., Leeman, S.E., and Perlman, R.L.: Somatostatin and Substance P Inhibit Catecholamine Secretion from Isolated Cells of Guinea-pig Adrenal Medulla, <i>Neurochem Int</i> 6, 1813, 1981 (643)
HBSS	Unsicker, K., Rieffert, B., and Ziegler, W.: Effects of Cell Culture Conditions, Nerve Growth Factor, Dexamethasone, and Cyclic AMP on Adrenal Chromaffin Cells <i>In Vitro</i> , <i>Adv Biochem Psychopharmacol</i> 255, 51, 1980 (713)
Kreb's-Ringer bicarb glucose buffer, CF	Hochman, J., and Perlman, R.L.: Catecholamine Secretion by Isolated Adrenal Cells, <i>Biochim Biophys Acta</i> 421, 168, 1976 (320)
Kreb's Ringer bicarbonate buffer	Liang, B.T., and Perlman, R.L.: Catecholamine Secretion by Hamster Adrenal Cells, <i>J Neurochem</i> 32, 927, 1979 (606)
Locke's solution	Jeon, Y., Baek, W., Chung, S., Shin, N., Kim, H., and Lee, S.: Cultured Human Chromaffin Cells Grafted in Spinal Subarachnoid Space Relieves Allodynia in a Pain Rat Model., <i>Korean J Anesthesiol Vol.</i> 60, 357, 2011 (10566)
Kreb's Ringer	Caroccia, B., Fassina, A., Seccia, T., Recarti, C., Petrelli, L., Belloni, A., Pelizzo, M. and Rossi, G.: Isolation of Human Adrenocortical Aldosterone-Producing Cells by a Novel Immunomagnetic Beads Method., <i>Endocrinology</i> 151, 1375, 2010 (10680)
Eagle's MEM	Tischler, A., DeLellis, R., Bailes, B., Nunnemacher, G., Carabba, V., and Wolfe, H.: Nerve Growth Factor-Induced Neurite Outgrowth from Normal Human Chromaffin Cells, <i>Lab Invest</i> 43, 399, 1980 (625)
Dulbecco's MEM w/10% calf serum	Folkman, J., Haudenschild, C. C., and Zetter, B. R.: Long-term Culture of Capillary Endothelial Cells, <i>Proc Natl Acad Sci U S A</i> 76, 5217, 1979 (653)
DMEM	Tian Jin-Hua, Wu Zheng-Xing, Unzicker Michael, Lu Li, Cai Qian, Li Culling, Schirra Claudia, Matti Ulf, Stevens David, Deng Chuxia, Rettig Jens, Sheng Zu-Hang: The role of Snapin in neurosecretion: snapin knock-out mice exhibit impaired calcium-dependent exocytosis of large dense-core vesicles in chromaffin cells, <i>J Neurosci</i> 25, 10546-55, 2005 (10118)
DMEM/Ham's F12	Valego, N. and Rose, J.: A Specific CRH Antagonist Attenuates ACTH-Stimulated Cortisol Secretion in Ovine Adrenocortical Cells., <i>Reprod Sci Vol.</i> 17, 477, 2010 (10562)
DMEM/Ham's F12	Valego, N., Su, Y., Carey, L., Young, S., Tatter, S., Wang, J. and Rose, J.: Hypothalamic-Pituitary Disconnection in Fetal Sheep Blocks the Peripartum Increases in Adrenal Responsiveness and Adrenal ACTH Receptor Expression., <i>Am J Physiol Regul Integr Comp Physiol</i> 289, R410, 2005 (10563)

Tissue Dissociation Guide

Species	Adrenal (con't)	Cell(s)	Enzyme(s)
Rat	Ovine, adult	Chromaffin cells	Collagenase Type 2: 0.2% Deoxyribonuclease I: 100 u/ml
	Ovine, 3 year	Anterior pituitary	Trypsin: 2.5% Deoxyribonuclease I: 0.004%
	Rat, SD	Chromaffin cells	Collagenase Type 1: 0.26% Deoxyribonuclease I: 0.015% Hyaluronidase: 0.015%
	Rat	Chromaffin	Collagenase Type 1: 0.26% Deoxyribonuclease I: 0.015% Hyaluronidase: 0.015%
	Rat, SD, male	Zona fasciculata/reticularis	Collagenase: 0.4%
	Rat, Wistar, newborn	Chromaffin cells	Collagenase Type 1: 0.025% Deoxyribonuclease I: 0.0015%
	Rat, SD, male	ZG ZFR	Collagenase Type 1: 0.2%
	Rat, Fischer, male, 10-16 weeks	Adrenocortical	Deoxyribonuclease I: 0.005%
	Rat, male, 120-160 g, Rat, SD, male, 400-450 g	Leydig Adrenal	Collagenase Type 2: 0.03% (adrenal)
	Rat, SD, 2-4 day old (also bovine)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%
	Rat, Long-Evans, female, 150-200 g	Glomerulosa	Collagenase: 0.2%
	Rat, Fischer, male, 1-10 months	Adrenocortical	Deoxyribonuclease I: 0.005%
	Rat, SD, male, 400-450 g	Decapular Capsular Glomerulosa	Deoxyribonuclease I: 0.01%
	Rat	Chromaffin	Trypsin: 0.10%
	Rat, Hanover-Wistar, 2nd postnatal week (also guinea-pig, cattle)	Chromaffin	Collagenase: 0.5%
	Rat, SD, female, 200 g	Glomerulosa	Deoxyribonuclease I: 0.05%
	Rat	Foreskin	Collagenase: 0.5%
	Rat, Wistar-Hanover, 7-12 day	Medullary	Trypsin: 0.125%
	Rat, SD, male	Cortical	Trypsin: 0.25%
	Rat, Holtzman, male, 180-250 g	Adrenal	Collagenase Type 1: 0.5%

Bone

Species		Cell(s)	Enzyme(s)
Bovine	Bovine	Chondrocytes	Collagenase Type 2: 0.4%
Chicken	Chick, day old	Osteoblasts	Trypsin: 0.03%
	Chick, Peterson/Arbor Acre, male, 4 weeks old (<i>Gallus domesticus</i>)	Chondrocytes	Trypsin: 0.25%
	Chick, embryo	Vertebrae chondroblasts	Trypsin: 0.25%

Tissue Dissociation Guide

Adrenal (con't)

Medium	Reference
Locke's solution	Keating, D., Rychkov, G., Adams, M., Holgert, H., McMillen, I.C. and Roberts, M.: Opioid Receptor Stimulation Suppresses the Adrenal Medulla Hypoxic Response in Sheep by Actions on Ca(2+) and K(+) Channels., <i>J Physiol</i> 555, 489, 2004 (10567)
DMEM	Canny B J, O'Farrell K A, Clarke I J, Tilbrook A J: The influence of sex and gonadectomy on the hypothalamo-pituitary-adrenal axis of the sheep, <i>J Endocrinol</i> 162, 215-25, 1999 (10324)
HBSS	Gilabert, J: Necessary Conditions to Maintain Rat Adrenal Chromaffin Cells in Primary Culture, <i>Cell Biology of the Chromaffin Cell</i> , Borges, R. and Gandia, L., Instituto Teofilo Hernando, 2004 (10564)
HBSS	Gilabert, J, Montalvo, G, and Artalejo A.: Rat Chromaffin cells primary cultures: Standardization and quality assessment for single-cell assays, <i>Nat Protoc</i> , 294, 2006 (10349)
Krebs-HEPES	Bruder EricD, Ball Dennis L, Goodfriend TheodoreL, Raff Hershel: An oxidized metabolite of linoleic acid stimulates corticosterone production by rat adrenal cells, <i>Am J Physiol Regul Integr Comp Physiol</i> 284, R1631-5, 2003 (10134)
DMEM	Zhang L, Castell A, Avila E, Drucker-Colin R, Escobar A: Immunocytochemical, ultrastructural and neurochemical evidences on synaptogenesis and dopamine release of rat chromaffin cells co-cultured with striatal neurons, <i>J Neuropathol Exp Neurol</i> 59, 170-4, 2000 (10247)
Kreb's	Sayed, S., Whitehouse, B., and Jones, P.: Phosphoserine/Threonine Phosphatases in the Rat Adrenal Cortex: A Role in the Control of Steroidogenesis, <i>J Endocrinol</i> 154, 449, 1997 (1072)
BSS	Roskelley, C.D. and Auersperg, N.: Density Separation of Rat Adrenocortical Cells: Morphology, Steroidogenesis, and P-450scc Expression in Primary Culture, <i>In Vitro Cell Dev Biol</i> 26, 493, 1990 (425)
Krebs Ringer bicarbonate buffer	Ng, T. and Liu, W.: Toxic Effect of Heavy Metals on Cells Isolated from the Rat Adrenal and Testis, <i>In Vitro Cell Dev Biol</i> 26, 24, 1990 (435)
25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, <i>In Vitro Cell Dev Biol</i> 26, 335, 1990 (438)
MEM-d-Val	Payet, N., Deziel, Y., and Lehoux, J.-G.: Vasopressin: A Potent Growth Factor in Adrenal Glomerulosa Cells in Culture, <i>J Steroid Biochem</i> 20, 449, 1984 (621)
BSS	Leonard, R.K., Auersperg, N., and Parkes, C.O.: Ascorbic Acid Accumulation by Cultured Rat Adrenocortical Cells, <i>In Vitro</i> 19, 46, 1983 (527)
Medium 199	Li, C.H., Ng, T.B., and Cheng, C.H.K.: Melanotropins: Aldosterone- and Corticosterone-Stimulating Activity in Isolated Rat Adrenal Cells, <i>Int J Pept Protein Res</i> 19, 361, 1982 (543)
Ham's F-12 w/HEPES	Englert, D.F.: An Optical Study of Isolated Rat Adrenal Chromaffin Cells, <i>Exp Cell Res</i> 125, 369, 1980 (389)
HBSS	Unsicker, K., Rieffert, B., and Ziegler, W.: Effects of Cell Culture Conditions, Nerve Growth Factor, Dexamethasone, and Cyclic AMP on Adrenal Chromaffin Cells, <i>Adv Biochem Psychopharmacol</i> 25, 51, 1980 (711)
Kreb's	Brale, L., Williams, G., and Bradwin, G.: The Effect of Unit Gravity Sedimentation on Adrenal Steroidogenesis by Isolated Rat Glomerulosa and Fasciculata Cells, <i>Endocrinology</i> 106 (1), 50, 1980 (769)
Dulbecco's MEM w/10% calf serum	Folkman, J., Haudenschild, C. C., and Zetter, B. R.: Long-term Culture of Capillary Endothelial Cells, <i>Proc Natl Acad Sci U S A</i> 76, 5217, 1979 (653)
HBSS	Unsicker, K., Krisch, B., Otten, U., and Thoenen, H.: Nerve Growth Factor-Induced Fiber Outgrowth From Isolated Rat Adrenal Chromaffin Cells: Impairment by Glucocorticoids, <i>Proc Natl Acad Sci U S A</i> 75 (7), 3498, 1978 (988)
Kreb's Ringer bicarbonate buffer	Barofsky, A., Feinstein, M., and Halkerston, I.: Enzymatic and Mechanical Requirements for the Dissociation of Cortical Cells From Rat Adrenal Glands, <i>Exp Cell Res</i> 79, 263, 1973 (1010)
Kreb's Ringer bicarbonate buffer	Kloppenborg, P., Island, D., Little, G., Michelakis, A., and Nicholson, W.: A Method of Preparing Adrenal Cell Suspensions and Its Applicability to the <i>In Vitro</i> Study of Adrenal Metabolism, <i>Endocrinology</i> 82, 1053, 1968 (383)

Bone

Medium	Reference
DMEM	Buschmann, M., Gluzband, Y., Grodzinsky, A., and Hunziker, E.: Mechanical Compression Modulates Matrix Biosynthesis in Chondrocyte/Agarose Culture, <i>J Cell Sci</i> 108, 1497, 1995 (1133)
DMEM	Gay, C., Lloyd, Q., and Gilman, V.: Characteristics and Culture of Osteoblasts Derived From Avian Long Bone, <i>In Vitro Cell Dev Biol</i> 30A, 379, 1994 (1036)
Ham's F12	Rossetol, G., Reginato, A.M., and Leach, R.M.: Development of a Serum-Free System to Study the Effect of Growth Hormone and Insulinlike Growth Factor-1 on Cultured Postembryonic Growth Plate Chondrocytes, <i>In Vitro Cell Dev Biol</i> 28A, 235, 1992 (481)
Simm's, CMF	Schiltz, J.R., Mayne R., and Holtzer, H.: The Synthesis of Collagen and Glycosaminoglycans by Dedifferentiated Chondroblasts in Culture, <i>Differentiation</i> 1, 97, 1973 (678)

Tissue Dissociation Guide

TISSUE TABLES

Species	Bone (con't)	Cell(s)	Enzyme(s)
Human	Human, 22-73 year	Osteoblasts	Trypsin: 0.5%
	Human, 60+ year	Bone cells, osteoblasts	Collagenase Type 2: 200-250 u/ml
	Human	Osteoblasts	Trypsin: 0.1%
	Human	Osteoblasts	Collagenase Type 4: 250 u/ml
Mouse	Mouse	Endosteal cells	Collagenase Type 1: 0.3%
	Mouse, male, 6-8 week old	Osteoclasts	Collagenase Type 3: 0.1%
	Mouse, 3-5 day, 6-8 week	Bone cells, osteoblasts	Collagenase Type 2: 200-250 u/ml
	Mouse, BALB/c	Osteoblastlike cells Stromal cell lines Hematopoietic blast cells	Trypsin: 0.1%
	Mouse, Swiss-Webster	Neonatal bone	Collagenase Type 2: 0.20%
Rat	Rat, fetal, 21 days of gestation	Calvaria	Collagenase: 0.2%
	Rat, newborn	Osteoblastlike cells	Collagenase Type 2: 0.3%

Brain

Species		Cell(s)	Enzyme(s)
Bovine	Bovine	Microvascular endothelial	Neutral Protease: 0.005%
	Bovine	Brain endothelial cells	Collagenase Type 2: 0.35%
	Bovine	Endothelial	Neutral Protease: 0.125%
	Bovine, adult	Cerebral artery Endothelial	Collagenase: 0.2%
	Bovine	Capillary endothelial	Collagenase: 0.1%
	Bovine	Endothelial	Neutral Protease: 0.5%
	Calf (also lamb)	Oligodendroglia Neural	Trypsin: 0.1%
Guinea-Pig	Guinea-pig, 200-400 g	Neurons	Trypsin: 0.06-0.08%
Human	Human	Tumor	Collagenase Type 4: 0.1% Hyaluronidase: 0.07% Deoxyribonuclease I: 0.04%
	Human	Microglia	Trypsin: 0.25% Deoxyribonuclease I: .005%
	Human	Microvessels	Collagenase Type 4: 0.1%
	Human	Neuronal	Deoxyribonuclease I: 10 µg/ml
	Human, 15-54 years	Microvessels	Collagenase: 0.1%
Insect	Drosophila	Neurons	Papain: 20 u/ml

Tissue Dissociation Guide

Medium	Reference
Basal Medium	Kneser U, Voogd A, Ohnolz J, Buettner O, Stangenberg L, Zhang YH, Stark GB, Schaefer DJ: Fibrin gel-immobilized primary osteoblasts in calcium phosphate bone cement: in vivo evaluation with regard to application as injectable biological bone substitute, <i>Cells Tissues Organs</i> 179, 158-69, 2005 (10316)
DMEM	Chen X., Qian H., Neff L., Satomura K., and Horowitz M.: Thy-1 antigen Expression by Cells in the Osteoblast Lineage, <i>J Bone Miner Res</i> 14, 362, 1999 (9811)
DMEM	Meikle, M., Boyd, S., Hembry, R., Compston, J., Croucher, P., and Reynolds, J.: Human Osteoblasts in Culture Synthesize Collagenase and Other Matrix Metalloproteinases in Response to Osteotropic Hormones and Cytokines, <i>J Cell Sci</i> 103, 1093, 1992 (1229)
DMEM	Fedarko, N.S., Termine, J.D., Young, M.F. and Robey, P.G.: Temporal Regulation of Hyaluronan and Proteoglycan Metabolism by Human Bone Cells in Vitro, <i>J Biol Chem</i> 265, 12200, 1990 (567)
DMEM	Nakamura, Y., Arai, F., Iwasaki, H., Hosokawa, K., Kobayashi, I., Gomei, Y., Matsumoto, Y., Yoshihara, H. and Suda, T.: Isolation and Characterization of Endosteal Niche Cell Populations that Regulate Hematopoietic Stem Cells., <i>Blood</i> 116, 1422, 2010 (10621)
DMEM	Sakai, E., Miyamoto, H., Okamoto, K., Kato, Y., Yamamoto, K., and Sakai, H.: Characterization of Phagosomal Subpopulations Along Endocytic Routes in Osteoclasts and Macrophages, <i>J Biochem (Tokyo)</i> 130, 823, 2001 (1145)
DMEM	Chen X., Qian H., Neff L., Satomura K., and Horowitz M.: Thy-1 antigen Expression by Cells in the Osteoblast Lineage, <i>J Bone Miner Res</i> 14, 362, 1999 (9811)
Eagle's MEM	Takanashi, H., Matsuishi, T., and Yoshizato, K.: Establishment and Characterization of Stromal Cell Lines That Support Differentiation of Murine Hematopoietic Blast Cells into Osteoblast-like Cells, <i>In Vitro Cell Dev Biol</i> 30A, 384, 1994 (1037)
Tris-buffered saline	Chen, T. and Feldman, D.: Regulation of 1,25-Dihydroxyvitamin D3 Receptors in Cultured Mouse Bone Cells, <i>J Biol Chem</i> 256, 5561, 1981 (554)
MEM	Owen, T., Aronow, M., Shalhoub, V., Barone, L., Wilming, L., Tassinari, M., Kennedy, M., Pockwinse, S., Lian, J., and Stein, G.: Progressive Development of the Rat Osteoblast Phenotype <i>In Vitro</i> : Reciprocal Relationships in Expression of Genes Associated with Osteoblast Proliferation and Differentiation During Formation of the Bone Extracellular Matrix, <i>J Cell Physiol</i> 143, 420, 1990 (1235)
MEM	Ernst, M., and Froesch, E.: Osteoblastlike Cells in a Serum-Free Methylcellulose Medium Form Colonies: Effects of Insulin and Insulinlike Growth Factor I, <i>Calcif Tissue Int</i> 40, 27, 1987 (1007)

Brain

Medium	Reference
Medium 199	Kanda, T., Yoshino, H., Ariga, T., Yamawaki, M., and Yu, R.: Glycosphingolipid Antigens in Cultured Microvascular Bovine Brain Endothelial Cells: Sulfoglucuronosyl Paragloboside as a Target of Monoclonal IgM in Demyelinative Neuropathy, <i>J Cell Biol</i> 126 (1), 235, 1994 (950)
DMEM	Wolburg H, Neuhaus J, Kniessel U, Krauss B, Schmid EM, Ocalan M, Farrell C, Risau W: Modulation of tight junction structure in blood-brain barrier endothelial cells. Effects of tissue culture, second messengers and cocultured astrocytes, <i>J Cell Sci</i> 107, 1347, 1994 (10048)
MEM	Miller, D., Audus, K., and Borchardt, R.: Application of Cultured Endothelial Cells of the Brain Microvasculature in the Study of the Blood-Brain Barrier, <i>J Tiss Cul Meth</i> 14, 217, 1992 (942)
HBSS	Machi, T., Kassell, N.F., and Scheld, W.M.: Isolation and Characterization of Endothelial Cells From Bovine Cerebral Arteries, <i>In Vitro Cell Dev Biol</i> 26, 291, 1990 (436)
DMEM	Estrada, C., Bready, J., Berliner, J., and Cancilla, P.: Choline Uptake by Cerebral Capillary Endothelial Cells in Culture, <i>J Neurochem</i> 54, 1467, 1990 (949)
MEM	Audus, K., and Borchardt, R.: Characterization of an <i>In Vitro</i> Blood-Brain Barrier Model System for Studying Drug Transport and Metabolism, <i>Pharm Res</i> 3 (2), 81, 1986 (855)
(see reference)	Poduslo, S., Miller, K., and McKhann, G.: Metabolic Properties of Maintained Oligodendroglia Purified from Brain, <i>J Biol Chem</i> 253, 1592, 1978 (552)
PIPES saline	Kay, A.R., and Wong, R.K.S.: Isolation of Neurons Suitable for Patch-Clamping from Adult Mammalian Central Nervous Systems, <i>J Neurosci Methods</i> 16, 227, 1986 (607)
(see reference)	Sauvageot, C., Weatherbee, J., Kesari, S., Winters, S., Barnes, J., Dellagatta, J., Ramakrishna, N., Stiles, C., Kung, A., Kieran, M. and Wen, P.: Efficacy of the HSP90 Inhibitor 17-AAG in Human Glioma Cell Lines and Tumorigenic Glioma Stem Cells., <i>Neuro Oncol Vol.</i> 11, 109, 2009 (10592)
DMEM/F12	Klegeris Andis, McGeer PatrickL: Chymotrypsin-like proteases contribute to human monocytic THP-1 cell as well as human microglial neurotoxicity, <i>Glia</i> 51, 56-64, 2005 (10112)
DMEM	Gerhart, D. Z., Broderius, M. A., and Drewes, L. R.: Cultured Human and Canine Endothelial Cells from Brain Microvessels, <i>Brain Res Bull</i> 21, 785, 1988 (344)
Tris-HCl, 50 mM, CaCl ₂ , 2 mM	Roher, A.E., Palmer, K.C., Chau, V., and Ball, M.J.: Isolation and Chemical Characterization of Alzheimer's Disease Paired Helical Filament Cytoskeletons: Differentiation from Amyloid Plaque Core Protein, <i>J Cell Biol</i> 107, 2703, 1988 (581)
Serum-free modified Lewis medium	Vinters, H.V., Reave, S., Costello, P., Girvin, J.P., and Moore, S.A.: Isolation and Culture of Cells Derived From Human Cerebral Microvessels, <i>Cell Tissue Res</i> 249, 657, 1987 (357)
Saline	Gu, H. and O'Dowd, D.: Whole Cell Recordings from Brain of Adult Drosophila., <i>J Vis Exp</i> 6, 248, 2007 (10651)

Tissue Dissociation Guide

Species	Brain (con't)	Cell(s)	Enzyme(s)
Monkey	Monkey, rhesus	Brain cells	Collagenase Type 2: 500 u/ml Deoxyribonuclease I: 28 u/ml
Mouse	Mouse, 1-2 day	Oligodendrocytes, dorsal root ganglia	Papain: 0.15% Deoxyribonuclease I: 0.006%
	Mouse, 6-12 week	Vascular smooth muscle cells	Papain: 0.05% Collagenase Type 4: 0.15% Elastase: 0.05%
	Mouse, 4-6 day	Neurons	PDS kit: with modifications
	Mouse, postnatal	Astrocytes	Trypsin: 0.25% Deoxyribonuclease I: 1,000 u/ml
	Mouse, neonatal	Neurons	Papain: 20 u/ml
	Mouse, embryonic and postnatal	Cortical neurons	Papain: 4-10 u/ml
	Mouse, 1 day	Neural progenitor	PDS kit: per instructions
	Mouse	Granule cell precursors, pre-neoplastic and tumor cells	Papain: 10 u/ml Deoxyribonuclease I: 250 u/ml
	Mouse	Cortical neurons	PDS kit: per instructions
	Mouse, embryonic	Cortical progenitors	PDS kit: per instructions
	Mouse, 1 day	Neurons and glia	PDS kit: per instructions
	Mouse	Neurospheres	PDS kit:
	Mouse, 3 day	Microglia	Papain: 90 u/ml Deoxyribonuclease I: 2000 u/ml
	Mouse, SD, 8-12 week	Microglia	Trypsin: 0.125% Collagenase Type 2: 0.01% Deoxyribonuclease I: .005%
	Mouse, 2-5 day	Postnal substantia nigra	PDS kit: per instructions
	Mouse	Cerebellar granule cell precursors	Papain: 0.435% Deoxyribonuclease I: 0.05%
	Mouse	Astrocytes	Trypsin: 0.25%
	Mouse, newborn	Hippocampal cells	Papain: 10 u/ml
	Mouse, SWR or CF1, 1-3 months	Papillae, taste receptor	Pronase E: 0.15%
	Mouse, 0-30 day	Neural	Trypsin NF 1:250: 50 0.25%
Ovine	Lamb (also calf)	Oligodendroglia Neural	Trypsin: 0.1%
Porcine	Mini pigs, Yucatan (Sus scrofa Yucatan), 4-6 months	Microvascular	Collagenase: 0.1%
Rat	Rat, SD, 19-21 day	Suprachiasmatic nucleus neurons	Papain: 100 u/ml
	Rat, Fisher, 7-21 month	Hippocampal neurons	Papain: 0.2%

Tissue Dissociation Guide

Medium	Reference
HBSS	Marcondes MC, Burudi EM, Huitron-Resendiz S, Sanchez-Alavez M, Watry D, Zandonatti M, Henriksen SJ, Fox HS: Highly activated CD8(+) T cells in the brain correlate with early central nervous system dysfunction in simian immunodeficiency virus infection, <i>J Immunol</i> 167, 5429-38, 2001 (10125)
DMEM	O'Meara, R., Ryan, S., Colognato, H. and Kothary, R.: Derivation of Enriched Oligodendrocyte Cultures and Oligodendrocyte/Neuron Myelinating Co-Cultures from Post-Natal Murine Tissues., <i>J Vis Exp</i> 54, 3324, 2011 (10650)
PBS	Chung, W., Farley, J., Swenson, A., Barnard, J., Hamilton, G., Chiposi, R. and Drummond, H.: Extracellular Acidosis Activates ASIC-like Channels in Freshly Isolated Cerebral Artery Smooth Muscle Cells., <i>Am J Physiol Cell Physiol</i> 298, C1198, 2010 (10575)
HBSS	Lee, H., Greene, L., Mason, C. and Manzini, M.: Isolation and Culture of Post-Natal Mouse Cerebellar Granule Neuron Progenitor Cells and Neurons., <i>J Vis Exp</i> 23, 990, 2009 (10652)
HBSS	Sher, F., Rosslor, R., Brouwer, N., Balasubramanian, V., Boddeke, E. and Copray, S.: Differentiation of Neural Stem Cells Into Oligodendrocytes: Involvement of the Polycomb Group Protein Ezh2., <i>Stem Cells</i> 26, 2875, 2008 (10507)
Neurobasal	Fasano, C., Thibault, D. and Trudeau, L.: Culture of Postnatal Mesencephalic Dopamine Neurons on an Astrocyte Monolayer, <i>Current Protocols in Neuroscience Vol. 44</i> , 3.21.1, 2008 (10687)
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(see reference)	Seaberg, R., Smukler, S. and Van der Kooy, D.: Intrinsic Differences Distinguish Transiently Neurogenic Progenitors from Neural Stem Cells in the Early Postnatal Brain., <i>Dev Biol</i> 278, 71, 2005 (10363)
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Neurobasal	Hernandez, F., Perez, M., Lucas, J., Mata, A., Bhat, R. and Avila, J.: Glycogen Synthase Kinase-3 plays a Crucial Role in Tau Exon 10 Splicing and Intranuclear Distribution of SC35. Implications for Alzheimer's disease., <i>J Biol Chem</i> 279, 3801, 2004 (10361)
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Eagle's MEM	Nishioku T, Hashimoto K, Yamashita K, Liou SY, Kagamiishi Y, Maegawa H, Katsube N, Peters C, von Figura K, Saffig P, Katunuma N, Yamamoto K, Nakanishi H: Involvement of cathepsin E in exogenous antigen processing in primary cultured murine microglia, <i>J Biol Chem</i> 277, 4816, 2002 (10043)
RPMI-1640	O'Donnell SL, Frederick TJ, Krady JK, Vannucci SJ, Wood TL: IGF-I and microglia/macrophage proliferation in the ischemic mouse brain, <i>Glia</i> 39, 85, 2002 (10050)
(see reference)	Smeyne Michelle, Smeyne RichardJ: Method for culturing postnatal substantia nigra as an in vitro model of experimental Parkinson's disease, <i>Brain Res Brain Res Protoc</i> 9, 105-11, 2002 (10274)
EBSS	Miyazawa K, Himi T, Garcia V, Yamagishi H, Sato S, and Ishizaki Y.: A role for p27/Kip1 in the control of cerebellar granule cell precursor proliferation, <i>J Neurosci</i> 20, 5756, 2000 (10060)
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DMKM	Jun K, Choi G, Yang SG, Choi KY, Kim H, Chan GC, Storm DR, Albert C, Mayr GW, Lee CJ, Shin HS: Enhanced hippocampal CA1 LTP but normal spatial learning in inositol 1,4,5-trisphosphate 3-kinase(A)-deficient mice, <i>Learn Mem</i> 5, 317-30, 1998 (10130)
Carbonate-Phosphate buffer (see reference)	Spielman, A., Mody, I., Brand, J., Whitney, G., MacDonald, J., and Salter, M.: A Method for Isolating and Patch-Clamping Single Mammalian Taste Receptor Cells, <i>Brain Res</i> 503, 326, 1989 (350)
BSS	Shrier, B., Wilson, S., and Nirenberg, M.: Cultured Cell Systems and Methods for Neurobiology, Vol. 32, 765, 1974 (637)
(see reference)	Poduslo, S., Miller, K., and McKhann, G.: Metabolic Properties of Maintained Oligodendroglia Purified from Brain, <i>J Biol Chem</i> 253, 1592, 1978 (552)
HBSS	Robinson, D.H., Kang, Y., Deschner, S.H., and Nielsen, T.B.: Morphologic Plasticity and Periodicity: Porcine Cerebral Microvascular Cells in Culture, <i>In Vitro Cell Dev Biol</i> 26, 169, 1990 (432)
MEM	Cao, R., Li, A., Cho, H., Lee, B. and Obrietan, K.: Mammalian Target of Rapamycin Signaling Modulates Photocentrin of the Suprachiasmatic Circadian Clock., <i>J Neurosci</i> 30, 6302, 2010 (10512)
Hibernate A	Chen, N., Newcomb, J., Garbuzova-Davis, S., Davis Sanberg, C., Sanberg, P. and Willing, A.: Human Umbilical Cord Blood Cells Have Trophic Effects on Young and Aging Hippocampal Neurons in Vitro., <i>Aging Dis</i> 1, 173, 2010 (10663)

Tissue Dissociation Guide

Species

Brain (con't)

Cell(s)

Enzyme(s)

Rat, SD, 7 day	Cerebellar granule neurons	PDS kit: per instructions
Rat, neonatal	Astrocytes	Papain: 20 u/ml
Rat, neonatal	Hippocampal neurons	Papain: 10 u/ml
Rat, E18	Hippocampal neurons	Papain: 0.2%
Rat, Wistar, 1-3 day	Hippocampal neurons	Trypsin: 0.05%
Rat, E19	Hippocampal neuron	Papain: 10 u/ml
Rat, Wistar, 14 day	Visual cortical	PDS kit:
Rat, Wistar, male	Cerebral endothelial	Collagenase Type 3: 0.2%
Rat, SD, E18	Cortical cells	Papain: 20 u/ml Deoxyribonuclease I: .005%
Rat, Wistar, 250-300 g	Cerebral artery smooth muscle cells	Papain: 1.5 mg/ml Collagenase Type 4: 1.5 mg/ml
Rat, SD, E16	Cortical neurons	PDS kit: with modifications
Rat, Wistar, neonatal	Hippocampal cells	Papain: 1 mg/ml
Rat, SD, Fisher	Hippocampal neurons	Papain: 0.2%
Rat, E18	Hippocampal and cortical neurons	PDS kit:
Rat, SD, newborn	Glial	Trypsin: 0.0625%
Rat, newborn	Astrocytes	Trypsin: 0.25%
Rat, 60-72 hours old	Fibroblasts	Trypsin: 0.2%
Rat, SD, adult	Endothelial	Collagenase Type 2: 0.5%
Rat, SD, female	Fetal rat brain	Collagenase Type 4: 0.1%
Rat, albino, adult and newborn	Cerebral cortices	Trypsin: 0.25%
Rat, Wistar, newborn	Germinal matrix	Trypsin: 0.25%
Rat, fetus, 18-20 day	Hippocampal neurons	Trypsin: 0.25%
Rat, fetus, 18 day	Hippocampi	Trypsin: 0.1%
Rat, SD, 19-20 days pregnant	Neural	Trypsin: 0.25%
Rat, SD, fetal	Cerebral cortex Hypothalamus	Deoxyribonuclease I: 0.001%
Rat, Wistar-Kyoto, male, 100 - 200 g	Endothelial Cerebral	Collagenase Type 2: 0.05%

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Medium	Reference
PBS	Tanaka, S., Shaikh, I., Chiocca, E. and Saeki, Y.: The Gs-Linked Receptor GPR3 Inhibits the Proliferation of Cerebellar Granule Cells During Postnatal Development., <i>PLoS ONE</i> 4, e5922, 2009 (10487)
EBSS	Shigetomi, E. and Khakh, B.: Measuring Near Plasma Membrane and Global Intracellular Calcium Dynamics in Astrocytes., <i>J Vis Exp</i> 26, 1142, 2009 (10656)
EBSS	Richler Esther, Chaumont Severine, Shigetomi Eiji, Sagasti Alvaro, Khakh Baljit S: Tracking transmitter-gated P2X cation channel activation in vitro and in vivo, <i>Nat Methods</i> 5, 87-93, 2008 (10319)
Hibernate	Jekabsons MB, Nicholls DG: Bioenergetic analysis of cerebellar granule neurons undergoing apoptosis by potassium/serum deprivation, <i>Cell Death Differ</i> 13, 1595-610, 2006 (10129)
DMEM	Velasco Myriam, Garca#a Esperanza, Onetti Carlos G: Glucose deprivation activates diversity of potassium channels in cultured rat hippocampal neurons, <i>Cell Mol Neurobiol</i> 26, 307-19, 2006 (10321)
MEM	Khakh Baljit S, Fisher James A, Nashmi Raad, Bowser David N, Lester Henry A: An angstrom scale interaction between plasma membrane ATP-gated P2X2 and alpha4beta2 nicotinic channels measured with fluorescence resonance energy transfer and total internal reflection fluorescence microscopy, <i>J Neurosci</i> 25, 6911-20, 2005 (10307)
EBSS	Mizoguchi Y, Kanematsu T, Hirata M, Nabekura J: A rapid increase in the total number of cell surface functional GABAA receptors induced by brain-derived neurotrophic factor in rat visual cortex, <i>J Biol Chem</i> 278, 44097, 2003 (10022)
MEM	Floris S, Van den Born J, van der Pol SM, Dijkstra CD, De Vries HE: Heparan sulfate proteoglycans modulate monocyte migration across cerebral endothelium, <i>J Neuropathol Exp Neurol</i> 62, 780, 2003 (10041)
EBSS	Behar, T, Smith, S, Kennedy, R, Mckenzie, J, Maric, I and Barker, J: GABA(B) receptors mediate motility signals for migrating embryonic cortical cells, <i>Cereb Cortex</i> 11, 744-53, 2001 (10116)
physiological salt solution	Brzezinska AK, Gebremedhin D, Chilian WM, Kalyanaraman B, Elliott SJ.: Peroxynitrite reversibly inhibits Ca(2+)-activated K(+) channels in rat cerebral artery smooth muscle cells, <i>Am J Physiol Heart Circ Physiol</i> 278, H1883, 2000 (10049)
Neurobasal medium	Varney MA, Cosford ND, Jachec C, Rao SP, Sacaan A, Lin FF, Bleicher L, Santori EM, Flor PJ, Allgeier H, Gasparini F, Kuhn R, Hess SD, Velicelebi G, Johnson EC: SIB-1757 and SIB-1893: selective, noncompetitive antagonists of metabotropic glutamate receptor type 5, <i>J Pharmacol Exp Ther</i> 290, 170, 1999 (10023)
DMEM	Boehm S, Betz H: Somatostatin inhibits excitatory transmission at rat hippocampal synapses via presynaptic receptors, <i>J Neurosci</i> 17, 4066, 1997 (10047)
HibernateA/B27	Brewer, G.J.: Isolation and culture of adult rat hippocampal neurons of any age, <i>J Neurosci Methods</i> 71, 143, 1997 (10067)
Neurobasal media	Naeve GS, Ramakrishnan M, Kramer R, Hevroni D, Citri Y, Theill LE: Neuritin: a gene induced by neural activity and neurotrophins that promotes neuritogenesis, <i>Proc Natl Acad Sci U S A</i> 94, 2648-53, 1997 (10107)
MEM, sterile	Pixley, S.K.: The Olfactory Nerve Contains Two Populations of Glia, <i>In Vitro</i> 5, 269, 1992 (536)
DMEM, HBSS	Holzwarth, J., Glaum, S., and Miller, R.: Activation of Endothelin Receptors by Sarafotoxin Regulates Ca2+ Homeostasis in Cerebellar Astrocytes, <i>Glia</i> 5, 239, 1992 (948)
HEPES buffered DMEM	Acheson, A., Barker, P., Alderson, R., Miller, F., and Murphy, R.: Detection of Brain-Derived Neurotrophic Factor-like Activity in Fibroblasts and Schwann Cells: Inhibition by Antibodies to NGF, <i>Neuron</i> 7, 265, 1991 (675)
Medium 199	Doron, D., Jacobowitz, D., Heldman, E., Feuerstein, G., Pollard, H., and Hallenbeck, J.: Extracellular Matrix Permits the Expression of Von Willebrand's Factor, Uptake of Di-I-Acetylated Low Density Lipoprotein and Secretion of Prostacyclin in Cultures of Endothelial Cells from Rat Brain Microvessels, <i>In Vitro Cell Dev Biol</i> 27A, 689, 1991 (860)
DMEM	Matsuda, M.: Serum Proteins Enhance Aggregate Formation of Dissociated Fetal Rat Brain Cells in an Aggregating Culture, <i>In Vitro Cell Dev Biol</i> 24 (10), 1031, 1988 (861)
PBS	Giulian, D. and Baker, T.J.: Characterization of Ameboid Microglia Isolated From Developing Mammalian Brain, <i>J Neurosci</i> 6, 2163, 1986 (616)
HBSS	Goldman, J.E., Geier, S.S., and Hirano, M.: Differentiation of Astrocytes and Oligodendrocytes From Germinal Matrix Cells in Primary Culture, <i>J Neurosci</i> 6, 52, 1986 (618)
HBSS, CMF	Bartlett, W. and Banker, G.: An Electron Microscopic Study of the Development of Axons and Dendrites by Hippocampal Neurons in Culture. I. Cells Which Develop Without Intercellular Contacts, <i>J Neurosci</i> 4, 1944, 1984 (613)
HBSS	Rothman, S.: Synaptic Release of Excitatory Amino Acid Neurotransmitter Mediates Anoxic Neuronal Death, <i>J Neurosci</i> 4 (7), 1884, 1984 (1011)
DMEM	Ahmed, Z., Walker, P., and Fellows, R.: Properties of Neurons from Dissociated Fetal Rat Brain in Serum-Free Culture, <i>J Neurosci</i> 3 (12), 2448, 1983 (1202)
HEPES	Peterfreund, R. and Vale, W.: High Molecular Weight Somatostatin Secretion by Cultured Rat Brain Cell, <i>Brain Res</i> 239, 463, 1982 (349)
HBSS	Diglio, C.A., Grammas, P., Filiberto Giacomelli, M.S., and Wiener, J.: Primary Culture of Rat Cerebral Microvascular Endothelial Cells, <i>Lab Invest</i> 46, 554, 1982 (626)

Tissue Dissociation Guide

Species

Brain (con't)

Cell(s)

Enzyme(s)

	Rat, fetus	Cortical	Trypsin: 0.25%
	Rat, Wistar, pregnant	Glial	Trypsin: 0.05%
	Rat, SD, one month old	Capillary endothelium Pericytes	Neutral Protease: 0.1%
	Rat, Wistar, male, 300-500 g	Microvessels Endothelial	Collagenase Type 2: 0.75%
	Rat, Wistar, adult, 170 g	Endothelial	Trypsin: 0.5%
	Rat, Holtzmann, 18 day	Hippocampal neurons	Trypsin: 0.1%
	Rat, SD, 2 days	Pineal	Trypsin: 0.25%
Shellfish	<i>Helisoma trivolvis</i>	Neurons Buccal ganglia	Trypsin: 0.2%

Cartilage

Species

Cell(s)

Enzyme(s)

Bovine	Bovine, 18-36 month	Chondrocytes	Collagenase Type 1: 0.1%
	Bovine, 6-8 week	Chondrocytes	Collagenase Type 2: 0.2%
	Steers, 1-2 years	Chondrocytes	Trypsin: 0.20%
	Calf, 1-14 days	Chondrocytes	Collagenase Type 2: 0.20%
Chicken	Chick embryos, HH stage	Mesenchymal	Trypsin: 0.1%
	Chicken, broiler strain, 8-10 weeks	Matrix vesicles Epiphyseal growth plate	Trypsin: 0.1%
	Chicken, broiler strain, 8-10 week	Matrix vesicles	Trypsin: 0.1%
	Chick embryos, White Leghorn, 19 day old	Fibroblasts Epithelial-like	Trypsin: 0.25%
	Chick embryos, 19 day old	Chondrocytes	Trypsin: 0.75%
	Chick embryos, White Leghorn	Wing buds	Trypsin: 0.1%
Human	Human, 52-82 years	Chondrocytes	Collagenase Type 2: 0.2% Pronase: 0.15%
	Human	Synoviocytes	Collagenase Type 1: 0.4%
	Human, 26-68 year	Meniscus and cartilage	Collagenase Type 2: 0.15%
	Human, 64-83 yr	Chondrocytes	Collagenase Type 2: 0.08%
	Human, adult	Synovial	Collagenase: 0.15% Hyaluronidase: 0.1% Deoxyribonuclease I: 0.015%
	Human, adult	Chondrocytes	Collagenase Type 2: 0.15%

Tissue Dissociation Guide

Medium	Reference
Puck's D1 (see reference)	Swaiman, K., Neale, E., Fitzgerald, S., and Nelson, P.: A Method for Large-scale Production of Mouse Brain Cortical Cultures, <i>Brain Res</i> 255, 361, 1982 (1281)
Eagle's MEM/DMEM	Abney, E., Bartlett, P., and Raff, M.: Astrocytes, Ependymal Cells, and Oligodendrocytes Develop on Schedule in Dissociated Cell Cultures of Embryonic Rat Brain, <i>Dev Biol</i> 83, 301, 1981 (858)
Medium 199	Bowman, P., Betz, A., Ar, D., Wolinsky, J., Penney, J., Shivers, R., and Goldstein, G.: Primary Culture of Capillary Endothelium From Rat Brain, <i>In Vitro</i> 17 (4), 353, 1981 (935)
Ringers-HEPES buffer	Williams, S., Gillis, J., Matthews, M., Wagner, R., and Bitensky, M.: Isolation and Characterization of Brain Endothelial Cells: Morphology and Enzyme Activity, <i>J Neurochem</i> 35 (2), 374, 1980 (885)
BSS	Phillips, P., Kumar, P., Kumar, S., and Waghe, M.: Isolation And Characterization of Endothelial Cells From Rat And Cow Brain White Matter, <i>J Anat</i> 129, 261, 1979 (708)
HBSS	Banker, G., and Cowan, M.: Rat Hippocampal Neurons in Dispersed Cell Culture, <i>Brain Res</i> 126, 397, 1977 (1004)
DMEM, MEM	Rowe, V., Neale, E., Avins, L., Guroff, G., and Schrier, B.: Pineal gland cells in culture. Morphology, Biochemistry, Differentiation, and co-culture with sympathetic neurons, <i>Exp Cell Res</i> 104, 345, 1977 (1311)
Saline, sterile	Hadley, R.D., Bodnar, D.A., and Kater, S.B.: Formation of Electrical Synapses Between Isolated, Cultured Helisoma Neurons Requires Mutual Neurite Elongation, <i>J Neurosci</i> 5, 3145, 1985 (615)

Cartilage

Medium	Reference
HBSS	White, R. and Gibson, J.: The Effect of Oxygen Tension on Calcium Homeostasis in Bovine Articular Chondrocytes., <i>J Orthop Surg Res Vol.</i> 5, 27, 2010 (10610)
DMEM	Hwang, Y., Sangaj, N. and Varghese, S.: Interconnected Macroporous Poly(ethylene glycol) Cryogels as a Cell Scaffold for Cartilage Tissue Engineering., <i>Tissue Eng Part A Vol.</i> 16,, , 3033-41, 2010 (10631)
HBSS	Mackintosh, D., and Mason, R.: Pharmacological Actions of 17 Beta-oestradiol on Articular Cartilage Chondrocytes and Chondrosarcoma Chondrocytes in the Absence of Oestrogen Receptors, <i>Biochim Biophys Acta</i> 964, 295, 1988 (334)
PBS	Klagsbrun, M.: Large Scale Preparation of Chondrocytes, <i>Vol.</i> 58,, , 560, 1979 (1263)
DMEM (see reference)	Wong, M., and Tuan, R.: Nuserum, A Synthetic Serum Replacement, Supports Chondrogenesis of Embryonic Chick Limb Bud Mesenchymal Cells in Micromass Culture, <i>In Vitro Cell Dev Biol</i> 29A, 917, 1993 (965)
Tris-buffered saline	Genge, B., Wu, L. and Wuthier, R.: Differential Fractionation of Matrix Vesicle Proteins: Further Characterization of the Acidic Phospholipid-dependent Ca ²⁺ -Binding Proteins, <i>J Biol Chem</i> 265, 4703, 1990 (569)
(see reference)	Genge, B.R., Wu, L.N.Y., and Wuthier, R.E.: Identification of Phospholipid-dependent Calcium-binding Proteins as Constituents of Matrix Vesicles, <i>J Biol Chem</i> 264, 10917, 1989 (564)
E 199 medium	Gionti, E., Capasso, O., and Cancedda, R.: The Culture of Chick Embryo Chondrocytes and the Control of Their Differentiated Functions <i>in Vitro</i> , <i>J Biol Chem</i> 258 (11), 7190, 1983 (982)
Coon's modified F-12	Capasso, O., Gionti, E., Pontarelli, G., Ambesi-Impioabato, F., Nitsch, L., Tajana, G., and Cancedda, R.: The Culture of Chick Embryo Chondrocytes and the Control of Their Differentiated Functions <i>In Vitro</i> , <i>Exp Cell Res</i> 142, 197, 1982 (983)
Saline G	Ahrens, P., Solorsh, M., and Reiter, R.: Stage-Related Capacity for Limb Chondrogenesis in Cell Culture, <i>Dev Biol</i> 60, 69, 1977 (967)
DMEM/F12	Pallu, S., Francin, P., Guillaume, C., Gegout-Pottie, P., Netter, P., Mainard, D., Terlain, B. and Presle, N.: Obesity Affects the Chondrocyte Responsiveness to Leptin in Patients with Osteoarthritis., <i>Arthritis Res Ther</i> 12, R112, 2010 (10619)
DMEM	Kim Wan-Uk, Kwok Seung-Ki, Hong Kyung-Hee, Yoo Seung-Ah, Kong Jin-Sun, Choe Jongseon, Cho Chul-Soo: Soluble Fas ligand inhibits angiogenesis in rheumatoid arthritis, <i>Arthritis Res Ther</i> 9, R42, 2007 (10173)
DMEM	Marsano A, Millward-Sadler SJ, Salter DM, Adesida A, Hardingham T, Tognana E, Kon E, Chiari-Grisar C, Nehrer S, Jakob M, Martin I: Differential cartilaginous tissue formation by human synovial membrane, fat pad, meniscus cells and articular chondrocytes, <i>Osteoarthritis Cartilage</i> 15, 48-58, 2007 (10338)
DMEM/F12	Tallheden T, Bengtsson C, Brantsing C, Sjogren-Jansson E, Carlsson L, Peterson L, Brittberg M, and Lindahl A.: Proliferation and Differentiation Potential of Chondrocytes from Osteoarthritic Patients, <i>Arthritis Res Ther</i> 7(3), R560, 2005 (9755)
DMEM	Liagre B, Vergne-Salle P, Corbiere C, Charissoux JL, and Beneytout JL.: Diosgenin, a Plant Steroid, Induces Apoptosis in Human Rheumatoid Arthritis Synoviocytes with Cyclooxygenase-2 Overexpression, <i>Arthritis Res Ther</i> 6(4), R373, 2004 (9815)
DMEM	Jakob M, Demartean O, Schafer D, Stumm M, Heberer M, and Martin I.: Enzymatic Digestion of Adult Human Articular Cartilage Yields a Small Fraction of the Total Available Cells, <i>Conn Tissue Res</i> 44, 173, 2003 (9812)

Tissue Dissociation Guide

Species

Cartilage (con't)

Cell(s)

Enzyme(s)

	Human, adults	Synoviocytes	Collagenase Type 1: 0.1%
	Human, 15-60 years	Chondrocytes	Collagenase Type 2: 0.2%
	Human	Synovial fibroblasts	Collagenase: 0.4%
	Human	Septal chondrocytes	Collagenase Type 2: 0.2% Hyaluronidase: 0.01% Deoxyribonuclease I: 0.015%
	Human w/ rheumatoid arthritis	Synovial tissue	Trypsin: 0.05%
	Human, 13-62 years	Articular chondrocytes	Trypsin: 0.2%
	Human, 26-84 years	Chondrocytes	Collagenase:
Mouse	Mouse, 1 day	Chondrocytes	Collagenase: 0.2%
Ovine	Sheep, 2 month	Chondrocytes	Collagenase Type 2: 0.3%
Porcine	Porcine, 1 year	Chondrocytes	Collagenase Type 1: 0.2%
	Porcine, 2-4 month	Chondrons	Neutral Protease: 0.3% Collagenase: 0.2%
Rabbit	Rabbit, New Zealand, 1.2-1.4 kg	Chondrocytes	Collagenase Type 2: 0.025% Pronase: 0.2%
	Rabbit, New Zealand, 1.8-2.3kg	Chondrocytes	Hyaluronidase: .05% Collagenase Type 2: 0.2% Trypsin: 0.2%
	Rabbit, New Zealand, white, 4-6 wk & 22-25 wk	Chondrocytes	Protease XIV: 5 mg/g of tissue
	Rabbit, white, male, 8 weeks	Chondrocytes	Trypsin: 0.2%
	Rabbit, New Zealand white or Dutch, 1 week (also human, newborn)	Chondrocytes	Trypsin: 0.1%
	Rabbit, New Zealand white, immature, 2.25 - 3.3 Kg	Articular chondrocytes Hyaline	Trypsin: 0.2%
	Rabbit, New Zealand white, male, 250-350 g	Epiphyseal Articular cartilage	Trypsin: 0.25%
Rat	Rat, SD, young, 100-120 g	Chondrocytes	Trypsin: 0.2%

Colon

Species

Cell(s)

Enzyme(s)

Human	Human	Colonic epithelial	Collagenase: 150 u/ml Neutral Protease: 0.04 mg/ml
	Human	Colorectal cancer	Collagenase Type 4: 1% Deoxyribonuclease I: 0.2%
	Human	Colonic epithelial	Collagenase Type 4: 0.1%
	Human	Colonic epithelial	Collagenase:Neutral Protease:0.3% Deoxyribonuclease I:0.05%

Medium	Reference	Cartilage (con't)
RPMI	McEvoy A., Murphy E., Ponnio T., Conneely O., Bresnihan B., FitzGerald O., and Murphy E.: Activation of Nuclear Orphan Receptor NURR1 Transcription by NF-kappa B and Cyclic Adenosine 5'-Monophosphate Response Element-binding Protein in Rheumatoid Arthritis Synovial Tissue, <i>J Immunol</i> 168(6), 2979, 2002 (9754)	
DMEM	Rotter N, Bonassar LJ, Tobias G, Lebl M, Roy AK, Vacanti CA: Age dependence of cellular properties of human septal cartilage: implications for tissue engineering, <i>Arch Otolaryngol Head Neck Surg</i> 127, 1248-52, 2001 (10219)	
DMEM	Sarkissian M, Lafyatis R: Integrin engagement regulates proliferation and collagenase expression of rheumatoid synovial fibroblasts, <i>J Immunol</i> 162, 1772-9, 1999 (10174)	
DMEM/F12	Dunham B P, Koch R J: Basic fibroblast growth factor and insulinlike growth factor I support the growth of human septal chondrocytes in a serum-free environment, <i>Arch Otolaryngol Head Neck Surg</i> 124, 1325-30, 1998 (10172)	
DMEM (see reference)	Dayer, J., Krane, S., Russell, R., and Robinson, D.: Production of Collagenase and Prostaglandins by Isolated Adherent Rheumatoid Synovial Cells, <i>Proc Natl Acad Sci U S A</i> 73 (3), 945, 1976 (780)	
BSS	Srivastava, V.M.L., Malemud, C.J., Hough, A.J., Bland, J.H., and Sokoloff, L.: Preliminary Experience with Cell Culture of Human Articular Chondrocytes, <i>Arthritis Rheum</i> 17, 165, 1974 (726)	
GBSS	Manning, W.K., and Bonner, W.M.: Isolation and Culture of Chondrocytes From Human Adult Articular Cartilage, <i>Arthritis Rheum</i> 10, 235, 1967 (727)	
DMEM	Terpstra, L, Prud'homme, J, Arabian, A, Takeda, S, Karsenty, G, Dedhar, S, and St-Arnaud, R.: Reduced Chondrocyte Proliferation and Chondrodysplasia in Mice Lacking the Integrin-linked Kinase in Chondrocytes, <i>J Cell Biol</i> 162, 139, 2003 (9756)	
Ham's F-12	Kojima Koji, Bonassar LawrenceJ, Roy AmitK, Mizuno Hirokazu, Cortiella Joaquin, Vacanti CharlesA: A composite tissue-engineered trachea using sheep nasal chondrocyte and epithelial cells, <i>FASEB J</i> 17, 823-8, 2003 (10216)	
DEMEM	Chowdhury, T., Schulz, R., Rai, S., Thuemmler, C., Wuestneck, N., Bader, A and Homandberg, G: Biomechanical Modulation of Collagen Fragment-Induced Anabolic and Catabolic Activities in Chondrocyte/Agarose Constructs., <i>Arthritis Res Ther</i> 12, R82, 2010 (10611)	
PBS	Graff RD, Lazarowski ER, Banes AJ, Lee GM: ATP release by mechanically loaded porcine chondrons in pellet culture, <i>Arthritis Rheum</i> 43, 1571-9, 2000 (10253)	
DMEM	Ju, X., Deng, M., Ao, Y., Yu, C., Wang, J., Yu, J., Cui, G. and Hu, Y.: Protective Effect of Sinomenine on Cartilage Degradation and Chondrocytes Apoptosis., <i>Yakugaku Zasshi Vol.</i> 130, 1053-60, 2010 (10604)	
Gey's solution	Mehraban F, Tindal MH, Proffitt MM, Moskowitz RW.: Temporal pattern of cysteine endopeptidase (cathepsin B) expression in cartilage and synovium from rabbit knees with experimental osteoarthritis: gene expression in chondrocytes in response to interleukin-1 and matrix depletion, <i>Ann Rheum Dis</i> 56, 108, 1997 (10031)	
Ham's F-12	Plaas, A., Sandy, J., and Kimura, J.: Biosynthesis of Cartilage Proteoglycan & Link Protein Articular Chondrocytes, <i>J Biol Chem</i> 263, 7560, 1988 (562)	
Gey's BSS	Benya, P.D., Padilla, S.R., and Nimni, M.E.: The Progeny of Rabbit Articular Chondrocytes Synthesize Collagen Types I and III and Type I Trimer, but Not Type II, <i>Biochem</i> 16, 865, 1977 (312)	
Saline G, CMF	Schindler, F.H., Ose, M.A., and Solursh, M.: The Synthesis of Cartilage Collagen by Rabbit and Human Chondrocytes in Primary Cell Culture, <i>In Vitro</i> 12, 44, 1976 (495)	
Gey's BSS	Green, J.R., and William, T.: Articular Cartilage Repair. Behavior of Rabbit Chondrocytes During Tissue Culture and Subsequent Allografting, <i>Clin Orthop Relat Res</i> , 237, 1976 (710)	
Eagle's basal medium	Bentley, G., and Greer, R.: Homotransplantation of Isolated Epiphyseal and Articular Cartilage Chondrocytes into Joint Surfaces of Rabbits, <i>Nature</i> 230, 385, 1971 (641)	
Ham's F-12 medium	Shimomura, Y., Yoneda, T., and Suzuki, F.: Osteogenesis by Chondrocytes from Growth Cartilage of Rat Rib, <i>Calcif Tissue Res</i> 19, 179, 1975 (351)	

Colon

Medium	Reference
Basal X media	Roig, A., Eskicak, U., Hight, S., Kim, S., Delgado, O., Souza, R., Spechler, S., Wright, W. and Shay, J.: Immortalized Epithelial Cells Derived from Human Colon Biopsies Express Stem Cell Markers and Differentiate In Vitro., <i>Gastroenterol</i> 138, 1012, 2010 (10560)
HBSS	Zhou, J., Belov, L., Huang, P., Shin, J., Solomon, M., Chapuis, P., Bokey, L., Chan, C., Clarke, C., Clarke, S. and Christopherson, R.: Surface Antigen Profiling of Colorectal Cancer Using Antibody Microarrays With Fluorescence Multiplexing., <i>J Immunol Methods</i> 355,
Not listed	Huang, E., Hynes, M., Zhang, T., Ginestier, C., Dontu, G., Appelman, H., Fields, J., Wicha, M. and Boman, B.: Aldehyde Dehydrogenase 1 is a Marker for Normal and Malignant Human Colonic Stem Cells (SC) and Tracks SC Overpopulation During Colon Tumorigenesis., <i>Cancer Res</i> 69, 3382-9, 2009 (10489)
RPMI 1640	Fukushima, K. and Fiocchi, C.: Paradoxical Decrease of Mitochondrial DNA Deletions in Epithelial Cells of Active Ulcerative Colitis Patients., <i>Am J Physiol Gastrointest Liver Physiol</i> Vol. 286, G804-13, 2004 (10355)

Tissue Dissociation Guide

Species

Colon (con't)

Cell(s)

Enzyme(s)

Mouse	Human	Epithelial and mucosal lymphocytes	Neutral Protease: 0.1% CLSPA: 0.02% Deoxyribonuclease I: 0.01%
	Human	Colonic epithelial	Neutral Protease: 1.2u/ml Collagenase Type 4: 50 u/ml
	Human	Cancer stem cell	Collagenase Type 3: 200 u/ml Deoxyribonuclease I: 100 u/ml
	Human	Colonic endothelial cells	Collagenase Type 2: 0.25%
	Human	Lamina propria lymphocytes	Collagenase: 25 u/ml
	Mouse, 6-8 week	Cancer stem cell	Collagenase Type 3: 200 u/ml Deoxyribonuclease I: 100 u/ml
	Mouse	Lamina propria	Collagenase Type 2: 0.1% Neutral Protease: 0.1% Deoxyribonuclease I: 0.004%
	Mouse	Dendric	Collagenase: 300 u/ml Deoxyribonuclease I: 0.002%
	Mouse	Lamina propria mononuclear cells	Collagenase: 0.05% Deoxyribonuclease I: 0.05% Neutral Protease: 0.3%
	Mouse	Lymphocytes	Collagenase/Dispase: 100 u/ml
Mouse, 6-8 week	Lamina propria lymphocytes	Collagenase Type 1: 0.2% Deoxyribonuclease I: 0.01%	
Mouse, 6-8 week	Lamina propria mononuclear cells	Collagenase Type 2: 0.015% Deoxyribonuclease I: 0.01%	

Endothelial

Species

Cell(s)

Enzyme(s)

Bovine	Bovine	Bovine umbilical cord (BUVEC)	Collagenase: 0.1%
	Bovine, (<i>Bos taurus</i>), calf	Endothelial Pulmonary	Collagenase: 1000 u/ml
	Calf	Endothelial	Trypsin: 0.25%
	Bovine, adult	Cerebral artery Endothelial	Collagenase: 0.2%
	Bovine	Endothelial Aortic	Trypsin: 0.05%
	Bovine	Endothelial, pulmonary artery	Collagenase: 0.1%
	Bovine	Endothelial Aorta	Collagenase Type 2: 0.1%
	Bovine	Endothelial Aortic	Collagenase Type 2: 0.1%
	Bovine	Endothelial Brain arteries	Collagenase Type 2: 0.2%
	Calf	Endothelial Smooth muscle	Collagenase: 0.75%
	Bovine	Endothelial Corneal	Trypsin: 0.2%

Tissue Dissociation Guide

Medium	Reference	Colon (con't)
RPMI 1640	Hisamatsu, T., Watanabe, M., Ogata, H., Ezaki, T., Hozawa, S., Ishii, H., Kanai, T. and Hibi, T.: Interferon-Inducible Gene Family 1-8U Expression in Colitis-Associated Colon Cancer and Severely Inflamed Mucosa in Ulcerative Colitis., <i>Cancer Res</i> 59, 5927-31, 1999 (10357)	
HBSS	Gibson, P., Rosella, O., Wilson, A., Mariadason, J., Rickard, K., Byron, K. and Barkla, D.: Colonic Epithelial Cell Activation and the Paradoxical Effects of Butyrate., <i>Carcinogenesis</i> 20, 539,1999 (10359)	
RPMI-1640	Dalerba Piero, Dylla Scott J, Park In-Kyung, Liu Rui, Wang Xinhao, Cho Robert W, Hoey Timothy, Gurney Austin, Huang Emina H, Simeone Diane M, Shelton Andrew A, Parmiani Giorgio, Castelli Chiara, Clarke Michael F: Phenotypic characterization of human colorectal cancer stem cells, <i>Proc Natl Acad Sci U S A</i> 104, 10158-63, 2007 (10221)	
HBSS/5%FBS	Wang D., Lehman R., Donner D., Matli M., Warren R., and Welton M.: Expression and Endocytosis of VEGF and Its Receptors in Human Colonic Vascular Endothelial Cells, <i>Am J Physiol/Gastro</i> 282, G1088, 2002 (9817)	
HBSS	Ueyama H, Kiyohara T, Sawada N, Isozaki K, Kitamura S, Kondo S, Miyagawa J, Kanayama S, Shinomura Y, Ishikawa H, Ohtani T, Nezu R, Nagata S, Matsuzawa Y: High Fas ligand expression on lymphocytes in lesions of ulcerative colitis, <i>Gut</i> 43, 48-55, 1998 (10244)	
RPMI-1640	Dalerba Piero, Dylla Scott J, Park In-Kyung, Liu Rui, Wang Xinhao, Cho Robert W, Hoey Timothy, Gurney Austin, Huang Emina H, Simeone Diane M, Shelton Andrew A, Parmiani Giorgio, Castelli Chiara, Clarke Michael F: Phenotypic characterization of human colorectal cancer stem cells, <i>Proc Natl Acad Sci U S A</i> 104, 10158-63, 2007 (10221)	
RPMI-1640	Atarashi, K., Nishimura, J., Shima, T., Umesaki, Y., Yamamoto, M., Onoue, M., Yagita, H., Ishii, N., Evans, R., Honda, K. and Takeda, K.: ATP Drives Lamina Propria T(H)17 Cell Differentiation., <i>Nature</i> 455, 808, 2008 (10685)	
RPMI-1640	Abe, K., Nguyen, K., Fine, S., Mo, J., Shen, C., Shenouda, S., Corr, M., Jung, S., Lee, J., Eckmann, L. and Raz, E.: Conventional Dendritic Cells Regulate the Outcome of Colonic Inflammation Independently of T Cells., <i>Proc Natl Acad Sci U S A</i> 104,17022, 2007 (10356)	
HBSS	Weigmann Benno, Tubbe Ingrid, Seidel Daniel, Nicolaev Alex, Becker Christoph, Neurath Markus F: Isolation and subsequent analysis of murine lamina propria mononuclear cells from colonic tissue, <i>Nat Protoc</i> 2, 2307-11, 2007 (10252)	
RPMI 1640	Annacker O, Coombes JL, Malmstrom V, Uhlig HH, Bourne T, Johansson-Lindbom B, Agace WW, Parker CM, Powrie F.: Essential role for CD103 in the T cell-mediated regulation of experimental colitis, <i>J Exp Med</i> 202, 1051, 2005 (10034)	
HBSS	Totsuka T, Kanai T, Uraushihara K, Iiyama R, Yamazaki M, Akiba H, Yagita H, Okumura K, Watanabe M: Therapeutic effect of anti-OX40L and anti-TNF-alpha MAbs in a murine model of chronic colitis, <i>Am J Physiol/Gastro</i> 284, G595-603, 2003 (10243)	
RPMI	Wirtz S., Becker C., Blumberg R., Galle P., and Neurath M.: Treatment of T cell-dependent experimental colitis in SCID mice by local administration of an adenovirus expressing IL-18 antisense mRNA, <i>J Immunol</i> 168(1), 411, 2002 (9826)	

Endothelial

Medium	Reference
Dulbecco's/Ham F-12	Ricken Albert M, Traenkner Anja, Merkwitz Claudia, Hummitzsch Katja, Grosche Jens, Spanel-Borowski Katharina: The short prolactin receptor predominates in endothelial cells of micro- and macrovascular origin, <i>J Vasc Res</i> 44, 19-30, 2007 (10302)
PBS, CMF	Del Vecchio, P.J., Siflinger-Birnboim, A., Belloni, P.N., Holleron, L.A., Lum, H., and Malik, A.B.: Culture and Characterization of Pulmonary Microvascular Endothelial Cell, <i>In Vitro Cell Dev Biol</i> 28A, 711, 1992 (487)
HEPES	Vender, R.: Role of Endothelial Cells in the Proliferative Response of Cultured Pulmonary Vascular Smooth Muscle Cells to Reduced Oxygen Tension, <i>In Vitro Cell Dev Biol</i> 28A, 403, 1992 (1146)
HBSS	Machi, T., Kassell, N.F., and Scheld, W.M.: Isolation and Characterization of Endothelial Cells From Bovine Cerebral Arteries, <i>In Vitro Cell Dev Biol</i> 26, 291, 1990 (436)
Krebs Ringer solution	DeNucci, G., Gryglewski, R.J., Warner, T.D., and Vane, J.R.: Receptor-Mediated Release of Endothelium-Derived Relaxing Factor and Prostacyclin From Bovine Aortic Endothelial Cells Is Coupled, <i>Proc Natl Acad Sci U S A</i> 85, 2334, 1988 (659)
CMF-Dulbecco's PBS	Martin, T.: Formation of Diacylglycerol by a Phospholipase D-phosphatidate Phosphatase Pathway Specific for Phosphatidylcholine in Endothelial Cells, <i>Biochim Biophys Acta</i> 962, 282, 1988 (333)
PBS	Carson, M.P. and Haudenschild, C.C.: Microvascular Endothelium and Pericytes: High Yield, Low Passage Cultures, <i>In Vitro Cell Dev Biol</i> 22, 344, 1986 (417)
DMEM	Kinsella, Michael G. and Wight, Thomas N.: Modulation of Sulfated Proteoglycan Synthesis by Bovine Aortic Endothelial Cells During Migration, <i>J Cell Biol</i> 102, 679, 1986 (576)
Dulbecco's PBS	Goetz, I., Warren, J., Estrada, C., Roberts, E., and Krause, D.: Long-Term Cultivation of Arterial and Capillary Endothelium From Adult Bovine Brain, <i>In Vitro Cell Dev Biol</i> 21, 172, 1985 (413)
DMEM	Voyta, J., Via, D., Butterfield, C., and Zetter, B.: Identification and Isolation of Endothelial Cells Based on Their Increased Uptake of Acetylated-Low Density Lipoprotein, <i>J Cell Biol</i> 99, 2034, 1984 (881)
PBS: DMEM	Scott, D., Murray, J., and Barnes, M.: Investigation of the Attachment of Bovine Corneal Endothelial Cells, <i>Exp Cell Res</i> 144, 472, 1983 (393)

Tissue Dissociation Guide

Species

Endothelial (con't)

Cell(s)

Enzyme(s)

	Bovine, 2-3 weeks	Endothelial Pulmonary artery	Collagenase Type 1: 0.2%
	Bovine	Endothelial, corneal	Trypsin: 0.05%
	Bovine	Endothelial Subclavian vein	Collagenase Type 1: 0.10%
	Bovine	Aortic Pulmonary artery	Collagenase Type 2: 0.10%
	Calf, fetal, 4-9 months	Endothelial	Collagenase Type 1: 0.25%
	Bovine, adult	Aorta	Collagenase Type 1: 125 u/ml
	Bovine, young	Pulmonary artery	Collagenase: 0.1%
	Bovine	Foreskin	Collagenase: 0.5%
	Bovine	Saphenous Vein Aorta	Collagenase: 0.01%
	Bovine	Endothelial Thoracic aorta Saphenous veins	Collagenase Type 2: 0.1%
	Bovine	Pulmonary artery	Collagenase Type 2: 0.25%
	Calf	Endothelial	Collagenase Type 2: 0.1%
Canine	Dog (also human)	Microvessels	Collagenase Type 4: 0.1%
	Dog, mongrel, adult	Endothelium Jugular vein	Trypsin: 0.1%
Guinea-Pig	Guinea pig, female, 300-350g	Coronary endothelial	Collagenase Type 2: 0.1%
Human	Human	Endothelial and vascular smooth muscle	Collagenase Type 1: 0.2%
	Human	Endothelial	Collagenase Type 1: 0.2%
	Human, 18-68 yr	Corneal endothelial	Collagenase: 0.2% Neutral Protease: 1.0%
	Human	HUVEC	Collagenase Type 4: 0.1%
	Human	Colonic endothelial cells	Collagenase Type 2: 0.25%
	Human	Pulmonary vascular endothelial cells	Neutral Protease: 1.18 u/ml Elastase: 10 u/ml
	Human	HUVEC	Collagenase Type 2: 0.1%
	Human	Endothelial	Trypsin: 2%
	Human	HUVEC, porcine pulmonary arterial endothelial cells	Collagenase Type 2: 0.2%
	Human	Vascular endothelial cells	Collagenase: 0.1%
	Human	Hepatic endothelial cells	Collagenase: 0.2%

Tissue Dissociation Guide

Medium	Reference	Endothelial (con't)
RPMI 1640 w/1% Fetal Bovine Serum	Lee, S., Douglas, W., Deneke, S., and Fanburg, B.: Ultrastructural Changes in Bovine Pulmonary Artery Endothelial Cells Exposed to 80% O ₂ , <i>In Vitro</i> , <i>In Vitro</i> 19, 714, 1983 (531)	
0.01M Phosphate buffer with 0.02% EDTA, 0.9% NaCl (see reference)	Robinson, J. and Gospodarowicz, D.: Glycosaminoglycans Synthesized by Cultured Bovine Corneal Endothelial Cells, <i>J Cell Physiol</i> 117, 368, 1983 (594)	
PBS	Olander, J., Marasa, J., Kimes, R., Johnston, G., and Feder, J.: An assay measuring the stimulation of several types of bovine endothelial cells by growth factor(s) derived from cultured human tumor cells, <i>In Vitro</i> 18, 99, 1982 (525)	
PBS	Makarski, J. S.: Stimulation of cyclic AMP production by vasoactive agents in cultured bovine aortic and pulmonary artery endothelial cells, <i>In Vitro</i> 17, 450, 1981 (513)	
PBS	Rosen, E., Mueller, S., Noveral, J., and Levine, E.: Proliferative Characteristics of Clonal Endothelial Cell Strains, <i>J Cell Physiol</i> 107, 123, 1981 (880)	
Dulbecco's PBS with calcium and magnesium	Cotta-Pereira, G., Sage, H., Bornstein, P., Ross, R., and Schwartz, S.: Studies of Morphologically Atypical ("Sprouting") Cultures of Bovine Aortic Endothelial Cells. Growth Characteristics and Connective Tissue Protein Synthesis, <i>J Cell Physiol</i> 102, 183, 1980 (592)	
Medium 199	Ryan, U., Mortara, M., and Whitaker, C.: Methods for Microcarrier Culture of Bovine Pulmonary Artery Endothelial Cells Avoiding the Use of Enzymes, <i>Tissue Cell</i> 12, 619, 1980 (670)	
Dulbecco's MEM w/10% calf serum	Folkman, J., Haudenschild, C. C., and Zetter, B. R.: Long-term Culture of Capillary Endothelial Cells, <i>Proc Natl Acad Sci U S A</i> 76, 5217, 1979 (653)	
PBS	Eskin, S., Sybers, H., Trevino, L., Lie, J., and Chimoskey, J.: Comparison of Tissue-Cultured Bovine Endothelial Cells from Aorta and Saphenous Vein, <i>In Vitro</i> 14, 903, 1978 (500)	
PBS	Schwartz, S.M.: Selection and Characterization of Bovine Aortic Endothelial Cells, <i>In Vitro</i> 14, 966, 1978 (501)	
Puck's solution	Ryan, U.S., Clements, E., Habliston, D., and Ryan, J.W.: Isolation And Culture of Pulmonary Artery Endothelial Cells, <i>Tissue Cell</i> 10, 535, 1978 (669)	
DMEM	Howard, B., Macarak, E., Gunson, D., and Kefalides, N.: Characterization of the Collagen Synthesized by Endothelial Cells in Culture, <i>Proc Natl Acad Sci U S A</i> 73 (7), 2361, 1976 (954)	
DMEM	Gerhart, D. Z., Broderius, M. A., and Drewes, L. R.: Cultured Human and Canine Endothelial Cells from Brain Microvessels, <i>Brain Res Bull</i> 21, 785, 1988 (344)	
Earle's PBS, CMF	Ford, J., Burkel, W., and Kahn, R.: Isolation of Adult Canine Venous Endothelium for Tissue Culture, <i>In Vitro</i> 17, 44, 1981 (512)	
(see reference)	Buxton I L, Kaiser R A, Oxhorn B C, Cheek D J: Evidence supporting the Nucleotide Axis Hypothesis: ATP release and metabolism by coronary endothelium, <i>Am J Physiol Heart Circ Physiol</i> 281, H1657-66, 2001 (10171)	
HBSS	Moss, S., Bates, M., Parrino, P. and Woods, TC.: Isolation of Endothelial Cells and Vascular Smooth Muscle Cells from Internal Mammary Artery Tissue., <i>Ochsner J</i> 7, 133, 2007 (10636)	
DMEM	Patel, V., Logan, A., Watkinson, J., Uz-Zaman, S., Sheppard, M., Ramsden, J. and Eggo, M.: Isolation and Characterization of Human Thyroid Endothelial Cells., <i>Am J Physiol Endocrinol Metab</i> Vol. 284, E168, 2003 (10586)	
DMEM/F12	Li Wei, Sabater Alfonso L, Chen Ying-Ting, Hayashida Yasutaka, Chen Szu-Yu, He Hua, Tseng Scheffer C G: A novel method of isolation, preservation, and expansion of human corneal endothelial cells, <i>Inv Ophthal Visual Sci</i> 48, 614-20, 2007 (10306)	
RPMI 1640	Silva AP, Kaufmann JE, Vivancos C, Fakan S, Cavadas C, Shaw P, Brunner HR, Vischer U, and Grouzmann E.: Neuropeptide Y expression, localization and cellular transducing effects in HUVEC, <i>Biol Cell</i> 97(6), 457, 2005 (9816)	
HBSS/5%FBS	Wang D., Lehman R., Donner D., Matli M., Warren R., and Welton M.: Expression and Endocytosis of VEGF and Its Receptors in Human Colonic Vascular Endothelial Cells, <i>Am J Physiol/Gastro</i> 282, G1088, 2002 (9817)	
M199	Muller AM, Hermanns MI, Skrzynski C, Nesslinger M, Muller KM, and Kirkpatrick CJ.: Expression of the endothelial markers PECAM-1, vWf, and CD34 in vivo and in vitro, <i>Exp Mol Pathol</i> 72, 221, 2002 (9823)	
PBS	Takano Manabu, Meneshian Avedis, Sheikh Emran, Yamakawa Yasuhiko, Wilkins Kirsten Bass, Hopkins Elise A, Bulkley Gregory B: Rapid upregulation of endothelial P-selectin expression via reactive oxygen species generation, <i>Am J Physiol Heart Circ Physiol</i> 283, H2054-61, 2002 (10311)	
PBS	Goolcharran, C., Cleland, J., Keck, R., Jones, A., and Borchardt, R.: Comparison of the Rates of Deamidation, Diketopiperazine Formation and Oxidation in Recombinant Human Vascular Endothelial Growth Factor and Model Peptides, <i>AAPS PharmSci</i> 2 (1), 5, 2000 (742)	
DMEM	Kwak HJ, Lee SJ, Lee YH, Ryu CH, Koh KN, Choi HY, and Koh GY.: Angiopoietin-1 inhibits irradiation- and mannitol-induced apoptosis in endothelial cells, <i>Circulation</i> 101(19), 2317, 2000 (9818)	
DMEM	Schonbeck U, Sukhova GK, Graber P, Coulter S, Libby P: Augmented expression of cyclooxygenase-2 in human atherosclerotic lesions, <i>Am J Pathol</i> 155, 1281-91, 1999 (10343)	
DMEM	Sanyal AJ, and Mirshahi F.: A simplified method for the isolation and culture of endothelial cells from pseudointima of transjugular intrahepatic portasystemic shunts, <i>Lab Invest</i> 78(11), 1469, 1998 (9819)	

Tissue Dissociation Guide

Species

Endothelial (con't)

Cell(s)

Enzyme(s)

Human	Foreskin microvascular endothelial	Trypsin: 0.3%
Human	Endothelial	Trypsin: 0.3.%
Human	Vascular endothelial	Neutral Protease: 0.15% Trypsin: 0.25%
Human	Umbilical vein HUVE	Collagenase: 0.1%
Human	Crypt cells	Collagenase: 125 u/ml
Human	Endothelial	Collagenase Type 1: 0.2%
Human	Human umbilical vein endothelial cells	Collagenase Type 2: 75 u/ml
Human	Microvessels	Collagenase Type 4: 0.1%
Human	Endothelial/HUVE Foreskin & umbilical cord	Trypsin: 0.3%
Human	Umbilical cord Smooth muscle	Collagenase: 0.1%
Human	Endothelial Saphenous vein	Collagenase Type 2: 0.1%
Human	Endothelial Dermal	Collagenase: 0.1%
Human	Fibroblasts Foreskin	Hyaluronidase: 0.10%
Human	Iliac arteries	Collagenase: 0.25%
Human, adult	Peripheral blood mononuclear Monocytes T cells Endothelial	Collagenase: 0.25%
Human	Microvascular endothelial Neonatal foreskins	Neutral Protease: at 1000 u/ml
Human (adult and child)	Foreskin	Collagenase: 0.5%
Human	Umbilical vein	Trypsin: 100 µg/ml
Human	Umbilical vein	Collagenase Type 1: 125 u/ml
Human	Umbilical cord	Collagenase: 0.1%
Human	Umbilical cord	Collagenase: 0.2%
Human	Umbilical cord	Trypsin NF 1:250: 0.125%
Human	Umbilical cord	Trypsin NF 1:250: 0.25%
Mouse	Mouse, 7-10 week Liver endothelial	Collagenase: 0.03%

Tissue Dissociation Guide

Medium	Reference	Endothelial (con't)
HBSS	Wojta J, Gallicchio M, Zoellner H, Filonzi E, Hamilton J, McGrath K: Interleukin-4 stimulates expression of urokinase-type-plasminogen activator in cultured human foreskin microvascular endothelial cells, <i>Blood</i> 81, 3285-92, 1993 (10262)	
HBSS (see reference)	Lee, K, Lawley, T, Xu, Y, and Swerlick, R.: VCAM-1-, ELAM-1-, and ICAM-1-Independent Adhesion of Melanoma Cells to Cultured Human Dermal Microvascular Endothelial Cells, <i>J Invest Dermatol</i> 98, 79, 1992 (960)	
M-199	Farber HW, Antonov AS, Romanov YA, Smirnov VN, Scarfo LM, Beer DJ: Cytokine secretion by human aortic endothelial cells is related to degree of atherosclerosis, <i>Am J Physiol</i> 262, H1088-95, 1992 (10152)	
Cord buffer (see reference)	Grant, D.S., Lelkes, P.I., Fukuda, K., Kleinman, H.K.: Intracellular Mechanisms Involved in Basement Membrane Induced Blood Vessel Differentiation <i>In Vitro</i> , <i>In Vitro Cell Dev Biol</i> 27, 327, 1991 (462)	
RMPI 1640	Whitehead, R., and Eeden, P.: A Method For the Prolonged Culture of Colonic Epithelial Cells, <i>J Tiss Cul Meth</i> 13, 103, 1991 (912)	
Medium 199	Fischer, E., Stingl, A., and Kirkpatrick, C.: Migration Assay for Endothelial Cells in Multiwells Application to Studies on the Effect of Opioids, <i>J Immunol Methods</i> 128, 235, 1990 (1080)	
M199	Muller WA, Ratti CM, McDonnell SL, Cohn ZA: A human endothelial cell-restricted, externally disposed plasmalemmal protein enriched in intercellular junctions, <i>J Exp Med</i> 170, 399-414, 1989 (10099)	
DMEM	Gerhart, D. Z., Broderius, M. A., and Drewes, L. R.: Cultured Human and Canine Endothelial Cells from Brain Microvessels, <i>Brain Res Bull</i> 21, 785, 1988 (344)	
HBSS/PBS, Medium 199 (see reference)	Kubota, Y., Kleinman, H., Martin, G., and Lawley, T.: Role of Laminin and Basement Membrane in Morphological Differentiation of Human Endothelial Cells into Capillary-like Structures, <i>J Cell Biol</i> 107, 1589, 1988 (580)	
HEPES	Hoshi, H., Kan, M., Chen, J., and McKeehan, W.: Comparative Endocrinology-Paracrinology-Autocrinology of Human Adult Large Vessel Endothelial and Smooth Muscle Cells, <i>In Vitro Cell Dev Biol</i> 24 (4), 309, 1988 (937)	
PBS, CMF	Sharefkin, J.B., Fairchild, K.D., Albus, R.A., Cruess, D.F., and Rich, N.M.: The Cytotoxic Effect of Surgical Glove Powder Particles on Adult Human Vascular Endothelial Cell Cultures: Implications for Clinical Uses of Tissue Culture Techniques, <i>J Surg Res</i> 41, 463, 1986 (725)	
HEPES	Hoshi, H., and McKeehan, W.: Isolation, Growth Requirements, Cloning, Prostacyclin Production and Life-Span of Human Adult Endothelial Cells in Low Serum Culture Medium, <i>In Vitro Cell Dev Biol</i> 22 (1), 51, 1986 (883)	
PBS	Marks, R.M., Czerniecki, M., and Penny, R.: Human Dermal Microvascular Endothelial Cells: An Improved Method for Tissue Culture and Description of Some Singular Properties in Culture, <i>In Vitro Cell Dev Biol</i> 21, 627, 1985 (415)	
DMEM	Gordon, P., Sussman, I., and Hatcher, V.: Long-Term Culture of Human Endothelial Cells, <i>In Vitro</i> 19, 661, 1983 (530)	
PBS w/Ca ⁺⁺ , Mg ⁺⁺ , & BSA (see reference)	Glassberg, M., Bern, M., Coughlin, S., Haudenschild, C., Hoyer, L., and Antoniades, H.: Cultured Endothelial Cells Derived from the Human Iliac Arteries, <i>In Vitro</i> 18, 859, 1982 (524)	
RPMI 1640	Ashida, E., Johnson, A., and Lipsky, P.: Human endothelial cell-lymphocyte interaction. Endothelial cells function as accessory cells necessary for mitogen-induced human T lymphocyte activation in vitro, <i>J Clin Invest</i> 67, 1490, 1981 (939)	
Konigsberg's modification of HBSS (see reference)	Sherer, G., Fitzharris, T., Faulk, W., and LeRoy, E.: Cultivation of Microvascular Endothelial Cells from Human Preputial Skin, <i>In Vitro</i> 16, 675, 1980 (509)	
Dulbecco's MEM w/10% calf serum	Folkman, J., Haudenschild, C. C., and Zetter, B. R.: Long-term Culture of Capillary Endothelial Cells, <i>Proc Natl Acad Sci U S A</i> 76, 5217, 1979 (653)	
Tris-HCl, 0.2 M	Jaffe, E.A., Minick, C.R., Adelman, B., Becker, C.G., and Nachman, R.: Synthesis of Basement Membrane Collagen By Cultured Human Endothelial Cells, <i>J Exp Med</i> 144, 209, 1976 (602)	
Dulbecco's PBS	Gimbrone Jr., M.A.: Culture of Vascular Endothelium, <i>Prog Hemost Thromb</i> 3, 1, 1976 (709)	
Dulbecco's PBS	Gimbrone, M.A., Cotran, R.S., and Folkman, J.: Human Vascular Endothelial Cells in Culture: Growth and DNA Synthesis, <i>J Cell Biol</i> 60, 673, 1974 (589)	
Cord buffer (see reference)	Jaffe, E., Nachman, R., Becker, C., and Minick, C.: Culture of Human Endothelial Cells Derived from Umbilical Veins. Identification by Morphologic and Immunologic Criteria, <i>J Clin Invest</i> 52, 2745, 1973 (598)	
Saline, normal	Lewis, L.J., Haok, J.C., Maca, R.D., and Fry, G.L.: Replication of Human Endothelial Cells in Culture, <i>Science</i> 181, 452, 1973 (666)	
CMF solution	Fryer, D.G., Birnbaum, G., and Luttrell, C.N.: Human Endothelium in Cell Culture, <i>J Atheroscler Res</i> 6, 151, 1966 (547)	
DMEM	Follenzi, A., Bente, D., Novikoff, P., Faulkner, L., Raut, S. and Gupta, S.: Transplanted Endothelial Cells Repopulate the Liver Endothelium and Correct the Phenotype of Hemophilia A Mice., <i>J Clin Invest</i> 118, 935, 2008 (10632)	

Tissue Dissociation Guide

Species

Endothelial (con't)

Cell(s)

Enzyme(s)

Porcine	Mouse, 4 week	Endothelial kidney	Collagenase Type 1: 0.1%
	Mouse, neonatal	Microvascular endothelial	Neutral Protease: 0.005% Collagenase Type 1: 4%
	Mouse, embryonic or yolk sac	Endothelial	Collagenase Type 3: 200 u/ml Deoxyribonuclease I: 0.001%
	Mouse, 4 week	Retinal endothelial cells	Collagenase Type 1: 0.1%
	Mouse, male	Endothelial cells from lymph node	Collagenase Type 1: 0.1%
	Porcine (also bovine)	Endothelial	Collagenase Type 1:
	Porcine	HUVEC, porcine pulmonary arterial endothelial cells	Collagenase Type 2: 0.2%
	Porcine, 6-7 month	Porcine pulmonary endothelial	Collagenase Type 1: 0.3%
	Porcine	Endothelial	Trypsin: 0.25%
	Porcine	Endothelial	Trypsin: 0.05%
	Porcine	Endothelial Aortic	Collagenase Type 2: 0.1%
	Porcine, 30-40 kg	Endothelia Aortic	Collagenase Type 4: 0.025%
	Rabbit	Porcine, 20-30 week	Endothelial Aortas Veins
Porcine, 60-100 days		Aorta	Trypsin: 0.1%
Rabbit		Corneal endothelial cells (CEC)	Hyaluronidase: 0.05%
Rat	Rabbit, 2-3Kg	Endothelial, aortic	Elastase: 0.2%
	Rat, SD, male, 250-300 g	Smooth muscle, aorta	Soybean Trypsin Inhibitor: 0.25%
	Rat, SD, male, 350 - 450 g	Lipocytes Kupffer Sinusoidal endothelial	Collagenase: 0.015%
	Rat, Wistar, male, 3 mo	Endothelial Kupffer Parenchymal	Pronase: 0.25%
	Rat, Wistar-Kyoto, male, 100 - 200 g	Endothelial Cerebral	Collagenase Type 2: 0.05%
	Rat, 300 G, and pig, 30-40 Kg	Endothelial Thoracic aorta	Trypsin: 0.05%
	Rat	Foreskin	Collagenase: 0.5%
	Rat, Wistar, adult, 170 g	Endothelial	Trypsin: 0.5%

Epithelial

Species

Cell(s)

Enzyme(s)

Bovine	Bovine, fetal	Epithelial Tracheal	Neutral Protease: 2%
Canine	Dog	Tracheal	Pronase: 0.1%

Tissue Dissociation Guide

Medium Reference **Endothelial (con't)**

DMEM	Kondo, S., Scheef, E., Sheibani, N. and Sorenson, C.: PECAM-1 Isoform-Specific Regulation of Kidney Endothelial Cell Migration and Capillary Morphogenesis., <i>Am J Physiol Cell Physiol</i> 292, C2070, 2007 (10549)
DMEM	Cha, S., Talavera, D., Demir, E., Nath, A. and Sierra-Honigmann, M.: A Method of Isolation and Culture of Microvascular Endothelial Cells from Mouse Skin., <i>Microvasc Res</i> 70, 198, 2005 (10635)
PBS	Braren Rickmer, Hu Huiqing, Kim YungHae, Beggs HilaryE, Reichardt LouisF, Wang Rong: Endothelial FAK is essential for vascular network stability, cell survival, and lamellipodial formation, <i>J Cell Biol</i> 172, 151-62, 2006 (10103)
DMEM	Su X, Sorenson CM, and Sheibani N.: Isolation and characterization of murine retinal endothelial cells, <i>Mol Vis</i> 9, 171, 2003 (9821)
PBS	Izawa D, Tanaka T, Saito K, Ogihara H, Usui T, Kawamoto S, Matsubara K, Okubo K, and Miyasaka M: Expression profile of active genes in mouse lymph node high endothelial cells, <i>Int Immunol</i> 11(12), 1989, 1999 (9822)
DMEM w/ 10% calf serum	Nugent, H., and Edelman, E.: Endothelial Implants Provide Long-Term Control of Vascular Repair in a Porcine Model of Arterial Injury, <i>J Surg Res</i> 99, 228, 2001 (1078)
DMEM	Kwak HJ, Lee SJ, Lee YH, Ryu CH, Koh KN, Choi HY, and Koh GY.: Angiotensin-1 inhibits irradiation- and mannitol-induced apoptosis in endothelial cells, <i>Circulation</i> 101(19), 2317, 2000 (9818)
RPMI 1640	Hill-Kapturczak N, Kapturczak MH, Block ER, Patel JM, Malinski T, Madsen KM, and Tisher CC.: Angiotensin II-stimulated nitric oxide release from porcine pulmonary endothelium is mediated by angiotensin IV, <i>J Am Soc Nephrol</i> 10(3), 481, 1999 (9881)
Medium 199	Shasby, S.: Endothelial Cells Grown On Permeable Membrane Supports, <i>J Tiss Cul Meth</i> 14, 247, 1992 (941)
DMEM	Vischer, P., and Buddecke, E.: Alteration of Glycosyltransferase Activities during Proliferation of Cultivated Arterial Endothelial Cells and Smooth Muscle Cells, <i>Exp Cell Res</i> 158, 15, 1985 (1056)
Dulbecco-Vogt MEM w/o serum	Dickinson, E. and Slakey, L.: Plasma-derived Serum as a Selective Agent to Obtain Endothelial Cultures from Swine Aorta, <i>In Vitro</i> 18, 63, 1982 (523)
Medium 199	Merrilees, M.J., and Scott, L.: Interaction of Aortic Endothelial and Smooth Muscle Cells in Culture, <i>Atherosclerosis</i> 39, 147, 1981 (307)
Medium 199 w/BSS & HEPES or NaHCO ₃	Slater, D.N., and Sloan, J.M.: The Porcine Endothelial Cell in Tissue Culture, <i>Atherosclerosis</i> 21, 259, 1975 (305)
Phosphate buffer (see reference)	Coulson, W.F.: The Effect Of Proteolytic Enzymes on the Tensile Strength of Whole Aorta and Isolated Aortic Elastin, <i>Biochim Biophys Acta</i> 237, 378, 1971 (319)
DMEM	Choi, J., Ko, M., and Kay, E.: Subcellular Localization of the Expressed 18 kDa FGF-2 Isoform in Corneal Endothelial Cells, <i>Mol Vis</i> 6, 222, 2000 (1077)
Hanks solution	Haley, N., Shio, H., Fowler, S.: Characterization of lipid-laden aortic cells from cholesterol-fed rabbits. I. Resolution of aortic cell populations by metrizamide density gradient centrifugation, <i>Lab Invest</i> 37, 287, 1977 (624)
HBSS with 0.2 mM Ca ⁺⁺	Schwertschlag, U.S., and Whorton, A.R.: Platelet-Activating Factor-Induced Homologous and Heterologous Desensitization In Cultured Vascular Smooth Muscle Cells, <i>J Biol Chem</i> 263, 13791, 1988 (560)
DMEM/Ham's F-12	Friedman, S. and Roll, F.: Isolation and Culture of Hepatic Lipocytes, Kupffer Cells, and Sinusoidal Endothelial Cells by Density Gradient Centrifugation with Stractan, <i>Anal Biochem</i> 161, 207, 1987 (301)
HBSS	Nagelkenke, J., Barto, K., and VanBerkel, T.: In vivo and in vitro uptake and degradation of acetylated low density lipoprotein by rat liver endothelial, Kupffer, and parenchymal cells, <i>J Biol Chem</i> 258, 12221, 1983 (557)
HBSS	Diglio, C.A., Grammas, P., Filiberto Giacomelli, M.S., and Wiener, J.: Primary Culture of Rat Cerebral Microvascular Endothelial Cells, <i>Lab Invest</i> 46, 554, 1982 (626)
Medium 199 and 0.01M EDTA	Merrilees, M.J., and Scott, L.: Interaction of aortic endothelial and smooth muscle cells in culture. Effect on glycosaminoglycan levels, <i>Atherosclerosis</i> 39, 147, 1981 (306)
Dulbecco's MEM w/10% calf serum	Folkman, J., Haudenschild, C. C., and Zetter, B. R.: Long-term Culture of Capillary Endothelial Cells, <i>Proc Natl Acad Sci U S A</i> 76, 5217, 1979 (653)
BSS	Phillips, P., Kumar, P., Kumar, S., and Waghe, M.: Isolation And Characterization of Endothelial Cells From Rat And Cow Brain White Matter, <i>J Anat</i> 129, 261, 1979 (708)

Epithelial

Medium	Reference
Dissociation medium, CMF	Schumann, B.L., Cody, T.E., Miller, M.L., Leikauf, G.D.: Isolation, Characterization, and Long-Term Culture of Fetal Bovine Tracheal Epithelial Cells, <i>In Vitro Cell Dev Biol</i> 24, 211, 1988 (422)
DMEM	Virmani, A., Naziruddin, B., Desai, V., Lowry, J., Graves, D., and Sachdev, G.: Evidence for Secretion of High Molecular Weight Mucins by Canine Tracheal Epithelial Cells in Primary Culture: Effects of Select Secretagogues on Mucin Secretion, <i>In Vitro Cell Dev Biol</i> 28A, 120, 1992 (1194)

Tissue Dissociation Guide

Species	Epithelial (con't)	Cell(s)	Enzyme(s)
Chicken	Chick, 5 day old	Intestinal mesenchymal and epithelial	Collagenase: 0.03%
Fish	Shark (<i>Squalus acanthias</i>)	Rectal gland	Collagenase: 0.2%
	Shark (<i>Squalus acanthias</i>)	Rectal gland	Collagenase: 0.2%
	Winter flounder, 200-500 g (<i>Pseudopleuronectes americanus</i>)	Renal tubule	Trypsin: 0.2%
Frog	Frog, <i>Xenopus laevis</i> , adult, female	Colonic epithelial	Collagenase Type 4: 0.1%
Guinea-Pig	Guinea-pig, Hartley, female, 200 g	Epithelial	Collagenase Type 1: 0.1%
	Guinea-pig, Hartley albino, 500-600 g	Endometrial	Collagenase: 0.25%
Hamster	Hamster, Syrian gold, male, 100-120 g	Tracheal	Pronase: 0.1%
	Hamster, Syrian golden (strain CR:RGH)	Tracheal	Trypsin: 0.1%
	Hamster (strain 1516 EHS and Lakeview), 8-12 weeks	Tracheal	Trypsin: 0.05%
	Hamster, Syrian gold, male, 6 weeks- 4 months	Tracheal	Trypsin: 0.25%
Human	Human	Colonic epithelial	Collagenase: Neutral Protease: 0.3% Deoxyribonuclease I: 0.05%
	Human	Intestinal epithelial	Collagenase Type 4: 72.5 u/ml
	Human	Human tracheal epithelium	Protease Type XIV: 0.04%
	Human	Corneal limbal epithelial sheet	Neutral Protease: 5%
	Human	Gastric	Collagenase Type 4: 0.01%
	Human	Nasal polyp epithelial	Neutral Protease: .004% Trypsin: 0.1%
	Human	Epithelial	Trypsin: 0.05%
	Human	Epithelial	Trypsin: 0.2%
	Human	Epithelial	Collagenase: 2.0%
	Human	Epithelial	Pronase: 0.1%
	Human	Epithelial Sweat gland	Collagenase Type 2: 0.2%
	Human (also bovine)	Endometrial epithelial	Trypsin:
	Human	Epithelial	Deoxyribonuclease I: 0.01%
	Human	Epithelial	Protease Type XIV: 0.1%
	Human, women, 27-49 years	Epithelial Ovary	Trypsin: 0.125%
Human, infant and neonate	Epithelial Prostate	Trypsin: 0.1%	

Tissue Dissociation Guide

Medium	Reference	Epithelial (con't)
DMEM	Simon-Assmann, P and Kedinger, M: Embryonic Gut-Dissaggregated Culture, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12A:3.1, 1995 (1271)	
Ringer's solution	Karnaky, Jr., K.J., Valentich, J.D., Currie, M.G., Oehlenschlager, W.F., and Kennedy, M.P.: Atriopeptin Stimulates Chloride Secretion in Cultured Shark Rectal Gland Cells, <i>Am J Physiol</i> 260, 1125, 1991 (287)	
Ringer's solution	Valentich, J.: , <i>J Tiss Cul Meth</i> 13, 149, 1991 (1265)	
CMF solution	Dickman, K.G., and Renfro, J.: Primary Culture of Flounder Renal Tubule Cells: Transepithelial Transport, <i>Am J Physiol</i> 251, 424, 1986 (295)	
Kreb's	Heinke, B, and Clauss, W.: Potassium Conductances in Isolated Single Cells from <i>Xenopus Laevis</i> Colonic Epithelium, <i>J Comp Physiol [B]</i> 169, 148, 1999 (1120)	
DMEM	Rutten, M.: Use of Commerically Available Cell Culture Inserts for Primary Culture and Electrophysiologic Studies of Guinea Pig Gastric Mucous Epithelial Cells, <i>J Tiss Cul Meth</i> 14, 235, 1992 (897)	
HBSS	Chaminadas, G., Alkhalaf, M., Remy-Martin, J.P., Propper, A.Y., and Adessi, G.L.: Specific Effect of Oestrone Sulphate on Protein Synthesis and Secretion by Cultured Epithelial Cells from Guinea-pig Endometrium, <i>J Endocrinol</i> 123, 233, 1989 (600)	
MEM with Hepes, CMF	Niles, R., Kim, K.C., Hyman, B., Christensen, T., Wasano, K., Brody, J.: Characterization Of Extended Primary And Secondary Cultures Of Hamster Tracheal Epithelial Cells, <i>In Vitro Cell Dev Biol</i> 24, 457, 1988 (423)	
Ham's F-12	McDowell, E., et al.: Differentiation of Tracheal Mucociliary Epithelium in Primary Cell Culture Recapitulates Normal Fetal Development and Regeneration Following Injury in Hamsters, <i>Am J Pathol</i> 129, 511, 1987 (283)	
Medium 199	Lee, T., Wu, R., Brody, A., Barrett, J., and Nettesheim, P.: Growth and Differentiation of Hamster Tracheal Epithelial Cells in Culture, <i>Exp Lung Res</i> 6, 27, 1984 (406)	
PBS with EDTA	Goldman, W.E., Baseman, J.B.: Selective Isolation and Culture of a Proliferating Epithelial Cell Population from the Hamster Trachea, <i>In Vitro</i> 16, 313, 1980 (506)	
RPMI 1640	Fukushima, K. and Fiocchi, C.: Paradoxical Decrease of Mitochondrial DNA Deletions in Epithelial Cells of Active Ulcerative Colitis Patients., <i>Am J Physiol Gastrointest Liver Physiol</i> Vol. 286, G804-13, 2004 (10355)	
HBSS	Fahlgren, A., Hammarstrom, S., Danielsson, A. and Hammarstrom, M.: Increased Expression of Antimicrobial Peptides and Lysozyme in Colonic Epithelial Cells of Patients with Ulcerative Colitis., <i>Clin Exp Immunol</i> 131, 90, 2003 (10358)	
DMEM/F12	Widdicombe JH, Sachs LA, Morrow JL, and Finkbeiner WE.: Expansion of cultures of human tracheal epithelium with maintenance of differentiated structure and function, <i>Biotechniques</i> 39(2), 249, 2005 (9824)	
(see reference)	Espana EM, Romano AC, Kawakita T, Di Pascuale M, Smiddy R, and Tseng SC: Novel enzymatic isolation of an entire viable human limbal epithelial sheet, <i>Inv Ophthal Visual Sci</i> 44(10), 4275, 2003 (9830)	
F-12 medium	Sarosiek, J, Marshall, B, Peura, D, Guerrant, L, McCallum, R and Little, C: The Isolation and Maintenance of Human Gastric Epithelial Cells in Primary Culture, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12B:10.1, 1995 (1273)	
(see reference)	Halbert CL, Alexander IE, Wolgamot GM, Miller AD: Adeno-associated virus vectors transduce primary cells much less efficiently than immortalized cells, <i>J Virol</i> 69, 1473-9, 1995 (10215)	
DMEM	Sabatini, L., Allen-Hoffmann, B, Warner, T., and Azen, E.: Serial Cultivation of Epithelial Cells from Human and Macaque Salivary Glands, <i>In Vitro Cell Dev Biol</i> 27A, 939, 1991 (1191)	
MEM, PBS	Robinson, C., and Wu, R.: Culture of Conducting Airway Epithelial Cells in Serum-Free Medium, <i>J Tiss Cul Meth</i> 13, 95, 1991 (1239)	
DMEM/Ham's F-12	Emerman, J. and Wilkinson, D.: Routine Culturing of Normal, Dysplastic and Malignant Human Mammary Epithelial Cells from Small Tissue Samples, <i>In Vitro Cell Dev Biol</i> 26, 1186, 1990 (429)	
PBS	Gruenert, D.C., Basbaum, C.B., and Widdicombe, J.H.: Long-Term Culture of Normal and Cystic Fibrosis Epithelial Cells Grown Under Serum-Free Conditions, <i>In Vitro Cell Dev Biol</i> 26, 411, 1990 (440)	
(see reference)	Wood, L. and Neufeld, E.: A Cystic Fibrosis Phenotype in Cells Cultured from Sweat Gland Secretory Coil. Altered Kinetics of ³⁶ Cl Efflux, <i>J Biol Chem</i> 265, 12796, 1990 (568)	
DMEM/Ham's F-12	Munson, L., Chandler, S., and Schlafer, D.: Cultivation of Bovine Fetal and Adult Endometrial Epithelial Cells, <i>J Tiss Cul Meth</i> 11 (3), 129, 1988 (913)	
HEPES with 5.9mM Glucose, 5mM DTT	Widdicombe, J.H., Coleman, D.L., Finkbeiner, W.E., and Tuet, I.K.: Electrical Properties of Monolayers Cultured From Cells of Human Tracheal Mucosa, <i>J Appl Physiol</i> 58, 1729, 1985 (545)	
Eagle's MEM	Yankaskas, J., Cotton, C., Knowles, M., Gatzky, J., and Boucher, R.: Culture of Human Nasal Epithelial Cells on Collagen Matrix Supports, <i>Am Rev Respir Dis</i> 132, 1281, 1985 (909)	
HBSS, CMF	Auersperg, N., Siemens, C.H., and Myrdal, S.E.: Human Ovarian Surface Epithelium In Primary Culture, <i>In Vitro</i> 20, 743, 1984 (535)	
HBSS	Lechner, J., Babcock, M., Marnell, M., Narayan, K., and Kaighn, M.: Normal Human Prostate Epithelial Cell Cultures, <i>Methods Cell Biol</i> 21, 195, 1980 (631)	

Tissue Dissociation Guide

Species

Epithelial (con't)

Cell(s)

Enzyme(s)

Mouse	Mouse, 11 week	Epithelial	Collagenase Type 3: 25 u/ml Hyaluronidase: 0.1% Protease XIV: 0.05% Deoxyribonuclease I: 0.04%
	Mouse, male, 8-16 week	Renal tubular epithelial	Collagenase: 200 u/ml Soybean Trypsin Inhibitor: see reference
	Mouse, 6-8 week	Lamina propria mononuclear cells	Collagenase Type 2: 0.015% Deoxyribonuclease I: 0.01%
	Mouse, female	Salivary gland epithelial	Collagenase Type 1: 750 u/ml Hyaluronidase: 500 u/ml
	Mouse, fetal 12-13 day	Intestinal mesenchymal and epithelial	Collagenase: 0.03%
	Mouse	Submandibular salivary	Collagenase Type 2 or 3: 0.16%
	Mouse	Esophageal	Trypsin: 0.25%
	Mouse, mature, female, 6-8-wk-old	Uterine	Trypsin: 0.2%
	Mouse, female, 18 - 20 days (also 20 - 22 days)	Uterine	Trypsin: 0.25%
	Mouse, BALB/c, male, 3-5 months	Epithelial Submandibular salivary gland	Collagenase Type 3: 0.16% , 1:1 v/v
	Mouse, female	Epithelial	Pepsin: 0.1%
	Mouse, BALB/cfC3H or BALB/c 8-12 day mid-pregnant	Epithelial Submandibular gland	Collagenase Type 3: 0.1%
	Mouse BALB/cfC3H	Mammary tumors Epithelial	Collagenase: 1.0%
	Mouse, C3H, 6-8 weeks	Epithelial	Collagenase: 0.10%
	Porcine	Porcine, 5-60 kg	Retinal pigment epithelial cells
Porcine		Trachea	Neutral Protease: 0.2%
Rabbit	Rabbit, New Zealand white, adult	Colon	Neutral Protease: 0.3%
	Rabbit, New Zealand white, male, 4-5lb.	Gastric Parietal and chief	Collagenase Type 2: 0.08%
	Rabbit, New Zealand white estrous, female, 4-5 months	Mesothelial and surface epithelial Ovaries	Trypsin: 0.125%-0.5%
	Rabbit, fetal	Epithelial Gastric	Collagenase Type 3: 0.10%
Rat	Rat	Mammary epithelial	Collagenase Type 3: 0.35%
	Rat, SD, adult, male, 8-10 weeks old	Seminiferous tubules	Trypsin: 0.05%
	Rat, male	Alveolar epithelial	Elastase: 40 u/ml
	Rat, embryonic	Tracheal epithelial	Collagenase Type 4: 0.05% Neutral Protease: Deoxyribonuclease I:

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Medium	Reference	Epithelial (con't)
DMEM/F12	Mueller, S., Clark, J., Myers, P. and Korach, K.: Mammary Gland Development in Adult Mice Requires Epithelial and Stromal Estrogen Receptor Alpha., <i>Endocrinology</i> 143, 2357, 2002 (10369)	
HBSS	Breggia, A. and Himmelfarb, J.: Primary Mouse Renal Tubular Epithelial Cells have Variable Injury Tolerance to Ischemic and Chemical Mediators of Oxidative Stress., <i>Oxid Med Cell Longev</i> Vol. 1, 33, 2008 (10554)	
RPMI	Wirtz S., Becker C., Blumberg R., Galle P., and Neurath M.: Treatment of T cell-dependent experimental colitis in SCID mice by local administration of an adenovirus expressing IL-18 antisense mRNA, <i>J Immunol</i> 168(1), 411, 2002 (9826)	
DMEM/F12	Ishimaru N, Saegusa K, Yanagi K, Haneji N, Saito I, Hayashi Y: Estrogen deficiency accelerates autoimmune exocrinopathy in murine Sjogren's syndrome through fas-mediated apoptosis, <i>Am J Pathol</i> 155, 173-81, 1999 (10269)	
DMEM	Simon-Assmann, P and Kedinger, M: Embryonic Gut-Dissaggregated Culture, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12A:3.1, 1995 (1271)	
DMEM	Durban, E: Submandibular Salivary Epithelial Cells, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12B:2.1, 1995 (1272)	
PBS, CMF	Katayama, M., Kan, M.: Heparin-Binding (Fibroblast) Growth Factors re Potential Autocrine Regulators of Esophageal Epithelial Cell Proliferation, <i>In Vitro Cell Dev Biol</i> 27, 533, 1991 (467)	
HBSS	Ghosh, D., Danielson, K., Alston, J., and Heyner, S.: Functional Differentiation of Mouse Uterine Epithelial Cells Grown on Collagen Gels or Reconstituted Basement Membranes, <i>In Vitro Cell Dev Biol</i> 27A, 713, 1991 (1195)	
HBSS	Fukamachi, H., and McLachlan, J.: Proliferation and Differentiation of Mouse Uterine Epithelial Cells in Primary Serum-Free Culture: Estradiol-17 β Suppresses Uterine Epithelial Proliferation Cultured on a Basement Membrane-Like Substratum, <i>In Vitro Cell Dev Biol</i> 27A, 907, 1991 (1196)	
DMEM with 15 mM HEPES	Durban, E.M.: Mouse Submandibular Salivary Epithelial Cell Growth and Differentiation in Long-Term Culture: Influence of the Extracellular Matrix, <i>In Vitro Cell Dev Biol</i> 26, 33, 1990 (437)	
HBSS	Reiser, M., Huff, B., and Medina, D.: Pepsin Can be Used to Subculture Viable Mammary Epithelial Cells, <i>In Vitro</i> 19 (9), 730, 1983 (1192)	
HBSS	Yang, J., Flynn, D., Larson, L., and Hamamoto, S.: Growth in Primary Culture of Mouse Submandibular Epithelial Cells, <i>In Vitro</i> 18, 435, 1982 (520)	
HBSS	Yang, J., Guzman, R., Richards, J., and Nandi, S.: Primary Cultures of Mouse Mammary Tumor Epithelial Cells Embedded in Collagen Gels, <i>In Vitro</i> 16, 502, 1980 (507)	
DMEM	Lillehaug, J., Mondal, S., and Heidelberger, C.: Establishment of Epithelial Cell Lines from Mouse Regenerating Liver, <i>In Vitro</i> 15, 910, 1979 (504)	
DMEM	Wiencke, A., Kiilgaard, J., Nicolini, J., Bundgaard, M., Ropke, C., and La Cour, M.: Growth of Cultured Porcine Retinal Pigment Epithelial Cells, <i>Acta Ophthalmol Scand</i> 81(2), 170, 2003 (9825)	
HBSS	De Buyscher, E., Kennedy, J., and Mendicino, J.: Synthesis of Mucin Glycoproteins by Epithelial Cells Isolated from Swine Trachea by Specific Proteolysis, <i>In Vitro</i> 20, 433, 1984 (534)	
PBS	Vidrich, A., Racindranath, R., Farsi, K., and Targan, S.: A Method for the Rapid Establishment of Normal Adult Mammalian Colonic Epithelial Cell Cultures, <i>In Vitro Cell Dev Biol</i> 24 (3), 188, 1988 (918)	
Sodium phosphate buffer	Chew, C.S., Brown, M.R.: Release of Intracellular Ca ²⁺ and Elevation of Inositol Triphosphate by Secretagogues in Parietal and Chief Cells Isolated from Rabbit Gastric Mucosa, <i>Biochim Biophys Acta</i> 888, 116, 1986 (326)	
Medium 199	Nicosia, S., Johnson, J., and Streibel, E.: Isolation and Ultrastructure of Rabbit Ovarian Mesothelium (Surface Epithelium), <i>Int J Gynecol Pathol</i> 3, 348, 1984 (542)	
HBSS	Logsdon, C.D., Bisbee, C.A., Rutten, M.J. and Machen, T.E.: Fetal Rabbit Gastric Epithelial Cells Cultured on Floating Collagen Gel, <i>In Vitro</i> 18, 233, 1981 (517)	
HBSS	Mei, N., McDaniel, L., Dobrovolsky, V., Guo, X., Shaddock, J., Mittelstaedt, R., Azuma, M., Shelton, S., McGarrity, L., Doerge, D. and Heflich, R.: The Genotoxicity of Acrylamide and Glycidamide in Big Blue Rats., <i>Toxicol Sci</i> 115, 412, 2010 (10638)	
Krebs-Ringer bicarbonate buffer (see reference)	Abou-Haila, A., and Tulsiani, D.: Acid Glycohydrolases in Rat Spermatozoa, Spermatozoa and Spermatozoa: Enzyme Activities, Biosynthesis and Immunolocalization, <i>Biol Proced Online</i> 3 (1), 35, 2001 (1074)	
DMEM	Planus, E., Galiacy, S., Matthay, M., Laurent, V., Gavrilovic, J., Murphy, G., Clerici, C., Isabay, D., Lafuma, C., and d'Ortho, M.: Role of Collagenase in Mediating in Vitro Alveolar Epithelial Wound Repair, <i>J Cell Sci</i> 112, 243, 1999 (9828)	
DMEM/F12	Shannon JM, Gebb SA, and Nielsen LD: Induction of alveolar type II cell differentiation in embryonic tracheal epithelium in mesenchyme-free culture, <i>Development</i> 126, 1675, 1999 (10012)	

Tissue Dissociation Guide

Species

Epithelial (con't)

Cell(s)

Enzyme(s)

Rat, fetal, 14-15 day	Intestinal mesenchymal and epithelial	Collagenase: 0.03%
Rat, 4-14 week	Retinal pigment epithelial cells	Collagenase Type 1: 65 u/ml Hyaluronidase: 220 u/ml
Rat, SD, adult, 90-120 day old, 350-450 g	Epididymal epithelial	Collagenase Type 2: 0.1%
Rat, 6 day old	Rat intestinal epithelial	Neutral Protease: 0.01% Collagenase: 300 u/ml
Rat, SD, male, 150 - 250 g	Epithelial Stomach	Pronase: 0.15%
Rat, adult (also hamster)	Interlobular duct fragments	Papain:
Rat, SD, 200 g	Colon	Deoxyribonuclease I: 10 µg/ml
Rat, Wistar, neonatal	Epithelial	Trypsin: 0.1%
Rodent, various (see reference)	Epithelial	Trypsin: 0.2%
Rat, 6-8 day	Retinal pigment epithelial	Neutral Protease: 2%
Rat (ACI/NMs X BUF/Mna) F1, male, 28 months Rat (ACI/MNs) male, 8 weeks	Epithelial	Collagenase Type 3: 0.1%
Rat, Fisher 344, male, 8 wks old, 250-300 g	Tracheal epithelial	Pronase: 0.5%
Rat, Fischer, male, 4-6 weeks	Epithelial Esophagus	Hyaluronidase: 0.1%
Rat, Fischer, Lewis and SD, male, 10-18 months	Epithelial	Trypsin: 0.05%
Rat, Wistar, 12 day	Epithelial	Trypsin: 0.05%
Rat, Fischer, adult, 200-250 g	Epithelial	Hyaluronidase: 0.0075%
Rat, Fischer, 10 day	Epithelial-like	Trypsin: 0.25%

Eye

Species

Cell(s)

Enzyme(s)

Bovine	Bovine	Pericyte	Collagenase: 0.2%
	Bovine	Microvascular endothelial	Collagenase/Dispase: 0.1%
Chicken	Chick, embryo, 6 day	Retinal cells	Trypsin: 0.005% Deoxyribonuclease I: 0.005%
	Chick embryo	Corneal epithelia	Collagenase: 0.08% Trypsin: 0.08%
	Chick embryo (also rat)	Retinal	Trypsin: 0.6%
	Chick, embryo, 10-14 day	Flat, retina	Trypsin: 0.1%
Human	Human	Corneal stromal stem	Neutral Protease: 1.2 u/ml Collagenase: 0.1%
	Human, 18-68 yr	Corneal endothelial	Collagenase: 0.2% Neutral Protease: 1.0%
	Human	Corneal limbal epithelial sheet	Neutral Protease: 5%

Tissue Dissociation Guide

Medium	Reference	Epithelial (con't)
DMEM	Simon-Assmann, P and Kedingner, M: Embryonic Gut-Dissaggregated Culture, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12A:3.1, 1995 (1271)	
CF Hanks with EDTA	Wang N, Koutz CA, and Anderson RE.: A method for the isolation of retinal pigment epithelial cells from adult rats, <i>Inv Ophthal Visual Sci</i> 34(1), 101, 1993 (9827)	
HBSS	Klinefelter, G.: A Novel System for the Co-Culture of Epididymal Epithelial Cells and Sperm From Adult Rats, <i>J Tiss Cul Meth</i> 14, 195, 1992 (898)	
DMEM	Evans GS, Flint N, Somers AS, Eyden B, and Potten CS.: The development of a method for the preparation of rat intestinal epithelial cell primary cultures, <i>J Cell Sci</i> 101, 219, 1992 (9829)	
Medium 199	Dial, E., Kao, Y., and Lichtenberger, L.: Effects of 16,16-Dimethyl Prostaglandin E2 On Glycoprotein And Lipid Synthesis of Gastric Epithelial Cells Grown in a Primary Culture, <i>In Vitro Cell Dev Biol</i> 27, 39, 1991 (464)	
DMEM/Ham's F-12	Heimann, T., and Githens, S.: Rat Pancreatic Duct Epithelium Cultured on a Porous Support Coated with Extracellular Matrix, <i>Pancreas</i> 6 (5), 514, 1991 (803)	
(see reference)	Yassin, R., Clearfield, H., Katz, S., and Murthy, S.: Gastrin Induction of mRNA Expression in Rat Colonic Epithelium In Vitro, <i>Peptides</i> 12, 63, 1991 (933)	
HBSS	Jassal, D., Han, R., Caniggia, I., Post, M., and Tanswell, A.: Growth of Distal Fetal Rat Lung Epithelial Cells in a Defined Serum-Free Medium, <i>In Vitro Cell Dev Biol</i> 27A, 625, 1991 (471)	
MEM, PBS	Robinson, C., and Wu, R.: Culture of Conducting Airway Epithelial Cells in Serum-Free Medium, <i>J Tiss Cul Meth</i> 13, 95, 1991 (1239)	
DMEM	Chang CW, Roque RS, Defoe DM, and Caldwell RB.: An improved method for isolation and culture of pigment epithelial cells from rat retina, <i>Curr Eye Res</i> 10(11), 1081, 1991 (9831)	
Eagle's MEM Serum-free	Masuda, A., Ohtsuka, K., and Matsuyama, M.: Establishment of Functional Epithelial Cell Lines from a Rat Thyoma and a Rat Thymus, <i>In Vitro Cell Dev Biol</i> 26, 713, 1990 (448)	
DMEM	Chang, L., Wu, R., and Nettesheim, P.: Morphological Changes in Rat Tracheal Cells During The Adaptive and Early Growth Phase in Primary Cell Culture, <i>J Cell Sci</i> 74, 283, 1985 (911)	
HEPES BSS	Babcock, M., Marino, M., Gunning, W., and Stoner, G.: Clonal Growth and Serial Propagation of Rat Esophageal Epithelial Cells, <i>In Vitro</i> 19, 403, 1983 (526)	
HBSS CMF	Herring, A., Raychaudhuri, R., Kelley, S., and Iybe, P.: Repeated Establishment of Diploid Epithelial Cell Cultures from Normal and Partially Hepatectomized Rats, <i>In Vitro</i> 19, 576, 1983 (528)	
HBSS, CMF	Malan-Shibley, L., and Iybe, P.: Influence of Cultures on Cell Morphology/Tyrosine Aminotransferase Levels, <i>Exp Cell Res</i> 131, 363, 1981 (391)	
KCI-NaCl HEPES Buffer	Williams, G., and Gunn, J.: Long-Term Culture of Adult Rat Liver Epithelial Cells, <i>Exp Cell Res</i> 89, 139, 1974 (405)	
PBS	Williams, G., Weisburger, E., and Weisburger, J.: Isolation and Long-Term Cell Culture of Epithelial-Like Cells from Rat Liver, <i>Exp Cell Res</i> 69, 106, 1971 (402)	

Eye

Medium	Reference	
MEM	Bryan, B. and D'Amore, P.: Pericyte Isolation and Use in Endothelial/Pericyte Coculture Models, <i>Meth Enzymol</i> 443, 315, 2008 (10682)	
MEM	Bowman, P., Betz, A., and Goldstein, G.: Primary Culture of Microvascular Endothelial Cells From Bovine Retina, <i>In Vitro</i> 18 (7), 626, 1982 (945)	
DMEM/F12	Jacob Vanessa, Rothermel Andrae, Wolf Peter, Layer Paul G: Rhodopsin, violet and blue opsin expressions in the chick are highly dependent on tissue and serum conditions, <i>Cell Death Differ</i> 180, 159-68, 2005 (10110)	
HBSS	Reenstra Wende R, Orlow Daniel L, Svoboda Kathy K H: ECM-stimulated signaling and actin reorganization in embryonic corneal epithelia are Rho dependent, <i>Inv Ophthal Visual Sci</i> 43, 3181-9, 2002 (10294)	
DMEM	Seigel, G.: The Golden Age of Retinal Cell Culture, <i>Mol Vis</i> 5, 4, 1999 (1085)	
Tyrode's solution, CMF	Moyer, M., Bullrich, F., and Sheffield, J.: Emergence of Flat Cells From Glia in Stationary Cultures of Embryonic Chick Neural Retina, <i>In Vitro Cell Dev Biol</i> 26, 1073, 1990 (427)	
DMEM	Du, Y., Roh, D., Funderburgh, M., Mann, M., Marra, K., Rubin, J., Li, X. and Funderburgh, J.: Adipose-Derived Stem Cells Differentiate to Keratocytes In Vitro., <i>Mol Vis</i> 16, 2680, 2010 (10602)	
DMEM/F12	Li Wei, Sabater Alfonso L, Chen Ying-Ting, Hayashida Yasutaka, Chen Szu-Yu, He Hua, Tseng Scheffer C G: A novel method of isolation, preservation, and expansion of human corneal endothelial cells, <i>Inv Ophthal Visual Sci</i> 48, 614-20, 2007 (10306)	
(see reference)	Espana EM, Romano AC, Kawakita T, Di Pascuale M, Smiddy R, and Tseng SC: Novel enzymatic isolation of an entire viable human limbal epithelial sheet, <i>Inv Ophthal Visual Sci</i> 44(10), 4275, 2003 (9830)	

Tissue Dissociation Guide

Species	Eye (con't)	Cell(s)	Enzyme(s)
Monkey	Human, 5-65 years	Retinal pigment epithelial (RPE)	Trypsin: 0.25%
	Macaques and baboon	Retinal	Papain: 20-40 u/ml Deoxyribonuclease I: 400 u/ml
	Monkey, cynomolgus, young adult	Conjunctival lymphocytes	Collagenase Type 1: 0.02%
Mouse	Mouse	Retinal ganglion	CLSPA: 240 u/ml Hyaluronidase: 0.2%
	Mouse, postnatal day 1	Retinal progenitor	Collagenase: 0.1%
	Mouse	Retinal	Collagenase: 0.1% Deoxyribonuclease I: 0.001%
	Mouse, 2-5 month	Mouse retinal and bipolar	Papain: 20 u/ml Deoxyribonuclease I: 200 u/ml
Porcine	Mouse, 4 week	Retinal endothelial cells	Collagenase Type 1: 0.1%
	Porcine, 5-60 kg	Retinal pigment epithelial cells	Collagenase: 2%
Rabbit	Rabbit, New Zealand	Corneal keratocytes	Trypsin: 0.25% Collagenase: 0.5%
	Rabbit, adult	Retinal neurons	Papain: 26 u/ml
Rat	Rabbit, New Zealand, adult, male, albino, 2 kg	Epithelial	Trypsin: 0.25%
	Rat, Fisher, adult	Retinal	PDS kit: per instructions
	Rat, Dark Agouti, 3-4 week	Ciliary-derived eye	PDS kit: with modifications
	Rat, embryonic 17 day	Retinal	Trypsin: 0.6%
	Rat, SD	Retina	Papain: 120 U
	Rat, 4-14 week	Retinal pigment epithelial cells	Collagenase Type 1: 65 u/ml Hyaluronidase: 220 u/ml
	Rat, 6-8 day	Retinal pigment epithelial	Neutral Protease: 2%
	Rat, Long Evans, 1-15 days	Neurons, visual cortex	Papain: 20 u/ml
	Rat, SD, pups	Retina	Trypsin: 0.25%
	Salamander	Salamanders	Neurons
Salamander, 18-25 cm		Retina	Papain: 14 u/ml
Salamander (<i>A. tigrinum</i>)		Photoreceptors, retina	Papain: 0.05%
Turtle	Turtle (<i>Pseudemys scripta elegans</i>)	Retinal	Papain: 0.1% (13.5 u/mg)
Heart			
Species		Cell(s)	Enzyme(s)
Bovine	Bovine (also rat)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%

Medium	Reference
HBSS	Von Recum, H., Okano, T., Kim, S, and Bernstein, P.: Maintenance of Retinoid Metabolism in Human Retinal Pigment Epithelium Cell Culture, <i>Exp Eye Res</i> 69, 97, 1999 (1185)
Ames' solution	Han ,Y., Jacoby, R. and Wu, S.:Morphological and Electrophysiological Properties of Dissociated Primate Retinal Cells., <i>Brain Res</i> 875, 175, 2000 (10573)
RPMI 1640	Whittum-Hudson JA, Taylor HR: Antichlamydial specificity of conjunctival lymphocytes during experimental ocular infection, <i>Infect Immun</i> 57, 2977, 1989 (10035)
Ames	Schmidt, T. and Kofuji, P.: An Isolated Retinal Preparation to Record Light Response from Genetically Labeled Retinal Ganglion Cells., <i>J Vis Exp</i> 47, 2367, 2011 (10659)
HBSS	Jiang, C. Klassen, H., Zhang, X. and Young, M.: Laser Injury Promotes Migration and Integration of Retinal Progenitor Cells into Host Retina., <i>Mol Vis</i> 16, 983, 2010 (10612)
RPMI	Amadi-Obi, A., Yu, C., Liu, X., Mahdi, R., Clarke, G., Nussenblatt, R, Gery, I., Lee, Y. and Egwuagu, C.: TH17 Cells Contribute to Uveitis and Scleritis and are Expanded by IL-2 and Inhibited by IL-27/STAT1., <i>Nat Med</i> 13, 711, 2007 (10684)
HBSS	Maxeiner Stephan, Dedek Karin, Janssen-Bienhold Ulrike, Ammermuller Josef, Brune Hendrik, Kirsch Taryn, Pieper Mario, Degen Joachim, Kruger Olaf, Willecke Klaus, Weiler Reto: Deletion of connexin45 in mouse retinal neurons disrupts the rod/cone signaling pathway between All amacrine and ON cone bipolar cells and leads to impaired visual transmission, <i>J Neurosci</i> 25, 566-76, 2005 (10100)
DMEM	Su X, Sorenson CM, and Sheibani N.: Isolation and characterization of murine retinal endothelial cells, <i>Mol Vis</i> 9, 171, 2003 (9821)
DMEM	Wiencke, A., Kiilgaard, J., Nicolini, J., Bundgaard, M., Ropke, C., and La Cour, M.: Growth of Cultured Porcine Retinal Pigment Epithelial Cells, <i>Acta Ophthalmol Scand</i> 81(2), 170, 2003 (9825)
PBS	Stramer Brian M, Kwok Michael G K, Farthing-Nayak Pamela J, Jung Jae-Chang, Fini M Elizabeth, Nayak Ramesh C: Monoclonal antibody (3G5)-defined ganglioside: cell surface marker of corneal keratocytes, <i>Inv Ophthal Visual Sci</i> 45, 807-12, 2004 (10242)
DMEM	Brockway LM, Zhou Z-H, Bubien JK, Jovov B, Benos DJ, Keyser KT: Rabbit retinal neurons and glia express a variety of ENaC/DEG subunits, <i>Am J Physiol Cell Physiol</i> 283, C126-34, 2002 (10228)
HBSS/DMEM	Johnson-Muller, B., and Gross, J.: Regulation of Corneal Collagenase Production: Epithelial-Stromal Cell Interactions, <i>Proc Natl Acad Sci U S A</i> 75 (9), 4417, 1978 (908)
MEM	Suzuki, T., Mandai, M., Akimoto, M., Yoshimura, N. and Takahashi, M.: The Simultaneous Treatment of MMP-2 Stimulants in Retinal Transplantation Enhances Grafted Cell Migration into the Host Retina., <i>Stem Cells</i> 24, 2406, 2006 (10515)
DMEM/F12	Akagi T, Mandai M, Ooto S, Hirami Y, Osakada F, Kageyama R, Yoshimura N, Takahashi M.: Otx2 homeobox gene induces photoreceptor-specific phenotypes in cells derived from adult iris and ciliary tissue, <i>Inv Ophthal Visual Sci</i> 45, 4570, 2004 (10024)
DMEM	Seigel, G.: The Golden Age of Retinal Cell Culture, <i>Mol Vis</i> 5, 4, 1999 (1085)
HBSS, PBS	Jing, S., Wen, D., Yu, Y., Holst, P., Luo, Y., Fang, M., Tamir, R., Antonio, L., Hu, Z., et al.: GDNF-Induced Activation of the Ret Protein Tyrosine Kinase is Mediated by GDNFR-a, a Novel Receptor for GDNF, <i>Cell</i> 85, 1113, 1996 (1122)
CF Hanks with EDTA	Wang N, Koutz CA, and Anderson RE.: A method for the isolation of retinal pigment epithelial cells from adult rats, <i>Inv Ophthal Visual Sci</i> 34(1), 101, 1993 (9827)
DMEM	Chang CW, Roque RS, Defoe DM, and Caldwell RB.: An improved method for isolation and culture of pigment epithelial cells from rat retina, <i>Curr Eye Res</i> 10(11), 1081, 1991 (9831)
BSS (see reference)	Huettnner, J., and Baughman, R.: Primary Culture of Identified Neurons From the Visual Cortex of Postnatal Rats, <i>J Neurosci</i> 6, 3044, 1986 (617)
Ham's F-12	Sarthy PV, Curtis BM, and Catterall WA.: Retrograde Labeling, Enrichment, and Characterization of Retinal Ganglion Cells from the Neonatal Rat, <i>J Neurosci</i> 3 (12), 2532, 1983 (1199)
Ringers	Clarke, R, Wang, J. and Townes-Anderson, E.: Using Laser Tweezers For Manipulating Isolated Neurons In Vitro., <i>J Vis Exp</i> 19, 911, 2008 (10688)
Saline	Townes-Anderson, E., MacLeish, P., and Raviola, E.: Rod Cells Dissociated from Mature Salamander Retina: Ultrastructure and Uptake of Horseradish Peroxidase, <i>J Cell Biol</i> 100, 175, 1985 (1200)
(see reference)	Bader, C., MacLeish, P., and Schwartz, E.: Responses to Light of Solitary Rod Photoreceptors Isolated From Tiger Salamander Retina, <i>Proc Natl Acad Sci U S A</i> 75, 3507, 1978 (652)
Kreb's Ringer	Lam, D.: Biosynthesis of Acetylcholine in Turtle Photoreceptors, <i>Proc Natl Acad Sci U S A</i> 69, 1987, 1972 (649)

Heart

Medium	Reference
25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, <i>In Vitro Cell Dev Biol</i> 26, 335, 1990 (438)

Tissue Dissociation Guide

Species	Heart (con't)	Cell(s)	Enzyme(s)
Canine	Canine, adult	Cardiomyocytes	Collagenase Type 2: 0.05% Protease: 0.008%
	Dog, mongrel, adult	Myocytes	Hyaluronidase: 0.1%
Chicken	Chicken embryos, 9-11 day incubated	Cardiomyocytes	Trypsin: 0.25%
	Chick, embryos, 10-11 day	Cardiomyocytes	Trypsin: 0.17%
	Chick, 10 day	Ventricular	Trypsin: 0.025%
	Chick, embryo, 11 day	Cardiomyocytes	Trypsin: 0.05%
	Chick, embryonic, 11-12-day-old	Myocytes	Trypsin: 0.025%
	Chick, 8 day embryo (also rat, neonate and mouse)	Myocytes	Trypsin NF 1:250: 0.25%
	Hen, White Leghorn	Heart	Trypsin: 0.025%
	Chick embryos, 5 day	Heart Liver	Trypsin: 3.0%
	Feline	Cat, mongrel, adult, 1.8-2.8 kg	Myocytes Ventricular
Fish	Octopus, <i>E. cirhosa</i> , 260-352 g	Systemic heart cardiomyocytes	Collagenase: 0.025% Trypsin: 0.02%
Frog	Frog (<i>Rana esculenta</i>)	Myocytes	Trypsin: 0.04%
Guinea-Pig	Guinea pig, adult	Cardiomyocytes	Collagenase Type 2: 100 u/ml
	Guinea pig, female, 300-350g	Coronary endothelial	Collagenase Type 2: 0.1%
	Guinea pig, 250-450 g	Endothelial	Collagenase Type 2: 0.15%
	Guinea-pig, male, 450-600 g	Myocytes	Hyaluronidase: 0.02%
	Guinea-pig	Cardiomyocytes	Collagenase: 0.04%
	Guinea-pig	Myocytes	Hyaluronidase: 0.10%
Human	Human	Coronary artery smooth muscle	Collagenase Type 2: 0.1% Elastase: 0.05%
	Human (n=16, age: 60 ±3 years)	Myocardial	Hyaluronidase: 0.05%
	Human	Cardiomyocytes	Collagenase Type 2: 0.05% Collagenase Type 1: 0.025% Protease XIV: 0.013%
	Human	Heart Myocytes	Collagenase Type 2: 200 u/ml
	Human, fetal, 14.5 week gestation	Myocytes	Hyaluronidase: 0.1%
	Human, both sexes, 18-81 years	Myocardial Atrial	Collagenase: 0.14%
	Human, adult	Myocytes	Trypsin: 0.25%
	Human	Thoracic aorta	Collagenase: 0.2%

Tissue Dissociation Guide

Medium	Reference	Heart (con't)
M199	Gavi, S., Yin, D., Shumay, E., Wang, H. and Malbon, C.: Insulin-Like Growth Factor-I Provokes Functional Antagonism and Internalization of Beta1-Adrenergic Receptors., <i>Endocrinology</i> 148, 2653-62, 2007 (10505)	
Joklik's MEM	Spanier, A. and Weglicki, W.: Ca ²⁺ -Tolerant Adult Canine Myocytes: Preparation and Response to Anoxia/Acidosis, <i>Am J Physiol</i> 243, H448, 1982 (291)	
PBS	Eschenhagen, T., Fink, C., Remmers, U., Scholz, H., Wattchow, J., Weil, J., et al.: Three-Dimensional Reconstitution of Embryonic Cardiomyocytes in a Collagen Matrix: A New Heart Muscle Model System, <i>FASEB J</i> 11, 683, 1997 (1110)	
HBSS, CMF	Wang, S., Greaser, M.L., Schultz, E., Bulinski, J.C., Lin, J.J., and Lessard, J.L.: Studies on Cardiac Myofibrillogenesis With Antibodies to Titin, Actin, Tropomyosin, and Myosin, <i>J Cell Biol</i> 107, 1075, 1988 (577)	
HBSS, CMF	Kim, D., Okada, A., and Smith, T.W.: Control of Cytosolic Calcium Activity During Low Sodium Exposure in Cultured Chick Heart Cells, <i>Circ Res</i> 61, 29, 1987 (365)	
HBSS, CMF	Jacob, R., Lieberman, M., Murphy, E., and Piwnica-Worms, D.: Effect of Sodium-Potassium Pump Inhibition and Low Sodium on Membrane Potential in Cultured Embryonic Chick Heart Cells, <i>J Physiol</i> 387, 549, 1987 (723)	
CMF solution	Murphy, E., Aiton, J., Russell, C., and Lieberman, M.: Calcium Elevation in Cultured Heart Cells: Its Role in Cell Injury, <i>Am J Physiol Cell Physiol</i> 245 (14), C316, 1983 (1188)	
Rinaldini's buffer solution, CF	Gross, W., Schopf-Ebner, E., and Bucher, O.: Technique for the Preparation of Homogeneous Cultures of Isolated Heart Muscle Cells, <i>Exp Cell Res</i> 53, 1, 1968 (397)	
Medium 199	Dehann, R.: Regulation of Spontaneous Activity and Growth of Embryonic Chick Heart Cells in Tissue Culture, <i>Dev Biol</i> 16, 216, 1967 (848)	
Tyrode's solution, CMF	Steinberg, M.: "ECM": Its Nature, Origin, And Function in Cell Aggregation, <i>Exp Cell Res</i> 30, 257, 1963 (396)	
Kreb's Henseleit, CF	Silver, L., Hemwall, E., Marino, T., and Houser, S.: Isolation and Morphology of Calcium-Tolerant Feline Ventricular Myocytes, <i>Am J Physiol</i> 245, H891, 1983 (293)	
(see reference)	Altimiras J., Hove-Madsen L., and Gesser H.: Ca(2+) Uptake in the Sarcoplasmic Reticulum from the Systemic Heart of Octopod Cephalopods, <i>J Exp Biol</i> 202, 2531, 1999 (9834)	
CF Ringer	Arrio-Dupont, M., and de Nay, D.: High Yield Preparation of Calcium-Tolerant Myocytes From Frog Ventricles, <i>Biol Cell</i> 54, 164, 1985 (339)	
M-199	Zorn-Pauly K, Schaffer P, Pelzmann B, Bernhart E, Lang P, and Koidl B: L-type and T-type Ca ²⁺ current in cultured ventricular guinea pig myocytes, <i>Physiol Res</i> 53(4), 369, 2004 (9865)	
(see reference)	Buxton I L, Kaiser R A, Oxhorn B C, Cheek D J: Evidence supporting the Nucleotide Axis Hypothesis: ATP release and metabolism by coronary endothelium, <i>Am J Physiol Heart Circ Physiol</i> 281, H1657-66, 2001 (10171)	
Perfusing solution (see reference)	Preisig-Muller, R., Mederos Y Schnitzler, M., Derst, C., and Daut J.: Separation of Cardiomyocytes and Coronary Endothelial Cells for Cell-Specific RT-PCR, <i>Am J Physiol</i> 277, H413, 1999 (1079)	
Krebs Henseleit bicarbonate buffer	Stemmer, P., Akera, T., Brody, T., Rardon, D., and Watanabe, A.: Isolation and Enrichment of Ca ²⁺ -Tolerant Myocytes for Biochemical Experiments from Guinea-Pig Heart, <i>Life Sci</i> 44, 1231, 1989 (628)	
Tyrode solution, CF	Ishihara, K., Mitsuiye, T., Noma, A., and Takano, M.: The Mg ²⁺ Block and Intrinsic Gating Underlying Inward Rectification of the K ⁺ Current in Guinea-Pig Cardiac Myocytes, <i>J Physiol</i> 419, 297, 1989 (721)	
Bicarbonate buffer, CF	Bridge, J., Spitzer, K., and Ershler, P.: Relaxation of Isolated Ventricular Cardiomyocytes by a Voltage-Dependent, <i>Science</i> 241, 823, 1988 (668)	
DMEM	Jensen, B., Swigart, P., Laden, M., DeMarco, T., Hoopes, C. and Simpson, P.: The Alpha-1D Is the Predominant Alpha-1-Adrenergic Receptor Subtype in Human Epicardial Coronary Arteries., <i>J Am Coll Cardiol</i> 54, 1137, 2009 (10580)	
Medium 199	Mukerjee, R., Multani, M., Sample, J., Dowdy, K., Zellner, J., Hoover, D., and Spinale, F.: Effects of adrenomedullin on human myocyte contractile function and beta-adrenergic response, <i>J Cardiovasc Pharmacol</i> 7 (4), 235, 2002 (1290)	
HEPES solution	Todor Anastassia, Sharov Victor G, Tanhehco Elaine J, Silverman Norman, Bernabei Alvise, Sabbah Hani N: Hypoxia-induced cleavage of caspase-3 and DFF45/ICAD in human failed cardiomyocytes, <i>Am J Physiol Heart Circ Physiol</i> 283, H990-5, 2002 (10283)	
Tyrode's solution	Hoppe, U., Jansen, E., Sudkamp, M., and Beukelmann, D.: Hyperpolarization-Activated Inward Current in Ventricular Myocytes From Normal and Failing Human Hearts, <i>Circulation</i> 97, 55, 1998 (745)	
HBSS with Calcium	Goldman, B., and Wurzel, J.: Effects of Subcultivation and Culture Medium on Differentiation of Human Fetal Cardiac Myocytes, <i>In Vitro Cell Dev Biol</i> 28, 109, 1992 (480)	
HBSS, CMF	Smith, D., Glover, J., Townsend, L., and Maupin, E.: A Method for the Harvest, Culture, and Characterization of Human Adult Atrial Myocardial Cells: Correlation with Age of Donor, <i>In Vitro Cell Dev Biol</i> 27, 914, 1991 (478)	
Joklik's MEM	Bugaisky, L.B.: Biology of Isolated Adult Cardiac Myocytes, <i>Isolation and Culture of Human Adult Cardiac Myocytes</i> , (Clark, Decker, Borg, eds), Elsevier Science Publishing Co., Inc., 1988 (679)	
Phosphate buffer w/NaCl	Hassler, O., Wiren, M., and Herbertsson, S.: The Elastic Coat of the Arterial Wall Studied with the Aid of Collagenase, <i>Acta Pathol Microbiol Scand</i> 57, 15, 1963 (695)	

Tissue Dissociation Guide

Species

Heart (con't)

Cell(s)

Enzyme(s)

Species	Heart (con't)	Cell(s)	Enzyme(s)
Mouse	Mouse, male, 6 week	Coronary endothelial cells	Collagenase Type 2: 0.1% Neutral Protease: 0.6 u/ml
	Mouse, adult	Ventricular myocytes	Collagenase: 0.1%
	Mouse	Cardiomyocytes	Collagenase Type 1: 0.17%
	Mouse, adult	Cardiomyocytes	Collagenase: see reference
	Mouse	Smooth muscle cells	Collagenase Type 2: 300 u/ml Elastase: 5 u/ml
	Mouse	Ventricular myocytes and mesenteric arterial SMC	Collagenase Type 2: 0.06% Papain: 0.175%
	Mouse, neonatal	Cardiomyocytes	Collagenase Type 1: 150 u/ml Trypsin: 0.01%
	Mouse, 1 day	Cardiomyocytes	NCIS kit:
	Mouse	Smooth muscle cells	Collagenase Type 1: 0.1% Elastase: 0.0125%
	Mouse, female, 14-18 g	Cardiomyocytes	Collagenase Type 2: 0.02% Elastase: 0.03% Pancreatin: 0.06%
	Mouse, 6-8 week	Vascular smooth muscle	Papain: 10 u/ml Elastase: .005% Collagenase: 0.05% Deoxyribonuclease I: 1000 u/ml PDS kit: with modifications
	Mouse	Cardiomyocytes	Collagenase Type 2: 0.2% Pancreatin: 0.06%
	Mouse, neonatal 2-4 day	Atrial myocytes	NCIS kit: with modifications see reference
	Mouse, neonatal and adult	Cardiomyocytes	Collagenase Type 2: 0.03-0.2%
	Mouse, NTg and Tg, 8 - 12 week	Myocytes	Collagenase Type 2: 150 u/ml
	Mouse, 1-6 day	Cardiomyocytes	Collagenase Type 2: 0.1% Deoxyribonuclease I: 0.002%
	Mouse, 2-4 mo	Ventricular myocytes	Collagenase Type 2: 0.05% Collagenase Type 4: 0.05% Protease XIV: 0.002%
	Mouse	Cardiomyocytes	Collagenase Type 2: 150 u/ml
	Mouse, 1-3 day	Cardiomyocytes	Trypsin: 0.25%
	Mouse, 1 day	Cardiomyocytes	NCIS kit: with modifications see reference
	Mouse, 3-7 month	Fibroblasts, mesangial, smooth muscle	Trypsin: 0.25% Collagenase: see reference Soybean Trypsin Inhibitor: .05%
	Mouse BALB/c, adult	Myocytes	Collagenase Type 2: 0.04%

Tissue Dissociation Guide

Medium	Reference
M199	Makino, A., Suarez, J., Wang, H., Belke, D., Scott, B. and Dillmann, W.: Thyroid Hormone Receptor-beta is Associated With Coronary Angiogenesis During Pathological Cardiac Hypertrophy., <i>Endocrinology</i> 150, 2008, 2009 (10645)
M199	Zhang, Y, Kanter, E., Laing, J., Aphys, C., Johns, D., Kardami, E. and Yamada, K.: Connexin43 Expression Levels Influence Intercellular Coupling and Cell Proliferation of Native Murine Cardiac Fibroblasts, <i>Cell Commun Adhes Vol. 15</i> , 289, 2008
PBS	Chen, H., Yong, W., Ren, S., Shen, W., He, Y., Cox, K., Zhu, W., Li, W., Soonpaa, M., Payne, RM, Franco, D., Field, L., Rosen, V., Wang, Y. and Shou, W.: Overexpression of Bone Morphogenetic Protein 10 in Myocardium Disrupts Cardiac Postnatal Hypertrophic Growth., <i>J Biol Chem</i> 281, 27481, 2006 (10354)
HBSS	O'Connell Timothy D, Rodrigo Manoj C, Simpson Paul C: Isolation and culture of adult mouse cardiac myocytes, <i>Methods Mol Biol</i> 357, 271-96, 2007 (10314)
F10 Ham	Verheye, S., Martinet, W., Kockx, M., Knaepen, M., Salu, K., Timmermans, J., Ellis, J., Kilpatrick, D., DeMayer, G.: Selective Clearance of Macrophages in Atherosclerotic Plaques by Autophagy, <i>J Am Coll Cardiol</i> 49, 706, 2007 (10335)
Krebs-Ringer	Lu Tong, Ye Dan, Wang Xiaoli, Seubert John M, Graves Joan P, Bradbury J Alyce, Zeldin Darryl C, Lee Hon-Chi: Cardiac and vascular KATP channels in rats are activated by endogenous epoxyeicosatrienoic acids through different mechanisms, <i>J Physiol</i> 575, 627-44, 2006 (10293)
M199	Takahashi N, Wang X, Tanabe S, Uramoto H, Jishage K, Uchida S, Sasaki S, Okada Y.: CIC-3-independent sensitivity of apoptosis to Cl- channel blockers in mouse cardiomyocytes, <i>Cell Physiol Biochem</i> 15, 263, 2005 (10033)
EBSS	Potts, Malia B., Vaughn, Allyson E., McDonough, Holly, Patterson, Cam, and Deshmukh, Mohanish: Reduced Apaf-1 levels in cardiomyocytes engage strict regulation of apoptosis by endogenous XIAP, <i>J Cell Biol</i> 171, 925, 2005 (10206)
DMEM	Fukumoto Yoshihiro, Deguchi Jun-o, Libby Peter, Rabkin-Aikawa Elena, Sakata Yasuhiko, Chin Michael T, Hill Christopher C, Lawler Patrick R, Varo Nerea, Schoen Frederick J, Krane Stephen M, Aikawa Masanori: Genetically determined resistance to collagenase action augments interstitial collagen accumulation in atherosclerotic plaques, <i>Circulation</i> 110, 1953-9, 2004 (10115)
(see reference)	Zhang Sui, Wang Dachun, Estrov Zeev, Raj Sean, Willerson James T, Yeh Edward T H: Both cell fusion and transdifferentiation account for the transformation of human peripheral blood CD34-positive cells into cardiomyocytes in vivo, <i>Circulation</i> 110, 3803-7, 2004 (10261)
EBSS	Qian, Q., Hunter, L., Li, M., Marin-Padilla, M., Prakash, Y., Somlo, S., Harris, P., Torres, V., and Sieck, G.: Pkd2 Haploinsufficiency Alters Intracellular Calcium Regulation in Vascular Smooth Muscle Cells, <i>Hum Mol Genet</i> 12(15), 1875, 2003 (9846)
DMEM/F-12	Rybkin, I., Markham, D., Yan, Z., Bassel-Duby, R., Williams, R., and Olson, E.: Conditional Expression of SV40 T-antigen in Mouse Cardiomyocytes Facilitates an Inducible Switch from Proliferation to Differentiation, <i>J Biol Chem</i> 278(18), 15927, 2003 (9854)
L-15	Bettahi, I., Marker, C., Roman, M., and Wickman, K.: Contribution of the Kir3.1 Subunit to the Muscarinic-gated Atrial Potassium Channel IKACH, <i>J Biol Chem</i> 277, 48282, 2002 (9987)
Joklik's MEM	Schreiber, K., Paquet, L., Allen, B., and Rindt, H.: Protein Kinase C Isoform Expression and Activity in the Mouse Heart, <i>Am J Physiol Heart Circ Physiol</i> 281(5), H2062, 2001 (9856)
Joklik's MEM	Nelson, D., Setser, E., Hall, D., Schwartz, S., Hewitt, T., Klevitsky, R., Osinska, H., Bellgrau, D., Duke, R., and Robbins, J.: Proinflammatory Consequences of Transgenic Fas Ligand Expression in the Heart, <i>J Clin Invest</i> 105 (9), 1199, 2000 (1067)
PBS	Watzka SB, Lucien J, Shimada M, Edwards V, Yeger H, Hannigan G, and Coles JG.: Selection of viable cardiomyocytes for cell transplantation using three-dimensional tissue culture, <i>Transplantation</i> 70, 1310, 2000 (10063)
MEM	Zhou YY, Wang SQ, Zhu WZ, Chruscinski A, Kobilka BK, Ziman B, Wang S, Lakatta EG, Cheng H, Xiao RP: Culture and adenoviral infection of adult mouse cardiac myocytes: methods for cellular genetic physiology, <i>Am J Physiol Heart Circ Physiol</i> 279, H429-36, 2000 (10117)
Joklik's MEM	Christensen G, Minamisawa S, Gruber PJ, Wang Y, Chien KR: High-efficiency, long-term cardiac expression of foreign genes in living mouse embryos and neonates, <i>Circulation</i> 101, 178-84, 2000 (10257)
FBS-MEM	Wang, G., and Kang, Y.: Inhibition of Doxorubicin Toxicity in Cultured Neonatal Mouse Cardiomyocytes with Elevated Metallothionein Levels, <i>J Pharmacol Exp Ther</i> 288(3), 938, 1999 (9864)
L-15	Lader, A., Kwiatkowski, D., and Cantiello, H.: Role of Gelsolin in the Actin Filament Regulation of Cardiac L-type Calcium Channels, <i>Am J Physiol</i> 277, C1277, 1999 (9988)
DMEM	Bradshaw AD, Francki A, Motamed K, Howe C, Sage EH: Primary mesenchymal cells isolated from SPARC-null mice exhibit altered morphology and rates of proliferation, <i>Mol Biol Cell</i> 10, 1569-79, 1999 (10136)
Tyrode's solution	Felzen, B., Shilkrut, M., Less, H., Sarapov, I., Maor, G., Coleman, R., Robinson, R., et al.: Fas (CD95/Apo-1)-Mediated Damage to Ventricular Myocytes Induced by Cytotoxic T Lymphocytes from Perforin-Deficient Mice, <i>Circ Res</i> 82, 438, 1998 (1134)

Tissue Dissociation Guide

Species

Heart (con't)

Cell(s)

Enzyme(s)

Porcine	Mouse, 1 day	Cardiomyocytes	NCIS kit: with modifications see reference	
	Mouse, P0-P1	Cardiomyocytes	Collagenase: 0.045%	
	Mouse, 6-9 or 21-41 day	Ventricular and atrial myocytes	Collagenase Type 2: 95 u/ml Hyaluronidase: 172.5 u/ml Trypsin: 0.002% Deoxyribonuclease I: 60u/ml	
	Mouse	Cardiomyocyte	Collagenase: 0.17 %	
	Mouse embryos	Myocytes	Collagenase Type 1: 0.1%	
	Mouse (also rat, neonate and chick, 8 day embryo)	Myocytes	Trypsin NF 1:250: 0.25%	
	Porcine	Coronary vascular smooth muscle (PCVSMCs)	Trypsin: 0.037%	
	Miniature swine, adult, female, 25-40 kg, familiarized with treadmill exercise	Coronary smooth muscle	Trypsin: 0.1%	
	Rabbit	Rabbit, New Zealand, 2.3-2.9 kg	Cardiomyocytes	Collagenase Type 1: 0.05% Hyaluronidase: see reference Protease: see reference
		Rabbit	Smooth muscle cells	Collagenase Type 2: 300 u/ml Elastase: 5 u/ml
Rabbit, New Zealand, 2 kg		Cardiomyocytes and fibroblasts	Collagenase: 0.06%	
Rabbit, New Zealand White, 2-3 kg		Myocytes	Collagenase Type 2: 0.1%	
Rabbit, New Zealand, white, either sex		Myocytes	Collagenase Type 2: 0.05%	
Rabbit, adult 1.5-2.5 kg		Tricuspid valve cells	Collagenase: 0.8 mg/ml	
Rabbit, New Zealand, white, male, 2-3 kg		Cardiomyocytes	Hyaluronidase: 0.5%	
Rabbit, adult, 2 kg		Myocytes	Hyaluronidase: 0.007%	
Rat		Rat, 3 day	Cardiomyocytes	Collagenase Type 2: 300 u/ml
		Rat, SD, male	Cardiomyocytes	Collagenase Type 2: 0.056%
	Rat, Wistar, male	Cardiomyocytes	NCIS kit: per instructions	
	Rat, neonatal	Cardiomyocytes	NCIS kit: per instructions	
	Rat, adult	Myocytes	Collagenase Type 2: 0.05% Protease XIV: 0.02%	
	Rat, SD, 200-250 g	Cardiomyocytes	Collagenase Type 1: 0.05% Hyaluronidase: see reference Protease: see reference	
	Rat, SD, 1-2 day	Cardiomyocytes	NCIS kit: per instructions	

Tissue Dissociation Guide

Medium	Reference
L-15	Lader, A., Xiao, Y., Ishikawa, Y., Cui, Y., Vatner, D., Vatner, S., Homcy, C., Cantiello, H.: Cardiac G α Overexpression Enhances L-type Calcium Channels Through an Adenylyl Cyclase Independent Pathway, <i>Proc Natl Acad Sci U S A</i> 95, 9669, 1998 (9989)
DMEM, Medium 199	Arber, S., Hunter, J., Ross, J., Hongo, M., Sansig, G., Borg, J., Perriard, J., Chien, K., and Caroni, P.: MLP-Deficient Mice Exhibit a Disruption of Cardiac Cytoarchitectural Organization, Dilated Cardiomyopathy, and Heart Failure, <i>Cell</i> 88, 393, 1997 (1108)
DMEM/Tyrodes	Valenzuela, D., Han, X., Mende, U., Fankhauser, C., Mashimo, H., Huang, P., Pfeffer, J., Neer, E., and Fishman, M.: G α (o) is Necessary for Muscarinic Regulation of Ca $^{2+}$ Channels in Mouse Heart, <i>Proc Natl Acad Sci U S A</i> 94(5), 1727, 1997 (9863)
PBS	Soonpaa, M., Kim, K., Pajak, aL., Franklin, M., and Field, L.: Cardiomyocyte DNA Synthesis and Binucleation During Murine Development, <i>Am J Physiol</i> 271, H2183, 1996 (1238)
DMEM	Wobus, A., Kleppisch, T., Maltsev, V., and Hescheler, J.: Cardiomyocyte-Like Cells Differentiated <i>In Vitro</i> From Embryonic Carcinoma Cells P19 are Characterized by Functional Expression of Adrenoceptors and Ca $^{2+}$ Channels, <i>In Vitro Cell Dev Biol</i> 30A, 425, 1994 (786)
Rinaldini's buffer solution, CF	Gross, W., Schopf-Ebner, E., and Bucher, O.: Technique for the Preparation of Homogeneous Cultures of Isolated Heart Muscle Cells, <i>Exp Cell Res</i> 53, 1, 1968 (397)
HEPES	Christ, M., Gunther, A., Heck, M., Schmidt, B., Falkenstein, E., and Wehling, M.: Aldosterone, Not Estradiol, Is the Physiological Agonist for Rapid Increases in cAMP in Vascular Smooth Muscle Cells, <i>Circulation</i> 99, 1485, 1999 (1068)
Krebs bicarbonate solution	Bowles, D., Hu, Q., Laughlin, M., and Sturek, M.: Exercise Training Increases L-type Calcium Current Density in Coronary Smooth Muscle, <i>Am J Physiol</i> 275 (44), H2159, 1998 (1069)
Krebs-Henseleit	Farkas, A., Acsai, K., Nagy, N., Toth, A., Fulop, F., Seprenyi, G., Birinyi, P., Nanasi, P., Forster, T., Csanady, M., Papp, J., Varro, A. and Farkas, A.: Na(+)/Ca(2+) Exchanger Inhibition Exerts a Positive Inotropic Effect in the Rat Heart, but Fails to Influence the Contractility of the Rabbit Heart., <i>Br J Pharmacol</i> 154, 93, 2008 (10509)
F10 Ham	Verheye, S., Martinet, W., Kockx, M., Knaepen, M., Salu, K., Timmermans, J., Ellis, J., Kilpatrick, D., DeMayer, G.: Selective Clearance of Macrophages in Atherosclerotic Plaques by Autophagy, <i>J Am Coll Cardiol</i> 49, 706, 2007 (10335)
Medium 199	Driesen Ronald B, Dispersyn Gerrit D, Verheyen Fons K, van den Eijnde Stefan M, Hofstra Leo, Thone Fred, Dijkstra Petra, Debie Wiel, Borgers Marcel, Ramaekers Frans C S: Partial cell fusion: a newly recognized type of communication between dedifferentiating cardiomyocytes and fibroblasts, <i>Cardiovasc Res</i> 68, 37-46, 2005 (10344)
HEPES	Spitzer, K., Ershler, P., Skolnick, R., and Vaughan-Jones, R.: Generation of Intracellular pH Gradients in Single Cardiac Myocytes with a Microperfusion System, <i>Am J Physiol</i> 278, H1371, 2000 (746)
EGTA-KB	Sedarat, F., Xu, L., Moore, E., and Tibbits, G.: Colocalization of Dihydropyridine and Ryanodine Receptors in Neonate Rabbit Heart Using Confocal Microscopy, <i>Am J Physiol Heart Circ Physiol</i> 279, H202, 2000 (748)
HEPES Tyrode solution	Anumonwo JM, Delmar M, and Jalife J.: Electrophysiology of single heart cells from the rabbit tricuspid valve, <i>J Physiol</i> 425, 145, 1990 (10066)
Eagle's MEM	Buxton, I., and Brunton, L.: Compartments of Cyclic AMP and Protein Kinase in Mammalian Cardiomyocytes, <i>J Biol Chem</i> 258 (17), 10233, 1983 (847)
Kreb's Ringer	Dani, A., Cittadini, A., Flamini, G., Festuccia, G., and Terranova, T.: Preparation and Some Properties of Isolated Beating Myocytes from Adult Rabbit Heart, <i>J Mol Cell Cardiol</i> 9, 777, 1977 (846)
PBS	Ye, K., Sullivan, K. and Black, L.: Encapsulation of Cardiomyocytes in a Fibrin Hydrogel for Cardiac Tissue Engineering., <i>J Vis Exp</i> 55, 3251, 2011 (10657)
Krebs	Mellor, K., Bell, J., Wendt, I., Davidoff, A., Ritchie, R. and Delbridge, L.: Fructose Modulates Cardiomyocyte Excitation-Contraction Coupling and Ca Handling <i>In Vitro.</i> , <i>PLoS ONE</i> 6, e25204, 2011 (10666)
L-15	Brinckmann, M., Kaschina, E., Alarache-Xifro, W., Curato, C., Timm, M., Grzesiak, A., Dong, J., Kappert, K., Kintscher, U., Unger, T. and Li, J.: Estrogen Receptor Alpha Supports Cardiomyocytes Indirectly Through Post-Infarct Cardiac c-kit+ Cells., <i>J Mol Cell Cardiol</i> 47, 66, 2009 (10538)
L-15	Smith, M., Huang, Y. and Deshmukh, M.: Skeletal Muscle Differentiation Evokes Endogenous XIAP to Restrict the Apoptotic Pathway., <i>PLoS ONE</i> 4, e5097, 2009 (10539)
Media 199	Xu, X. and Colecraft, H.: Primary Culture of Adult Rat Heart Myocytes., <i>J Vis Exp</i> 28, 1308, 2009 (10689)
Krebs-Henseleit	Farkas, A., Acsai, K., Nagy, N., Toth, A., Fulop, F., Seprenyi, G., Birinyi, P., Nanasi, P., Forster, T., Csanady, M., Papp, J., Varro, A. and Farkas, A.: Na(+)/Ca(2+) Exchanger Inhibition Exerts a Positive Inotropic Effect in the Rat Heart, but Fails to Influence the Contractility of the Rabbit Heart., <i>Br J Pharmacol</i> 154, 93, 2008 (10509)
L-15	Kim, M., Oh, J., Sakata, S., Liang, I., Park, W., Hajjar, R. and Lebeche, D.: Role of Resistin in Cardiac Contractility and Hypertrophy., <i>J Mol Cell Cardiol</i> 45, 270, 2008 (10540)

Tissue Dissociation Guide

Rat, SD, 2 day	Cardiomyocytes	Collagenase Type 2: 0.1%
Rat, SD, male, 225-250 g	Cardiomyocytes	Collagenase Type 2: 0.1%
Rat, 2-3 day	Cardiomyocytes	Collagenase Type 2: 0.05% Pancreatin: 0.1%
Rat, neonatal	Cardiomyocytes	NCIS kit: per instructions
Rat, Wistar, neonatal	Cardiomyocytes	NCIS kit: per instructions
Rat, SD, male, 250-300 g	Cardiomyocytes	Collagenase Type 2: 250 u/ml
Rat, Wistar, 1-3 day	Cardiomyocytes	NCIS kit: per instructions
Rat, neonatal	Cardiomyocytes	Collagenase Type 2: 0.1%
Rat, SD, 300-350 g	Cardiomyocytes	Collagenase Type 2: 0.05% Hyaluronidase: 0.02%
Rat, SD, male	Ventricular myocytes	Collagenase: 0.13% Hyaluronidase: 0.06%
Rat, SD, 200-250 g	Ventricular myocytes and mesenteric arterial SMC	Collagenase Type 2: 0.06% Papain: 0.175%
Rat, 1-2 day	Cardiomyocytes	NCIS kit: per instructions
Rat, SD, 2-4 day	Cardiomyocytes, fibroblasts	Trypsin: 0.1% Collagenase: 0.1%
Rat, SD, 1-2 day	Cardiomyocytes	NCIS kit: per instructions
Rat, 1-2 day	Cardiomyocytes	NCIS kit: per instructions
Rat, 1 day	Cardiomyocytes	NCIS kit: per instructions
Rat, neonatal	Cardiac myocytes	Collagenase Type 2: 0.5%
Rat, male, 250-300 g	Cardiomyocytes	Collagenase Type 1: 0.03% Protease: 0.01%
Rat, fetal or 3-day	Cardiomyocytes	Collagenase Type 2: 0.1%
Rat, adult, 220-280g	Cardiomyocytes	Collagenase Type 2: 178 u/ml Hyaluronidase: 0.01%
Rat, 50 g	Ventricular myocytes	Collagenase: 223 u/ml Hyaluronidase: 0.01% Trypsin: 0.002%

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Medium	Reference
DMEM/F-12	Jang, J., Ku, S., Kim, J., Choi, K., Kim, Y., Kim, H., Oh, S., Lee, E., Cho, H., Song, Y., Lee, S., Lee, S., Suh, C., Kim, S., Moon, S. and Choi, Y.: Notch Inhibition Promotes Human Embryonic Stem Cell-Derived Cardiac Mesoderm Differentiation., <i>Stem Cells</i> 26, 2782, 2008 (10546)
J-MEM	Kubli, D., Quinsay, M., Huang, C., Lee, Y., Gustafsson, A.: Bnip3 Functions as a Mitochondrial Sensor of Oxidative Stress During Myocardial Ischemia and Reperfusion., <i>Am J Physiol Heart Circ Physiol</i> 295, H2025, 2008 (10596)
DMEM	LaFramboise, W., Scalise, D., Stoodley, P., Graner, S., Guthrie, R., Magovern, J and Becich, M.: Cardiac Fibroblasts Influence Cardiomyocyte Phenotype in Vitro., <i>Am J Physiol Cell Physiol</i> 292, C1799-808, 2007 (10506)
L-15	Robinet, A., Millart, H., Oszust, F., Hornebeck, W. and Bellon, G.: Binding of Elastin Peptides to S-Gal Protects the Heart Against Ischemia/Reperfusion Injury by Triggering the RISK Pathway., <i>FASEB J</i> 21, 1968, 2007 (10537)
L-15	Butler, T., Au, C., Yang, B., Egan, J., Tan, Y., Hardeman, E., North, K., Verkman, A. and Winlaw, D.: Cardiac Aquaporin Expression in Humans, Rats, and Mice., <i>Am J Physiol Heart Circ Physiol</i> 291, H705, 2006 (10541)
Cardioplegic solution	Head, B., Patel, H., Roth, D., Lai, N., Niesman, I., Farquhar, M. and Insel, P.: G-Protein-Coupled Receptor Signaling Components Localize in Both Sarcolemmal and Intracellular Caveolin-3-Associated Microdomains in Adult Cardiac Myocytes., <i>J Biol Chem</i> 280, 31036-44, 2005 (10372)
L-15	Castillo, A., Ruzmetov, N., Harvey, K., Stillwell, W., Zaloga, G. and Siddiqui, R.: Docosahexaenoic Acid Inhibits Protein Kinase C Translocation/Activation and Cardiac Hypertrophy in Rat Cardiomyocytes., <i>J Mol Genet Med</i> 1, 18, 2005 (10642)
DMEM	Tamamori-Adachi, M., Hayashida, K., Nobori, K., Omizu, C., Yamada, K., Sakamoto, N., Kamura, T., Fukuda, K., Ogawa, S., Nakayama, K. and Kitajima, S.: Down-Regulation of p27 Promotes Cell Proliferation of Rat Neonatal Cardiomyocytes Induced by Nuclear Expression of Cyclin D1 and CDK4, <i>J Biol Chem</i> 279, 50429, 2004 (10497)
DMEM	Niederbichler Andreas D, Hoesel Laszlo M, Westfall Margaret V, Gao Hongwei, Ipaktchi Kyros R, Sun Lei, Zetoune Firas S, Su Grace L, Arbabi Saman, Sarma J Vidya, Wang StewartC, Hemmila MarkR, Ward PeterA: An essential role for complement C5a in the pathogenesis of septic cardiac dysfunction, <i>J Exp Med</i> 203, 53-61, 2006 (10159)
DMEM	Stagg Mark A, Coppen Steven R, Suzuki Ken, Varela-Carver Anabel, Lee Joon, Brand Nigel J, Fukushima Satsuki, Yacoub Magdi H, Terracciano Cesare M: Evaluation of frequency, type, and function of gap junctions between skeletal myoblasts overexpressing connexin43 and cardiomyocytes: relevance to cell transplantation, <i>FASEB J</i> 20, 744-6, 2006 (10275)
Krebs-Ringer	Lu Tong, Ye Dan, Wang Xiaoli, Seubert John M, Graves Joan P, Bradbury J Alyce, Zeldin Darryl C, Lee Hon-Chi: Cardiac and vascular KATP channels in rats are activated by endogenous epoxyeicosatrienoic acids through different mechanisms, <i>J Physiol</i> 575, 627-44, 2006 (10293)
DMEM/F-12	Pedram, A., Razandi, M., Aitkenhead, M., and Levin, E: Estrogen Inhibits Cardiomyocyte Hypertrophy in vitro. Antagonism of Calcineurin-related Hypertrophy through Induction of MCIP1, <i>J Biol Chem</i> 280, 26339, 2005 (9993)
Medium 199	Entcheva, E., Bien, H, Yin L., Chung, C., Farrell, M., and Kostov, Y.: Functional Cardiac Cell Constructs on Cellulose-based Scaffolding, <i>Biomaterials</i> 25(26), 5753, 2004 (9840)
L-15	Muller-Bore, B., Cascio, W., Anderson, P., Snowwaert, J., Frye, J., Desai, N., Esch, G., Brackham, J., Bagnell, C., Coleman, W., Grisham, J., and Malouf, N.: Adult-derived Liver Stem Cells Acquire a Cardiomyocyte Structural and Functional Phenotype ex vivo, <i>Am J Pathol</i> 165, 135, 2004 (9991)
DMEM	Natarajan AR, Rong Q, Katchman AN, and Ebert SN.: Intrinsic cardiac catecholamines help maintain beating activity in neonatal rat cardiomyocyte cultures, <i>Pediatr Res</i> 56, 411, 2004 (9992)
F-10	Chen, Z., Ge, Y., and Kang J.: Down-regulation of the M6P/IGF-II Receptor Increases Cell Proliferation and Reduces Apoptosis in Neonatal Rat Cardiac Myocytes, <i>BMC Cell Biol</i> 5, 15, 2004 (9995)
HBSS	Chen Hsiao-Huei, Mullett Steven J, Stewart Alexandre F R: Vgl-4, a novel member of the vestigial-like family of transcription cofactors, regulates alpha1-adrenergic activation of gene expression in cardiac myocytes, <i>J Biol Chem</i> 279, 30800-6, 2004 (10138)
HBSS	Hunton DaciaL, Zou LuYun, Pang Yi, Marchase Richard B: Adult rat cardiomyocytes exhibit capacitative calcium entry, <i>Am J Physiol Heart Circ Physiol</i> 286, H1124-32, 2004 (10155)
HEPES	Tamamori-Adachi Mimi, Ito Hiroshi, Sumrejkanchanakij Piyamas, Adachi Susumu, Hiroe Michiaki, Shimizu Masato, Kawauchi Junya, Sunamori Makoto, Marumo Fumiaki, Kitajima Shigetaka, Ikeda Masa-Aki: Critical role of cyclin D1 nuclear import in cardiomyocyte proliferation, <i>Circ Res</i> 92, e12-9, 2003 (10104)
Krebs-Henseleit, CF	Gordon Jennifer M, Dusting Gregory J, Woodman Owen L, Ritchie Rebecca H: Cardioprotective action of CRF peptide urocortin against simulated ischemia in adult rat cardiomyocytes, <i>Am J Physiol Heart Circ Physiol</i> 284, H330-6, 2003 (10101)
Medium 199	Aberle II NS, and Ren J.: Experimental Assessment of the Role of Acetaldehyde in Alcoholic Cardiomyopathy, <i>Biol Proced Online</i> 5, 1, 2003 (9835)

Tissue Dissociation Guide

Species

Heart (con't)

Cell(s)

Enzyme(s)

Rat, neonatal	Cardiomyocytes	NCIS kit: per instructions
Rat, SD, 200-300 g	Cardiomyocytes	Collagenase Type 2: 0.05-0.1%
Rat, SD, 175-200g	Cardiomyocytes	Collagenase Type 2: 0.05% Deoxyribonuclease I: 0.02%
Rat, SD, pregnant female	Peritoneal mast	Hyaluronidase: 100 u/ml
Rat, neonatal, adult, 17 day	Ventricular cardiomyocytes	Collagenase Type 2: 0.7-1%
Rat, 1 day	Cardiomyocytes	NCIS kit: per instructions
Rat, female, 250-300 g	Ventricular myocytes	Collagenase Type 2: 0.1%
Rat, Wistar, 1 day	Cardiomyocytes	Collagenase Type 2: 80u/ml
Rat, male, 6-12 months old	Cardiac myocytes	Protease: 0.55 u/ml
Rat, Harlan SD, pup	Cardiomyocytes	Trypsin:
Rat, SD, newborn	Cardiac myocytes	NCIS kit: per instructions
Rat, adult	Ventricular myocytes	Collagenase: 223 u/ml Hyaluronidase: 0.01% Trypsin: 0.002%
Rat, SD, male	Cardiomyocytes	Collagenase Type 2: 140 u/ml
Rat, neonatal	Cardiomyocytes	NCIS kit: per instructions
Rat, Wistar, female	Ventricular myocytes	Collagenase Type 2: 0.1%
Rat, SD, 1-2 day	Cardiomyocytes	Trypsin: 0.01% Collagenase Type 2: 0.08%
Rat, newborn	Atrial	Trypsin: 0.06%
Rat, 1-2 day	Cardiomyocytes	Collagenase Type 1: 84 u/ml Pancreatin: 0.05%
Rat, Wistar, 1-2 day	Ventricular myocytes	Collagenase Type 2: 0.08%
Rat, SD, adult female	Heart microvascular cells	CLSPA: 250u/ml Papain: 5 u/ml Elastase: 0.8 u/ml
Rat, SD, male, 250-300g	Cardiomyocytes	Collagenase Type 2: 0.06%
Rat, Wistar, 2 day	Cardiomyocytes	NCIS kit: per instructions
Rat, neonatal	Cardiomyocytes	NCIS kit: per instructions
Rat, Lewis, adult male, 300-350-g	Aorta, smooth muscle	Trypsin: 0.025%

Tissue Dissociation Guide

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Medium	Reference
L-15	Li TS, Ito H, Kajiwara K, and Hamano K.: Long-term survival of xenografted neonatal cardiomyocytes by adenovirus-mediated CTLA4-Ig expression and CD40 blockade, <i>Circulation</i> 108, 1760, 2003 (9990)
Joklik's MEM	Swift Luther, McHowat Jane, Sarvazyan Narine: Inhibition of membrane-associated calcium-independent phospholipase A2 as a potential culprit of anthracycline cardiotoxicity, <i>Cancer Res</i> 63, 5992-8, 2003 (10280)
Krebs-Ringer	Tardif Annie, Julien Nathalie, Chiasson Jean-Louis, Coderre Lise: Stimulation of glucose uptake by chronic vanadate pretreatment in cardiomyocytes requires PI 3-kinase and p38 MAPK activation, <i>Am J Physiol/Endo</i> 284, E1055-64, 2003 (10282)
DMEM (see reference)	DeAlmeida, A., Mustin, D., Forman, M., Brower, G., Janicki, J., and Carver, W.: Effects of mast cells on the behavior of isolated heart fibroblasts: modulation of collagen remodeling and gene expression, <i>J Cell Physiol</i> 191, 51, 2002 (1296)
DMEM	Lam ML, Bartoli M, and Claycomb WC.: The 21-day postnatal rat ventricular cardiac muscle cell in culture as an experimental model to study adult cardiomyocyte gene expression, <i>Mol Cell Biochem</i> 229, 51, 2002 (9842)
L-15	Yang Y, Liao H, Ke Q, Cai J, Xiao YF, and Morgan JP: Enhancement of nitric oxide production by methylecgonidine in cultured neonatal rat cardiomyocytes, <i>Br J Pharmacol</i> 135, 188, 2002 (9994)
Ringer solution	Liu Qinghang, Hofmann Polly A: Antiadrenergic effects of adenosine A(1) receptor-mediated protein phosphatase 2a activation in the heart, <i>Am J Physiol Heart Circ Physiol</i> 283, H1314-21, 2002 (10175)
M199	Shimizu Tatsuya, Yamato Masayuki, Isoi Yuki, Akutsu Takumitsu, Setomaru Takeshi, Abe Kazuhiko, Kikuchi Akihiko, Umezumi Mitsuo, Okano Teruo: Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces, <i>Circ Res</i> 90, e40, 2002 (10212)
Tyrode's solution	Kitta, K., Day, R., Ikeda, T., and Suzuki, Y.: Hepatocyte Growth Factor Protects Cardiac Myocytes Against Oxidative Stress-Induced Apoptosis, <i>Free Radic Biol Med</i> 31 (7), 902, 2001 (1061)
DMEM/Ham's F-12	Schwartzbauer, G., and Robbins, J.: The Tumor Suppressor Gene PTEN Can Regulate Cardiac Hypertrophy and Survival, <i>J Biol Chem</i> 276, 35786, 2001 (1140)
L-15	Rahman A, Alam M, Rao S, Cai L, Clark LT, Shafiq S, and Siddiqui MA: Differential effects of doxorubicin on atrial natriuretic peptide expression in vivo and in vitro, <i>Biol Res</i> 34(3-4), 195, 2001 (9849)
Tyrode solution	Ren J, and Wold LE.: Measurement of Cardiac Mechanical Function in Isolated Ventricular Myocytes from Rats and Mice by Computerized Video-Based Imaging, <i>Biol Proced Online</i> 3, 43, 2001 (9852)
Krebs-Henseleit	Dai L, Brookes PS, Darley-Usmar VM, and Anderson PG.: Bioenergetics in cardiac hypertrophy: mitochondrial respiration as a pathological target of NO, <i>Am J Physiol/Heart</i> 281, H2261-H2269, 2001 (10065)
DMEM/M199	Guo K, Searfoss G, Krolkowski D, Pagnoni M, Franks C, Clark K, Yu KT, Jaye M, Ivashchenko Y: Hypoxia induces the expression of the pro-apoptotic gene BNIP3, <i>Cell Death Differ</i> 8, 367-76, 2001 (10153)
Ringer solution	Pyle, W, Lester, J, and Hofmann, P.: Effects of Kappa-opioid receptor activation of myocardium, <i>Am J Physiol Heart Circ Physiol</i> 281, H669, 2001 (10176)
HBSS	Arutunyan A, Webster D, Swift L, Sarvazyan N: Localized injury in cardiomyocyte network: a new experimental model of ischemia-reperfusion arrhythmias, <i>Am J Physiol Heart Circ Physiol</i> 280, H1905-15, 2001 (10315)
DMEM	Kim, D., and Pleumsamran, A.: Cytoplasmic Unsaturated Free Fatty Acids Inhibit ATP-Dependent Gating of the G Protein-gated K ⁺ Channel, <i>J Gen Physiol</i> 115, 287, 2000 (1137)
M199 medium	Bueno OF, De Windt LJ, Tymitz KM, Witt SA, Kimball TR, Klevitsky R, Hewett TE, Jones SP, Lefer DJ, Peng CF, Kitsis RN, and Molkentin JD.: The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice, <i>EMBO J</i> 19(23), 6341, 2000 (9836)
DMEM	Maki T, Horio T, Yoshihara F, Suga S, Takeo S, Matsuo H, and Kangawa K: Effect of neutral endopeptidase inhibitor on endogenous atrial natriuretic peptide as a paracrine factor in cultured cardiac fibroblasts, <i>Br J Pharmacol</i> 131(6), 1204, 2000 (9844)
L-15	Puri S, Bansal DD, Uskokovic MR, and MacGregor RR.: Induction of tissue plasminogen activator secretion from rat heart microvascular cells by fM 1,25(OH)(2)D(3), <i>Am J Physiol/Endo</i> 278(2), E293, 2000 (9845)
M199	Sun L, Chang J, Kirchhoff SR, and Knowlton AA.: Activation of HSF and selective increase in heat-shock proteins by acute dexamethasone treatment, <i>Am J Physiol Heart Circ Physiol</i> 278(4), H1091, 2000 (9861)
DMEM-F12	Cowan DB, Poutias DN, Del Nido PJ, and McGowan FX Jr: CD14-independent activation of cardiomyocyte signal transduction by bacterial endotoxin, <i>Am J Physiol Heart Circ Physiol</i> 279, H619, 2000 (9996)
L-15	Webster DR, and Patrick DL.: Beating rate of isolated neonatal cardiomyocytes is regulated by the stable microtubule subset, <i>Am J Physiol Heart Circ Physiol</i> 278, H1653, 2000 (9997)
Medium 199 with 20% FBS medium	Kim, B., Nikolovski, J., Bonadio, J., Smiley, E., and Mooney, D.: Engineered Smooth Muscle Tissues: Regulating Cell Phenotype with the Scaffold, <i>Exp Cell Res</i> 251, 318, 1999 (1063)

Tissue Dissociation Guide

Rat, 10-15 newborn, 1-2 days old	Atrial	Trypsin: 0.03%
Rat, 1-3 day	Neonatal rat cardiomyocytes	Collagenase Type 2: 80u/ml Pancreatin: 0.06%
Rat, 1-2 day	Cardiomyocytes	Pancreatin: 0.06% Collagenase Type 2: 95 u/ml
Rat, adult and neonatal	Cardiomyocytes	Collagenase Type 4: 0.12%-adult Collagenase Type 2: 0.05%-neonatal
Rat, Wistar, male	Cardiomyocytes	Collagenase Type 2: 228 u/ml
Rat, 1-2 day	Cardiomyocytes	NCIS kit: per instructions
Rat	Thoracic aorta, smooth muscle	Elastase: 0.02%
Rat, SD, 1 day	Cardiomyocytes	NCIS kit:
Rat, SD, 300-350 g	Cardiomyocytes	Collagenase Type 2: 0.04%
Rat, SD, female, 200g	Ventricular cardiac myocytes	Collagenase Type 2: 0.5% Hyaluronidase: 0.2%
Rat, Wistar, 1 day	Cardiomyocytes	Collagenase Type 2: 80 u/ml
Rat, neonatal, 5-day	Cardiomyocytes	Trypsin: 0.05%
Rat, SD, 250-300 g	Cardiomyocytes	Collagenase Type 2: 0.05%
Rat, SD, fetal and neonatal	Cardiomyocytes	Collagenase: 0.1% Trypsin: 0.2%
Rat, SD, male, 200-250 g	Myocytes Ventricles	Collagenase: 0.07%
Rat, SD, female, 60-90 days	Myocardial	Collagenase Type 2: 0.1%
Rat, SD, adult, female, 180-220 g	Cardiomyocytes	Collagenase: 0.11%
Rat, neonatal, 1-4 days	Cardiomyocytes	Collagenase Type 2: 0.12%
Rat (also bovine)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%
Rat, adult, female	Myocytes	Collagenase Type 2: 0.05%
Rats, AW, late-gestation pregnant females	Ventricular Cardiomyocytes	Collagenase Type 2: 0.05 - 0.08%
Rat, SD, albino, 10-14 weeks	Myocytes	Collagenase Type 1: 100 u/ml and 150 u/ml
Rat, SD, adult, female, 175 - 225 g	Myocytes	Collagenase: 0.1%
Rat, SD, male, 250 - 350 g	Myocytes, ventricular	Collagenase Type 1: 90 and 100 u/ml
Rat, SD, 2-4 day old	Cardiomyocytes Fibroblasts	Trypsin: 0.1%

Tissue Dissociation Guide

Medium	Reference
HBSS or EBSS	Nemec, J., Wickman, K., and Clapham, D.: GBy Binding Increases the Open Time of I_{KACH} : Kinetic Evidence for Multiple GBy Binding Sites, <i>Biophys J</i> 76, 246, 1999 (1066)
DMEM/F12	Adderley SR, and Fitzgerald DJ.: Oxidative damage of cardiomyocytes is limited by extracellular regulated kinases 1/2-mediated induction of cyclooxygenase-2, <i>J Biol Chem</i> 274, 5038, 1999 (9833)
DMEM	Reinecke H, Zhang M, Bartosek T, and Murry CE.: Survival, integration, and differentiation of cardiomyocyte grafts: a study in normal and injured rat hearts, <i>Circulation</i> 100(2), 193, 1999 (9851)
DMEM/medium 199	Richards SM, Jaconi ME, Vassort G, and Puceat M.: A spliced variant of AE1 gene encodes a truncated form of Band 3 in heart: the predominant anion exchanger in ventricular myocytes, <i>J Cell Sci</i> 112, 1519, 1999 (9853)
Joklik MEM	Sambandam N, Abrahani MA, St Pierre E, Al-Atar O, Cam MC, and Rodrigues B.: Localization of lipoprotein lipase in the diabetic heart: regulation by acute changes in insulin, <i>Arterioscler Thromb Vasc Biol</i> 19(6), 1526, 1999 (9855)
RPMI	Matsui, T., Li, Ling, Del Monte, F., Fukui, Y., Franke, T., Hajjar, R., Rosenzweig, A.: Adenoviral gene transfer of activated phosphatidylinositol 3'-kinase and Akt inhibits apoptosis of hypoxic cardiomyocytes in vitro, <i>Circulation</i> 100, 2373-9, 1999 (10121)
(see reference)	Zilberman, A., Dave, V., Miano, J., Olson, E., and Periasamy, M.: Evolutionary Conserved Promoter Region Containing CArg-Like Elements is Crucial for Smooth Muscle Myosin Heavy Chain Gene Expression, <i>Circ Res</i> 82, 566, 1998 (1064)
DMEM/F-12	Wagner DR, Combes A, McTiernan C, Sanders VJ, Lemster B, Feldman AM: Adenosine inhibits lipopolysaccharide-induced cardiac expression of tumor necrosis factor-alpha, <i>Circ Res</i> 82, 47-56, 1998 (10213)
(see reference)	Yu L, Netticadan T, Xu YJ, Panagia V, Dhalla NS: Mechanisms of lysophosphatidylcholine-induced increase in intracellular calcium in rat cardiomyocytes, <i>J Pharmacol Exp Ther</i> 286, 1-8, 1998 (10289)
Krebs-Henseleit buffer	Westfall M V, Rust E M, Metzger J M: Slow skeletal troponin I gene transfer, expression, and myofilament incorporation enhances adult cardiac myocyte contractile function, <i>Proc Natl Acad Sci U S A</i> 94, 5444-9, 1997 (10178)
HBSS	Kinugawa, K., Kohmoto, O., Yao, A., Serizawa, T., and Takahashi, T.: Cardiac inducible nitric oxide synthase negatively modulates myocardial function in cultured rat myocytes, <i>Am J Physiol</i> 272, H35-47, 1997 (10209)
DMEM	Vanwinkle, W., Snuggs, M., and Buja, L.: Cardiogel: A Biosynthetic Extracellular Matrix for Cardiomyocyte Culture, <i>In Vitro Cell Dev Biol Anim</i> 32, 478, 1996 (1172)
Joklik's	Sharma VK, Colecraft HM, Wang DX, Levey AI, Grigorenko EV, Yeh HH, and Sheu SS: Molecular and functional identification of m1 muscarinic acetylcholine receptors in rat ventricular myocytes, <i>Circ Res</i> 79(1), 86, 1996 (9857)
PBS	Li RK, Mickle DA, Weisel RD, Zhang J, Mohabeer MK: In vivo survival and function of transplanted rat cardiomyocytes, <i>Circ Res</i> 78, 283-8, 1996 (10304)
Joklik's MEM	Laughlin, M., Schaeffer, M., and Sturek, M.: Effect of Exercise Training on Intracellular Free Ca^{2+} Transients in Ventricular Myocytes of Rats, <i>Am J Physiol</i> 73, 1141, 1992 (773)
Joklik's MEM, Krebs-Henseleit buffer, CF	Welder, A.A., Grant, R., Bradlaw, J., and Acosta, D.: A Primary Culture System of Adult Rat Heart Cells for the Study of Toxicologic Agents, <i>In Vitro Cell Dev Biol</i> 27, 921, 1991 (479)
(see reference)	Fischer, Y., Rose, H., and Kammermeier, H.: Highly Insulin-Responsive Isolated Rat Heart Muscle Cells Yielded By a Modified Isolation Method, <i>Life Sci</i> 49, 1679, 1991 (1173)
Krebs-Henseleit Buffer, CF	Marino, T.A., Walter, R., Cobb, E., Palasiuk, M., Parsons, T., and Mercer, W.E.: Effects of Norepinephrine on Neonatal Rat Cardiocyte Growth and Differentiation, <i>In Vitro Cell Dev Biol</i> 26, 229, 1990 (433)
25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, <i>In Vitro Cell Dev Biol</i> 26, 335, 1990 (438)
Joklik's MEM	Nag, A.C., Lee, M., and Kosiur, J.R.: Adult Cardiac Muscle Cells in Long-Term Serum-Free Culture: Myofibrillar Organization and Expression of Myosin Heavy Chain Isoforms, <i>In Vitro Cell Dev Biol</i> 26, 464, 1990 (442)
(see reference)	Engelmann, G., McTiernan, C., Gerrity, R., and Samarel, A.: , <i>Technique</i> 2 (6), 279, 1990 (1292)
Krebs Ringer bicarbonate buffer	Berg, I., Guse, A.H., and Gercken, G.: Carbamoylcholine-Induced Accumulation of Inositol Mono-, Bis-, Tris-, And Tetrakisphosphates In Isolated Cardiac Myocytes From Adult Rats, <i>Biochim Biophys Acta</i> 1010, 100, 1989 (315)
Joklik's medium	Bugaisky, L.B. and Zak, R.: Differentiation of Adult Rat Cardiac Myocytes in Cell Culture, <i>Circ Res</i> 64, 493, 1989 (367)
Joklik's solution with and without calcium	De Young, M., Giannattasio, B., and Scarpa, A.: Isolation of Calcium-Tolerant Atrial And Ventricular Myocytes From Adult Rat Heart, <i>Methods Enzymology</i> , Vol. 173, 662, 1989 (634)
HBSS	Toraason, M., Luken, M.E., Breitenstein, M., Krueger, J.A., and Biagini, R.E.: Comparative Toxicity of Allylamine and Acrolein in Cultured Myocytes and Fibroblasts from Neonatal Rat Heart, <i>Toxicology</i> 56, 107, 1989 (672)

Tissue Dissociation Guide

Species

Heart (con't)

Cell(s)

Enzyme(s)

	Rat, SD, male	Aortic smooth muscle	Trypsin: 0.05%
	Rat, adult, 150-200 g	Myocytes, atria	Collagenase Type 1: 100 u/ml
	Rat, SD	Myocytes	Collagenase Type 2: 0.1%
	Rat, SD, male, 250-350 g	Myocytes, heart	Hyaluronidase: 0.03%
	Rat, Wistar, 2-4 day neonates	Myoblast, cardiac	Trypsin: 0.1%
	Rat, SD, timid-pregnant	Myocytes	Trypsin: 2.4 u/ml
	Rat, SD, female, 12 weeks	Myocytes	Collagenase Type 1: 0.06%
	Rat, male/female, 200-250 g	Muscle	Hyaluronidase: 0.1%
	Rat, female, adult, 200 - 300 g	Myocytes	Collagenase Type 2: 0.1%
	Rat, SD, adult, male, 200-250 g	Myocytes	Hyaluronidase: 0.10%
	Rat, SD, female, 12 wks, 250 g	Myocytes Ventricular myocardium	Collagenase Type 1: 0.20%
	Rat, 10 wk	Myocytes	Collagenase Type 1: 0.1%
	Rat, male, 200-300 g	Myocytes	Hyaluronidase: 82 u/ml
	Rat, female, albino, 200-250 g	Myocytes	Hyaluronidase: 0.1%
	Rat, adult	Myocytes	Collagenase: 0.1%
	Rat, SD, albino, male, 180-230 g	Heart ventricles, beating	Collagenase: 0.05-0.2%
	Rat, Hebrew Un strain, 3 & 17 months, 150-210 g	Myocytes	Trypsin: 0.05%
	Rat, 2-5 day	Myocytes	Trypsin: 0.01%
	Rat, SD, male, 150-200 g	Myocytes	Hyaluronidase: 0.05%
	Rat, albino, male, 250 g	Myocytes	Hyaluronidase: 0.20%
	Rat, adult, male, 250 g	Myocytes	Trypsin: 0.1%
	Rat, Wistar, 1-4 day old	Myocytes	Trypsin: 0.125%
Shellfish	Pacific Oysters	Haemocytes	Trypsin:
	Clams, 3.0 to 4.5 cm	Myocytes Fibroblasts	Collagenase Type 1: 2%

Intestine

Species

Cell(s)

Enzyme(s)

Canine	Dog, adult	Intestinal L-cells	Collagenase Type 1: 75 u/ml
Human	Human	Intestinal epithelial	Collagenase Type 4: 72.5 u/ml

Heart (con't)

Medium	Reference
DMEM	Cornwell, T., and Lincoln, T.: Regulation of Intracellular Ca ²⁺ Levels in Cultured Vascular Smooth Muscle Cells, <i>J Biol Chem</i> 264 (2), 1146, 1989 (867)
Kreb's Ringer bicarbonate - HEPES buffer	McMahon, K.K.: A Study of Adult Rat Atrial Myocyte Attachment to Extracellular Matrix Components and Long Term Culture, <i>Biology of Isolated Adult Cardiac Myocytes</i> , (Clark, Decker, Borg, Eds), 318, 1988 (680)
Kreb's Ringer w/ calcium	Buxton, I.L.O. and Doggwiler, K.O.: Alpha1-Adrenergic Receptor Signal Transduction in the Adult Rat Cardiac Myocyte, <i>Biology of Isolated Adult Cardiac Myocytes</i> , (Clark, Decker, Borg, eds), , 298, 1988 (712)
Bicarbonate-buffered medium	Kim, D. and Smith, T.W.: Temporal Variation in Contractile State And [Ca ⁺⁺] in Isolated Adult Rat And Guinea Pig Cardiac Myocytes, <i>Biology of Isolated Adult Cardiac Myocytes</i> , (Clark, Decker, Borg, eds), 370, 1988 (728)
Standardized Medium (see reference)	Grynberg, A., Degois, M., Guenet, L., and Athias, P.: Primary Rat Cardiac Cell Culture: Diet of the Mother Rats as a Determinant Parameter of Cardiomyoblast Production from Neonates, <i>Biol Cell</i> 57, 89, 1986 (340)
DMEM	Freerksen, D., Schroedl, N., and Hartzell, C.: Control of Enzyme Activity Levels by Serum and Hydrocortisone in Neonatal Rat Heart Cells Cultured in Serum-Free Medium, <i>J Cell Physiol</i> 120, 126, 1984 (1230)
Krebs Ringer bicarbonate buffer	Piper, H.M., Probst, I., Schwartz, P., Hutter, F.J., and Spieckermann, P.G.: Culturing of Calcium Stable Adult Cardiac Myocytes, <i>J Mol Cell Cardiol</i> 14, 397, 1982 (718)
Krebs Ringer phosphate buffer, CMF	Nag, A. and Cheng, M.: Adult Mammalian Cardiac Muscle Cells in Culture, <i>Tissue Cell</i> 13, 515, 1981 (671)
Joklik's MEM	Claycomb, W.C., and Palazzo, M.C.: Culture of The Terminally Differentiated Adult Cardiac Muscle Cell: A Light and Scanning Electron Microscope Study, <i>Dev Biol</i> 80, 466, 1980 (370)
Joklik MEM, CF	Frangakis, C., Bahl, J., McDaniel, H., and Bressler, R.: Tolerance to Physiological Calcium by Isolated Myocytes from the Adult Rat Heart; An Improved Cellular Preparation, <i>Life Sci</i> 27, 815, 1980 (627)
Kreb's Ringer bicarbonate buffer	Powell, T., Terrar, D.A., and Twist, V.W.: Electrical Properties of Individual Cells Isolated From Adult Rat Ventricular Myocardium, <i>J Physiol</i> 302, 131, 1980 (719)
Saline	Bishop, S., Oparil, S., Reynolds, R., and Drummond, J.: Regional Myocyte Size in Normotensive and Spontaneously Hypertensive Rats, <i>Hypertension</i> 1 (4), 378, 1979 (1190)
Kreb's Henseleit buffer	Farmer, B., Harris, R., Jolly, W., Hathaway, D., Katzberg, A., Watanabe, A., Whitlow, A., and Besch, H.: Isolation and Characterization of Adult Rat Heart Cells, <i>Arch Biochem Biophys</i> 179, 545, 1977 (851)
Kreb's Ringers phosphate	Nag, A., Fischman, D., Aumont, M., and Zak, R.: Studies of Isolated Adult Rat Heart Cells: The Surface Morphology and the Influence of Extracellular Calcium Ion Concentration on Cellular Viability, <i>Tissue Cell</i> 9 (3), 419, 1977 (854)
Perfusing solution	Moustafa, E., Skomedal, T., Osnes, J., and Oye, I.: Cyclic Amp Formation and Morphology of Myocardial Cells Isolated from Adult Heart: Effect of Ca ²⁺ and Mg ²⁺ , <i>Biochim Biophys Acta</i> 421, 411, 1976 (1171)
Phosphate buffer	Glick, M., Burns, A., and Reddy, W.: Dispersion and Isolation of Beating Cells from Adult Rat Heart, <i>Anal Biochem</i> 61, 32, 1974 (302)
Versene buffer	Bierman, E.L., Stein, O., and Stein, Y.: Lipoprotein Uptake and Metabolism by Rat Aortic Smooth Muscle Cells in Tissue Culture, <i>Circ Res</i> 35, 136, 1974 (363)
Saline A	Speicher, D.W., and McCarl, R.L.: Pancreatic Enzyme Requirements for the Dissociation of Rat Hearts for Culture, <i>In Vitro</i> 10, 30, 1974 (494)
(see reference)	Pretlow II, T., Glick, M., and Reddy, W.: Separation of Beating Cardiac Myocytes from Suspensions of Heart Cells, <i>Am J Pathol</i> 67 (2), 215, 1972 (1175)
Hank's solution, CF	Berry, M., Friend, D., and Scheuer, J.: Morphology and Metabolism of Intact Muscle Cells Isolated from Adult Rat Heart, <i>Circ Res</i> 26, 679, 1970 (362)
Saline A	Vahouny, G.V., Wei, R., Starkweather, R., and Davis, C.: Preparation of Beating Heart Cells From Adult Rats, <i>Science</i> 167, 1616, 1970 (664)
Gey's BSS	Mark, G., and Strasser, F.: Pacemaker Activity and Mitosis in Cultures of Newborn Rat Heart Ventricle Cells, <i>Exp Cell Res</i> 44, 217, 1966 (853)
L15 medium	Le Duff, R., Lipart, C., and Renault, T.: Primary Culture of Pacific Oyster, <i>Crassostrea gigas</i> , heart cells, <i>J Tiss Cul Meth</i> 16, 67, 1994 (752)
L15 medium	Wen, C., Kou, G., and Chen, S.: Cultivation of Cells From the Heart of the Hard Clam, <i>Merretrix lusoria</i> (Roding), <i>J Tiss Cul Meth</i> 15, 123, 1993 (751)

Intestine

Medium	Reference
HBSS	Damholt AB, Buchan AM, Kofod H: Glucagon-like-peptide-1 secretion from canine L-cells is increased by glucose-dependent-insulinotropic peptide but unaffected by glucose, <i>Endocrinology</i> 139, 2085-91, 1998 (10141)
HBSS	Fahlgren, A., Hammarstrom, S., Danielsson, A. and Hammarstrom, M.: Increased Expression of Antimicrobial Peptides and Lysozyme in Colonic Epithelial Cells of Patients with Ulcerative Colitis., <i>Clin Exp Immunol</i> 131, 90, 2003 (10358)

Tissue Dissociation Guide

Species	Intestine (con't)	Cell(s)	Enzyme(s)
Mouse	Human	Lamina propria mononuclear cells	Collagenase: 0.02%
	Human	Mucosal mononuclear cells	Collagenase Type 3: 0.01% Deoxyribonuclease I: 0.01% Soybean Trypsin Inhibitor: 0.01%
	Mouse, 7 week	Interstitial cells	Collagenase: 0.04% Trypsin: 0.02%
	Mouse, 9-13 day	Interstitial cells of Cajal	Collagenase Type 2: 0.13%
	Mouse, 0-15 day	Interstitial cells of Cajal	Collagenase Type 2: 0.13%
	Mouse	Lamina propria lymphocytes	Collagenase Type 4: 300 u/ml
	Mouse, F14	Intestinal mesenchymal	Collagenase Type 2: 0.03%
Rat	Rat, male, 18-24 day	Intestinal epithelial	Collagenase Type 1: 0.1%

Kidney

Species		Cell(s)	Enzyme(s)
Avian	Avian, chicken	Tubule and glomeruli	Collagenase Type 1: 0.1%
	Avian, house sparrows	Tubule segments	Collagenase Type 2: 0.1%
Bovine	Bovine	Papillary duct	Hyaluronidase: 0.2%
Canine	Canine	Renal adipose derived cells	Collagenase Type 1: 0.3%
	Dog, 1-5 months	Proximal tubules	Collagenase Type 1: 0.15%
	Dog, mongrel, 20 Kg	Proximal tubular	Deoxyribonuclease I: 0.0125%
Fish	Winter flounder (<i>Pseudopleuronectes americanus</i>) 200 - 500 g	Renal tubule	Trypsin: 0.2%
Guinea-Pig	Guinea-pig, male, 100 g	Single cells	Trypsin: 0.25%
Hamster	Hamster (also rat, SD and Wistar, 150-225 g, rabbit, bovine)	Papillary duct	Hyaluronidase: 0.2%
Human	Human	Renal adipose derived cells	Collagenase Type 1: 0.3%
	Human, adult	Renal proximal tubule and cortical fibroblasts	Collagenase Type 2: 383 u/ml
	Human	Renal cortex	Trypsin: 0.1%
	Human	Papillary duct	Collagenase: 400 u/ml
	Human	Mesangial	Trypsin: 0.25%
	Human, adult	Tubular	Collagenase: 250 u/ml
	Human, adult, 14-66 years	Tubular	Collagenase: 100 u/ml
	Human	Malignant Stromal	Papain: 0.009%
	Human, 24 newborn and stillborn (also rabbit)	Renal	Trypsin: 0.25%

Tissue Dissociation Guide

Medium	Reference	Intestine (con't)
HBSS	Kanai Takanori, Totsuka Teruji, Uraushihara Koji, Makita Shin, Nakamura Tetsuya, Koganei Kazutaka, Fukushima Tsuneo, Akiba Hisaya, Yagita Hideo, Okumura Ko, Machida Utako, Iwai Hideyuki, Azuma Miyuki, Chen Lieping, Watanabe Mamoru: Blockade of B7-H1 suppresses the development of chronic intestinal inflammation, <i>J Immunol</i> 171, 4156-63, 2003 (10232)	
RPMI	Stallmach A, Schaffer F, Hoffmann S, Weber S, Muller-Molaian I, Schneider T, Kohne G, Ecker KW, Feifel G, Zeitz M: Increased state of activation of CD4 positive T cells and elevated interferon gamma production in pouchitis, <i>Gut</i> 43, 499-505, 1998 (10240)	
(see reference)	Goto Kazunori, Matsuoka Satoshi, Noma Akinori: Two types of spontaneous depolarizations in the interstitial cells freshly prepared from the murine small intestine, <i>J Physiol</i> 559, 411-22, 2004 (10145)	
HBSS	Ordag Tamas, Redelman Doug, Miller Lisa J, Horvath Viktor J, Zhong Qiao, Almeida-Porada Graca, Zanjani Esmail D, Horowitz Burton, Sanders Kenton M: Purification of interstitial cells of Cajal by fluorescence-activated cell sorting, <i>Am J Physiol Cell Physiol</i> 286, C448-56, 2004 (10236)	
Hanks	Lee Young Mee, Kim Byung Joo, Kim Hyun Jin, Yang Dong Ki, Zhu Mei Hong, Lee Kyu Pil, So Insuk, Kim Ki Whan: TRPC5 as a candidate for the nonselective cation channel activated by muscarinic stimulation in murine stomach, <i>Am J Physiol/Gastro</i> 284, G604-16, 2003 (10233)	
PBS	Wu Y, Wang X, Csencsits KL, Haddad A, Walters N, Pascual DW: M cell-targeted DNA vaccination, <i>Proc Natl Acad Sci U S A</i> 98, 9318-23, 2001 (10271)	
HBSS	Sakagami Y, Inaguma Y, Sakakura T, Nishizuka Y: Intestine-like remodeling of adult mouse glandular stomach by implanting of fetal intestinal mesenchyme, <i>Cancer Res</i> 44, 5845-9, 1984 (10146)	
DMEM	Quaroni A, Wands J, Trelstad RL, Isselbacher KJ: Epithelioid cell cultures from rat small intestine. Characterization by morphologic and immunologic criteria, <i>J Cell Biol</i> 80, 248-65, 1979 (10144)	

Kidney

Medium	Reference
RPMI 1640	Reich, C. and Bonar, R.: Separation of Avian Kidney Tubules and Glomeruli for In Vitro Culture, <i>Meth Cell Sci</i> 7, 97, 1982 (10676)
HBSS	Goldstein D., Reddy V., and Plaga K.: Second Messenger Production in Avian Medullary Nephron Segments in Response to Peptide Hormones, <i>Am J Physiol</i> 276, R847, 1999 (9873)
Kreb's buffer HEPES buffered saline	Husted, R., Hayashi, M., and Stokes, J.: Characteristics of Papillary Collecting Duct Cells in Primary Culture, <i>Am J Physiol</i> 255, F1160, 1988 (298)
DMEM	Basu, J., Genheimer, C., Sangha, N., Quinlan, S., Guthrie, K., Kelley, R., Ilagan, R., Jain, D., Bertram, T. and Ludlow, J.: Organ Specific Regenerative Markers in Peri-Organ Adipose: Kidney., <i>Lipids Health Dis</i> Vol. 10, 171, 2011 (10665)
Krebs Ringer bicarbonate buffer	States, B., Reynolds, R., Lee, J., and Segal, S.: Cystine Uptake By Cultured Cells Originating From Dog Proximal Tubule Segments, <i>In Vitro Cell Dev Biol</i> 26, 105, 1990 (426)
(see reference)	Yau, C., Rao, L., and Silverman, M.: Sugar Uptake Into a Primary Culture of Dog Kidney Proximal Tubular Cells, <i>Can J Physiol Pharmacol</i> 63, 417, 1985 (707)
CMF solution (see reference)	Dickman, K. and Renfro, J.: Primary Culture of Flounder Renal Tubule Cells: Transepithelial Transport, <i>Am J Physiol</i> 251, 424, 1986 (297)
CF salt solution	Phillips, H.: Dissociation of Single Cells from Lung or Kidney Tissue with Elastase, <i>In Vitro</i> 8, 101, 1972 (538)
Kreb's buffer HEPES buffered saline	Husted, R., Hayashi, M., and Stokes, J.: Characteristics of Papillary Collecting Duct Cells in Primary Culture, <i>Am J Physiol</i> 255, F1160, 1988 (298)
DMEM	Basu, J., Genheimer, C., Sangha, N., Quinlan, S., Guthrie, K., Kelley, R., Ilagan, R., Jain, D., Bertram, T. and Ludlow, J.: Organ Specific Regenerative Markers in Peri-Organ Adipose: Kidney., <i>Lipids Health Dis</i> Vol. 10, 171, 2011 (10665)
DMEM/F-12	Johnson, D., Saunders, H., Johnson, F., Huq, S., Field, M., and Pollock, C.: Cyclosporin exerts a direct fibrogenic effect on human tubulointerstitial cells: roles of insulin-like growth factor I, transforming growth factor beta1, and platelet-derived growth factor, <i>J Pharmacol Exp Ther</i> 289, 535-42, 1999 (10158)
Tissue Culture Grade Water	McAteer, J, Kempson, S., and Evan, A: Culture of Human Renal Cortex Epithelial Cells, <i>J Tiss Cul Meth</i> 13, 143, 1991 (1266)
Eagle's MEM-HEPES buffer w/L-glutamine	Trifillis, A. and Kahng, M.: Characterization of an In Vitro System of Human Renal Papillary Collecting Duct Cells, <i>In Vitro Cell Dev Biol</i> 26, 441, 1990 (441)
DMEM/Ham's F-12	Heieren, M., van der Woude, F., and Balfour Jr., H.: Cytomegalovirus Replicates Efficiently in Human Kidney Mesangial Cells, <i>Proc Natl Acad Sci U S A</i> 85, 1642, 1988 (657)
PBS	Yang, A., Gould-Kostka, J., and Oberley, T.: In Vitro Growth and Differentiation of Human Kidney Tubular Cells on a Basement Membrane Substrate, <i>In Vitro Cell Dev Biol</i> 23 (1), 34, 1987 (972)
Joklik's MEM	Trifillis, A., Regec, A., and Trump, B.: Isolation, Culture, and Characterization of Human Renal Tubular Cells, <i>J Urol</i> 133, 324, 1985 (622)
Sacks solution	Hemstreet, G., Enoch, P., and Pretlow, T.: Tissue Disaggregation of Human Renal Cell Carcinoma with Further Isopyknic and Isokinetic Gradient Purification, <i>Cancer Res</i> 40, 1043, 1980 (973)
(see reference)	Montes De Oca, H., and Malinin, T.: Dispersion and Cultivation of Renal Cells After Short-Term Storage of Kidneys, <i>J Clin Microbiol</i> 2 (3), 243, 1975 (986)

Tissue Dissociation Guide

Species

Kidney (con't)

Cell(s)

Enzyme(s)

Monkey	Monkey	Kidney	Trypsin: 0.01% - 0.00001%	
Mouse	Mouse	Proximal tubule	Collagenase Type 2: 0.013%	
	Mouse, 4 week	Endothelial kidney	Collagenase Type 1: 0.1%	
	Mouse, male, 12-16 week	Renal proximal tubule cells	Collagenase Type 2: 0.1% Soybean Trypsin Inhibitor: 0.25%	
	Mouse, male, 2 month	Cortical collecting duct	Collagenase: 54-178 u/ml Protease: see reference	
	Mouse, male, 8-16 week	Renal tubular epithelial	Collagenase: 200 u/ml Soybean Trypsin Inhibitor: see reference	
	Mouse, adult	Cortex, proximal tubule	Collagenase: 0.15%	
	Mouse, 6 week	Glomerular mesangial cells	Collagenase Type 4: 0.1%	
	Mouse, 3-7 month	Fibroblasts, mesangial, smooth muscle	Trypsin: 0.25% Collagenase: see reference Soybean Trypsin Inhibitor: .05%	
	Mouse	Epithelial	Collagenase Type 4: 1%	
	Mouse, naive SJL (H-2S)	Proximal tubular epithelial	Deoxyribonuclease I: 15 µg/ml	
	Rabbit	Rabbit, New Zealand	Proximal tubule cells	Collagenase Type 4: 0.005% Soybean Trypsin Inhibitor: 0.00025%
		Rabbit, New Zealand White, female	Nephron segments	Collagenase Type 2: 0.05% Deoxyribonuclease I: 50 u/ml
		Rabbit, New Zealand white, male, 1.5 - 2.0 Kg	Duct	Soybean Trypsin Inhibitor: 0.025%
Rabbit, New Zealand white, female, 2-3 Kg		Renal proximal tubules	Deoxyribonuclease I: 70 u/ml	
Rabbit (also rat, SD and Wistar, 150-225 g, hamster, bovine)		Papillary duct	Hyaluronidase: 0.2%	
Rabbit, New Zealand, white, 2-3 kg		MTALH cells RCCT cells	Collagenase: 0.1%	
Rabbit, New Zealand, white		Collecting tubule	Trypsin: 0.05%	
Rabbit, New Zealand, white, female, 2.5 Kg		Papillary collecting duct	Collagenase: 0.2%	
Rat	Rat, Lewis, male	Renal adipose derived cells	Collagenase Type 1: 0.3%	
	Rat	Proximal tubule	Collagenase Type 2: 0.1%	
	Rat, Wistar, male, 300-350 g	Renal proximal tubules	Collagenase Type 2: 0.2%	
	Rat, SD, male, 200-250g	Medullary thick ascending limb	Collagenase: 0.1%	

Tissue Dissociation Guide

Medium	Reference
Eagle's MEM	Melnick, J., and Wallis, C.: Problems Related to the Use of Serum and Trypsin in the Growth of Monkey Kidney Cells, <i>Dev Biol</i> 37, 77, 1976 (706)
(see reference)	Wright, J., Morales, M., Sousa-Menzes, J., Ornellas, D., Sipes, J., Cui, Y., Cui, I., Hulamm, P., Cebotaru, V., Cebotaru, L., Guggino, W. and Guggino, S.: Transcriptional Adaptation to Cln5 Knockout in Proximal Tubules of Mouse Kidney., <i>Physiol Genomics</i> Vol. 33, 341, 2008 (10589)
DMEM	Kondo, S., Scheef, E., Sheibani, N. and Sorenson, C.: PECAM-1 Isoform- Specific Regulation of Kidney Endothelial Cell Migration and Capillary Morphogenesis., <i>Am J Physiol Cell Physiol</i> 292, C2070, 2007 (10549)
DMEM/F-12	Cunningham, R., Xiaofei, E., Steplock, D., Shenolikar, S. and Weinman, E.: Defective PTH Regulation of Sodium-Dependent Phosphate Transport in NHERF-1/-Renal Proximal Tubule Cells and Wild-Type Cells Adapted to Low-Phosphate Media., <i>Am J Physiol Renal Physiol</i> Vol. 289, F933, 2005 (10626)
MEM	Sindic, A., Velic, A., Basoglu, C., Hirsch, J., Edemir, B., Kuhn, M. and Schlatter, E.: Uroguanylin and Guanylin Regulate Transport of Mouse Cortical Collecting Duct Independent of Guanylate Cyclase C., <i>Kidney Int</i> Vol. 68, 1008, 2005 (10627)
HBSS	Breggia, A. and Himmelfarb, J.: Primary Mouse Renal Tubular Epithelial Cells have Variable Injury Tolerance to Ischemic and Chemical Mediators of Oxidative Stress., <i>Oxid Med Cell Longev</i> Vol. 1, 33, 2008 (10554)
DMEM	Syal Ashu, Schiavi Susan, Chakravarty Sumana, Dwarakanath Vangipuram, Quigley Raymond, Baum Michel: Fibroblast growth factor-23 increases mouse PGE2 production in vivo and in vitro, <i>Am J Physiol/ Renal</i> 290, F450-5, 2006 (10281)
RPMI 1640	Radeke HH, Janssen-Graalfs I, Sowa EN, Chouchakova N, Skokowa J, Loscher F, Schmidt RE, Heeringa P, Gessner JE.: Opposite regulation of type II and III receptors for immunoglobulin G in mouse glomerular mesangial cells and in the induction of anti-glomerular basement membrane (GBM) nephritis, <i>J Biol Chem</i> 277(30), 27535, 2002 (9874)
DMEM	Bradshaw AD, Francki A, Motamed K, Howe C, Sage EH: Primary mesenchymal cells isolated from SPARC-null mice exhibit altered morphology and rates of proliferation, <i>Mol Biol Cell</i> 10, 1569-79, 1999 (10136)
DMEM /F-12	Taub, M: Renal Tubule Cells, <i>Cell & Tissue Culture: Laboratory Procedures</i> Vol. 2, Doyle, A., Griffiths, J., and Newell, D., John Wiley & Sons, Ltd., 14B:6.1, 1995 (1279)
RPMI 1640	Haverty, T., Kelly, C., Hines, W., Amenta, P., Watanabe, M., Harper, R., Kefalides, N., and Neilson, E.: Characterization of a Renal Tubular Epithelial Cell Line Which Secretes the Autologous Target Antigen of Autoimmune Experimental Interstitial Nephritis, <i>J Cell Biol</i> 107, 1359, 1988 (578)
DMEM/F-12	Taub, M.: Primary Kidney Proximal Tubule Cells., <i>Methods Mol Biol</i> 290, 231, 2005 (10628)
Eagle's MEM	Schafer, J., Watkins, M., Li, L., Herter, P., Haxelmans, S., and Schlatter, E.: A Simplified Method for Isolation of Large Numbers of Defined Nephron Segments, <i>Am J Physiol</i> 273, F650, 1997 (9871)
Hank's Solution with calcium and HEPES	Naray-Fejes-Toth, A., and Fejes-Toth, G.: Immunoelection and Culture of Cortical Collecting Duct Cells, <i>J Tiss Cul Meth</i> 13, 179, 1991 (1267)
Modified DME-F12	Rodeheaver, D., Aleo, M., and Schnellmann, R.: Differences in Enzymatic and Mechanical Isolated Rabbit Renal Proximal Tubules: Comparison in Long-Term Incubation, <i>In Vitro Cell Dev Biol</i> 26, 898, 1990 (454)
Kreb's buffer HEPES buffered saline	Husted, R., Hayashi, M., and Stokes, J.: Characteristics of Papillary Collecting Duct Cells in Primary Culture, <i>Am J Physiol</i> 255, F1160, 1988 (298)
DMEM	Allen, M., Nakao, A., Sonnenburg, W., Burnatowska-Hledin, M., Speilman, W., and Smith, W.: Immunodissection of Cortical and Medullary Thick Ascending Limb Cells From Rabbit Kidney, <i>Am J Physiol</i> 255, F704, 1988 (971)
Kreb's Ringer buffer	Grenier, F., and Smith, W.: Formation of 6-keto-PGF _{1α} by Collecting Tubule Cells Isolated from Rabbit Renal Papillae, <i>Prostaglandins</i> 16, 759, 1978 (705)
(see reference)	Dworzack, D., and Grantham, J.: Preparation of Renal Papillary Collecting Duct Cells for Study, <i>Nat Neurosci</i> 8, 191, 1975 (704)
DMEM	Basu, J., Genheimer, C., Sangha, N., Quinlan, S., Guthrie, K., Kelley, R., Ilagan, R., Jain, D., Bertram, T. and Ludlow, J.: Organ Specific Regenerative Markers in Peri-Organ Adipose: Kidney., <i>Lipids Health Dis</i> Vol. 10, 171, 2011 (10665)
(see reference)	Panico, C., Luo, Z., Damiano, S., Artigiano, F., Gill, P. and Welch, W.: Renal Proximal Tubular Reabsorption is Reduced in Adult Spontaneously Hypertensive Rats: Roles of Superoxide and Na ⁺ /H ⁺ Exchanger 3., <i>Hypertension</i> 54, 1291, 2009 (10590)
DMEM/F-12	Deng, A., Miracle, C., Suarez, J., Lortie, M., Satriano, J., Thomson, S., Munger, K. and Blantz, R.: Oxygen Consumption in the Kidney: Effects of Nitric Oxide Synthase Isoforms and Angiotensin II., <i>Kidney Int</i> Vol. 68, 723, 2005 (10578)
HEPES-saline	Silva, G., Beierwaltes, W., and Garvin, J.: Extracellular ATP stimulates NO production in rat thick ascending limb, <i>Hypertension</i> 47, 563-7, 2006 (10177)

Tissue Dissociation Guide

Species

Kidney (con't)

Cell(s)

Enzyme(s)

Rat, Wistar, 300g	Renal proximal tubules	Collagenase Type 2: 0.2%
Rat	Cortical tubule cells	Collagenase: 0.6%
Rat, SD, male 275-300 g	Glomerular mesangial cells	Collagenase Type 1: 250 u/ml
Rat, SD, 1 week	Cortical collection duct	Collagenase Type 2: 0.15%
Rat, SD, 250-300 g	Microvessels	Collagenase Type 2: 0.1%
Rat, SD, 250-300 g	Renal	Collagenase Type 2: 0.2%
Rat, SD, male, 5-6 week	Renal tubules	Collagenase: 0.1%
Rat, SD, 225-250 g	Glomerular mesangial cells	Collagenase Type 1: 250 u/ml
Rat, SD, male	Proximal tubule suspensions	Collagenase Type 4: 0.1% Pronase E: 2 u/ml
Rat, SD, male	Nephron segments	Collagenase Type 2: 0.05% Deoxyribonuclease I: 50 u/ml
Rat, 12-weeks-old	Renal	Collagenase: 0.1%
Rat, male, 150-200 g	Epithelial Proximal tubule	Protease: 0.1%
Rat, adult	Inner medullary collecting duct Papillae	Collagenase Type 2: 0.1%
Rat, Wistar, female, 100-150 g	Glomerular mesangial	Collagenase: 0.025%
Rat, Wistar, male, 150 - 200 g	Renal target	Collagenase: 1.0% (also 0.7%)
Rat, Wistar, male, adult	Proximal tubules	Collagenase: 0.2%
Rat, Wistar, 300-400 g	Proximal tubules	Collagenase: 0.15 %
Rat	Fibroblasts Kidney	Trypsin: up to 0.25%

Liver

Species

Cell(s)

Enzyme(s)

Avian	Ducklings, 7 day	Hepatocytes	Collagenase: 0.05% Hyaluronidase: 0.05%
Canine	Dog, 13 years old (also rat, guinea-pig, rabbit, human)	Hepatocytes	Collagenase: 90 u/ml
	Dog, adult	Hepatocytes	Trypsin: 0.1%
Chicken	Chicken, adult	Hepatocytes	Collagenase: 0.02%
	Chicken (also rat, SD, 150-250 g)	Hepatocytes	Collagenase Type 4: 6000 units
	Chicken, Leghorn, white, 10-15 day	Hepatocytes	Deoxyribonuclease I: 0.00125%

Tissue Dissociation Guide

Medium	Reference
DMEM/F12	Deng Aihua, Miracle Cynthia M, Lortie Mark, Satriano Joseph, Gabbai Francis B, Munger Karen A, Thomson Scott C, Blantz Roland C: Kidney oxygen consumption, carbonic anhydrase, and proton secretion, <i>Am J Physiol/Renal</i> 290, F1009-15, 2006 (10305)
DMEM	Arystarkhova, E., Wetzell R., and Sweadner K.: Distribution and Oligomeric Association of Splice Forms of Na(+)-K(+)-ATPase Regulatory Gamma-subunit in Rat Kidney, <i>Am J Physiol/Renal</i> 282(3), F393, 2002 (9872)
DMEM	Amiri Farhad, Shaw Sean, Wang Xiaodan, Tang Jie, Waller Jennifer L, Eaton Douglas C, Marrero Mario B: Angiotensin II activation of the JAK/STAT pathway in mesangial cells is altered by high glucose, <i>Kidney Int Suppl</i> 61, 1605-16, 2002 (10250)
DMEM/Ham's F12	Valencia L., Bidet M., Martial S., Sanchez E., Melendez E., Tauc M., Poujeol C., Martin D., Namorado M., Reyes J., and Poujeol P.: Nifedipine-activated Ca(2+) Permeability in Newborn Rat Cortical Collecting Duct Cells in Primary Culture, <i>Am J Physiol Cell Physiol</i> 280(5), C1193, 2001 (9875)
PSS	Li, N., Tegatz, E., Li, P., Allaire, R., and Zou, A.: Formation and Actions of Cyclic ADP-Ribose in Renal Microvessels, <i>Microvasc Res</i> 60, 149, 2000 (1083)
(see reference)	Mattson D., and Wu F.: Nitric Oxide Synthase Activity and Isoforms in Rat Renal Vasculature, <i>Hypertension</i> 35, 337, 2000 (9877)
(see reference)	Miyata, N., Park, F., Li, X., and Cowley, A.: Distribution of Angiotensin AT1 and AT2 Receptor Subtypes in the Rat Kidney, <i>Am J Physiol</i> 277, F437, 1999 (9876)
DMEM	Amiri F, Garcia R: Regulation of angiotensin II receptors and PKC isoforms by glucose in rat mesangial cells, <i>Am J Physiol</i> 276, F691-9, 1999 (10251)
HEPES buffer	Eitle E, Hiranyachattada S, Wang H, Harris PJ: Inhibition of proximal tubular fluid absorption by nitric oxide and atrial natriuretic peptide in rat kidney, <i>Am J Physiol</i> 274, C1075-80, 1998 (10254)
Eagle's MEM	Schafer, J., Watkins, M., Li, L., Herter, P., Haxelmans, S., and Schlatter, E.: A Simplified Method for Isolation of Large Numbers of Defined Nephron Segments, <i>Am J Physiol</i> 273, F650, 1997 (9871)
DMEM	Ishikawa, S., Kusaka, I., Higashiyama, M., Nagasaka, S., Saito, T., Honda, K., and Saito, T.: Cellular Signaling and Proliferative Action of AVP in Mesangium of SHR: Effect of Low Density Lipoprotein, <i>Nat Neurosci</i> 50, 1506, 1996 (1187)
HBSS/CMF	Elliget, K., and Trump, B.: Primary Cultures of Normal Rat Kidney Proximal Tubule Epithelial Cells for Studies of Renal Cell Injury, <i>In Vitro Cell Dev Biol</i> 27, 739, 1991 (476)
PBS	Brion, L., Schwartz, J., Lachman, H., Zavilowitz, B., and Schwartz, G.: Development of H+ Secretion by Cultured Renal Inner Medullary Collecting Duct Cells, <i>Am J Physiol</i> 257, F486, 1989 (300)
RPMI 1640	Wang, J., Kester, M., and Dunn, M.: The Effects of Endotoxin on Platelet-Activating Factor Synthesis in Cultured Rat Glomerular Mesangial Cells, <i>Biochim Biophys Acta</i> 969, 217, 1988 (338)
Eagle's MEM	Barlet-Bas, C., Khadouri, C., Marsey, S., and Doucet, A.: Sodium-Independent <i>In Vitro</i> Induction of Na+,K+-ATPase by Aldosterone in Renal Target Cells: Permissive Effect of Triiodothyronine, <i>Proc Natl Acad Sci U S A</i> 85, 1707, 1988 (658)
Krebs-Henseleit buffer	Gesek, F., Wolff, D., and Strandhöv, J.: Improved Separation Method for Rat Proximal and Distal Renal Tubules, <i>Am J Physiol</i> 253, F358, 1987 (969)
Krebs Henseleit solution	Vinay, P., Gougoux, A., and Lemieux, G.: Isolation of a Pure Suspension of Rat Proximal Tubules, <i>Am J Physiol</i> 241, F403, 1981 (289)
Dulbecco-Vogt MEM	Wallach, D., Anderson, W., and Pastan, I.: Activation of Adenylate Cyclase in Cultured Fibroblasts by Trypsin, <i>J Biol Chem</i> 253, 24, 1978 (553)

Liver

Medium	Reference
DMEM/ F12	Lee J., Culvenor J., Angus P., Smallwood R., Nicoll A., and Locarnini S.: Duck Hepatitis B Virus Replication in Primary Bile Duct Epithelial Cells, <i>J Virol</i> 75(16), 7651, 2001 (9883)
CF EGTA perfusate	Reese, J. and Byard, J.: Isolation And Culture of Adult Hepatocytes from Liver Biopsies, <i>In Vitro</i> 17, 935, 1981 (412)
HBSS, CMF	Vickrey, H., Ramon, J., and McCann, D.: Continuous Culture of Normal Adult Mammalian Hepatocytes Exhibiting Parenchymal Functions, <i>In Vitro</i> 15, 120, 1979 (502)
HEPES, CF	Fraslin, J., Touquette, L., Douaire, M., Menezo, Y., Guillemot, J., and Mallard, J.: Isolation and Long Term Maintenance of Differentiated Adult Chicken Hepatocytes in Primary Culture, <i>In Vitro Cell Dev Biol</i> 28, 615, 1992 (486)
Medium A	Roseman, S., Weigel, P., Schnaar, R., Kuhlenschmidt, M., Schmell, E., Lee, R., and Lee, Y.: Adhesion of Hepatocytes to Immobilized Sugars. A Threshold Phenomenon, <i>J Cell Biol</i> 254, 10830, 1979 (582)
PBS	Tarlow, D., Watkins, P., Reed, R., Miller, R., Zwergel, E., and Lane, M.: Lipogenesis and the Synthesis and Secretion of Very Low Density Lipoprotein by Avian Liver Cells in Nonproliferating Monolayer Culture, <i>J Cell Biol</i> 73, 332, 1974 (590)

Tissue Dissociation Guide

Species

Liver (con't)

Cell(s)

Enzyme(s)

Equine	Chick embryos, 5 day	Heart Liver	Trypsin: 3.0%
	Chick embryonic	Various tissues (heart, liver, skeletal, cardiac)	Trypsin: various grades
	Equine, 4-13 yr	Hepatocytes	Collagenase Type 4: 0.1%
Fish	Rainbow trout (<i>Oncorhynchus mykiss</i>), male & female, 120-600 g	Hepatocytes	Collagenase Type 2: 0.045%
	Trout, male, 100 - 200 g	Hepatocytes	Collagenase Type 2: 0.045%
	Rainbow trout, (<i>Salmo gairdneri</i>), male, 150-200 g	Hepatocytes	Collagenase: 100 u/ml
Guinea-Pig	Trout 100g - 5kg	Hepatocytes	Hyaluronidase: 0.08%
	Trout (<i>Salmo gairdneri</i>), male, 150-200 g Catfish (<i>Ictalurus punctatus</i>), male, 100 - 150 g	Hepatocytes	Collagenase: 100u/ml
	Guinea-pig, Hartley, male, 250-300 g	Hepatocytes	Collagenase Type 2:
Human	Guinea-pig, young	Hepatocytes	Collagenase: 90 u/ml
	Human	Hepatic stem cells and hepatoblasts	Collagenase Type 4: 0.014-0.06%
	Human	Hepatic side population	Collagenase: 0.02-0.05%
	Human, fetal	Epithelial progenitor	Collagenase: 0.03%
	Human	Hepatocytes	Collagenase: 0.05%
	Human	Hepatocytes	Collagenase: 0.05%
	Human, adult	Hepatocytes	Collagenase: 0.6%
	Human	Hepatocytes	Collagenase Type 4: 0.05%
	Human (also rat)	Hepatocytes	Collagenase Type 1: 0.05%
	Human	Hepatocytes	Collagenase: 0.05%
	Human	Hepatocytes	Collagenase: 0.05%
	Human, male, 17-40 yrs.	Hepatocytes	Collagenase: 0.05%
	Human, 4 male kidney donors, age between 17 and 31 (also adult rats, male, SD, 180-200 g)	Hepatocytes	Collagenase: 0.05% & 0.025%
	Human, 3 human kidney donors, age 20, 23, 25	Hepatocytes	Collagenase: 0.05%
	Human, 51-75 years old (also rat, guinea-pig, rabbit, dog)	Hepatocytes	Collagenase: 90 u/ml
Human	Hepatocytes	Trypsin: 0.1%	

Tissue Dissociation Guide

Medium	Reference
Tyrode's solution, CMF	Steinberg, M.: "ECM": Its Nature, Origin, And Function in Cell Aggregation, <i>Exp Cell Res</i> 30, 257, 1963 (396)
CMF Tyrode's solution	Rinaldini, L.: An Improved Method for the Isolation and Quantitative Cultivation of Embryonic Cells, <i>Exp Cell Res</i> 16, 477, 1959 (394)
HBSS	Bakala A., Karlik W., and Wiechetek M.: Preparation of Equine Isolated Hepatocytes, <i>Toxicol In Vitro</i> 17(5-6), 615, 2003 (9880)
Kreb's-Ringer bicarbonate buffer, CF	Blair, J.B., Miller, M.R., Pack, D., Barnes, R., Teh, S.J. and Hinton, D.E.: Isolated Trout Liver Cells: Establishing Short-Term Primary Cultures Exhibiting Cell-to-Cell Interactions, <i>In Vitro Cell Dev Biol</i> 26, 237, 1990 (434)
HBSS with 0.05M HEPES	Lipsky, M., Sheridan, T., Bennett, R., and May, E.: Comparison of Trout Hepatocyte Culture on Different Substrates, <i>In Vitro Cell Dev Biol</i> 22, 360, 1986 (418)
HBSS/CMF	Klaunig, J., Ruch, R., and Goldblat, P.: Trout Hepatocyte Culture: Isolation and Primary Culture, <i>In Vitro Cell Dev Biol</i> 21, 221, 1985 (414)
(see reference)	Bailey, G., Taylor, M., and Selivonchick, D.: Aflatoxin B1 Metabolism and DNA Binding in Isolated Hepatocytes From Rainbow Trout <i>Salmo gairdner</i> , <i>Carcinogenesis</i> 3, 511, 1982 (1264)
HBSS	Klaunig, J.: Establishment of Fish Hepatocyte Cultures For Use in <i>In Vitro</i> Carcinogenicity Studies, <i>Natl Cancer Inst Monogr</i> 65, 163, 1981 (703)
Kreb's Ringer bicarbonate buffer	Arinze, I. and Kawai, Y.: Adrenergic Regulation of Glycogenolysis in Isolated Guinea-Pig Hepatocytes: Evidence that B_2 -Receptors Mediate Catecholamine Stimulation of Glycogenolysis, <i>Arch Biochem Biophys</i> 225, 196, 1983 (304)
CF EGTA perfusate	Reese, J. and Byard, J.: Isolation And Culture of Adult Hepatocytes from Liver Biopsies, <i>In Vitro</i> 17, 935, 1981 (412)
various	Wauthier, E., Schmelzer, E., Turner, W., Zhang, L., LeCluyse, E., Ruiz, J., Turner, R., Furth, M., Kubota, H., Lozoya, O., Barbier, C., McClelland, R., Yao, H., Moss, N., Bruce, A., Ludlow, J. and Reid, L.: Hepatic Stem Cells and Hepatoblasts: Identification, Isolation, and Ex Vivo Maintenance., <i>Methods Cell Biol</i> 86, 137, 2008 (10557)
HBSS	Hussain, S., Strom, S., Kirby, M., Burns, S., Langemeijer, S., Ueda, T., Hsieh, M. and Tisdale, J.: Side Population Cells Derived from Adult Human Liver Generate Hepatocyte-Like Cells In Vitro., <i>Dig Dis Sci Vol. 50</i> , 1755, 2005 (10608)
DMEM	Malhi, H., Irani, A., Gagandeep, S. and Gupta, S.: Isolation of Human Progenitor Liver Epithelial Cells with Extensive Replication Capacity and Differentiation into Mature Hepatocytes., <i>J Cell Sci</i> 115, 2679, 2002 (10368)
HEPES	Pichard L, Raulet E, Fabre G, Ferrini JB, Ourlin JC, and Maurel P: Human hepatocyte culture, <i>Methods Mol Biol</i> 320, 283, 2006 (10056)
EBSS	Hughes, R., Mitry, R., Dhawan, A., Lehec, S., Girlanda, R., Rela, M., Heaton, N., and Muiesan, P.: Isolation of Hepatocytes from Livers from Non-Heart-Beating Donors for Cell Transplantation, <i>Liver Transpl</i> 12, 713, 2006 (10205)
RPMI 1640	Cho JJ, Joseph B, Sappal BS, Giri RK, Wang R, Ludlow JW, Furth ME, Susick R, and Gupta S.: †Analysis of the functional integrity of cryopreserved human liver cells including xenografting in immunodeficient mice to address suitability for clinical applications, <i>Liver Int</i> 24, 361, 2004 (10055)
Williams E	Duanmu Z., Locke D., Smigelski J., Wu W., Dahn M., Falany C., Kocarek T., and Runge-Morris M.: Effects of Dexamethasone on Aryl (SULT1A1)- and hydroxysteroid (SULT2A1)-Sulfotransferase Gene Expression in Primary Cultured Human Hepatocytes, <i>Drug Metab Dispos</i> 30(9), 997, 2002 (9879)
Leffert's buffer	Dandri, M., Burda, M., Torok, E., Pollok, J., Iwanska, A., Sommer, G., et al.: Repopulation of Mouse Liver with Human Hepatocytes and <i>In Vivo</i> Infection with Hepatitis B Virus, <i>Hepatology</i> 33, 981, 2001 (1102)
Williams E	Donato M, Viitala P, Rodriguez-Antona C, Lindfors A, Castell J, Raunio H, Gomez-Lechon M, Pelkonen O: CYP2A5/CYP2A6 expression in mouse and human hepatocytes treated with various in vivo inducers, <i>Drug Metab Dispos</i> 28, 1321-6, 2000 (10267)
HEPES buffer (see reference)	Gomez-Lechon, M., Lopez, P., Donato, T., Montoya, A., Larrauri, A., Gimenez, P., Trullenque, R., Fabra, R., and Castell, J.: Culture of Human Hepatocytes From Small Surgical Liver Biopsies: Biochemical Characterization And Comparison With <i>in vivo</i> , <i>In Vitro Cell Dev Biol</i> 26, 67, 1990 (445)
HEPES buffer	Begue, J., Baffet, G., Campion, J., and Guillouzo, A.: Differential Response of Primary Cultures of Human and Rat Hepatocytes to Aflatoxin B1-Induced Cytotoxicity and Protection by the Hepatoprotective agent(+)-Cyanidanol-3, <i>Biol Cell</i> 63, 327, 1988 (341)
HEPES (see reference)	LeBot, M., Begue, J., Kernaleguen, D., Robert, J., Ratanasavanh, D., Airiau, J., Riche, C., and Guillouzo, A.: Different Cytotoxicity and Metabolism of Doxorubicin, Daunorubicin, Epirubicin, Esorubicin, and Idarubicin in Cultured Human and Rat Hepatocytes, <i>Biochem Pharmacol</i> 37 (20), 3877, 1988 (823)
HEPES	Gugen-Guillouzo, C., Campion, J., Brissot, P., Glaise, D., Launois, B., Bourel, M., and Guillouzo, A.: High Yield Preparation of Isolated Human Adult Hepatocytes by Enzymatic Perfusion of the Liver, <i>Cell Biol Int Rep</i> 6 (6), 625, 1982 (819)
CF EGTA perfusate	Reese, J. and Byard, J.: Isolation And Culture of Adult Hepatocytes from Liver Biopsies, <i>In Vitro</i> 17, 935, 1981 (412)
HBSS, CMF	Kaighn, M.: Human Liver Cells, <i>Tissue Culture Methods / Applications</i> , (Kruse, P., Patterson, M. eds), 54, 1973 (702)

Tissue Dissociation Guide

Species

Liver (con't)

Cell(s)

Enzyme(s)

Monkey	Monkey, (<i>Macaca mulatta</i>), 3-5.5 kg	Hepatocytes	Collagenase Type 1: 129 u/ml
	Monkey (<i>Macaca fascicularis</i>), adult, 5-6 Kg	Hepatocytes	Trypsin: 160 u/ml
Mouse	Mouse	Hepatocytes & non-parenchymal liver cells	Collagenase: 0.03-0.05%
	Mouse	Hepatocytes	HIS kit: with modifications
	Mouse	Hepatocytes	Collagenase Type 1: 0.05%
	Mouse, male, 12-14 week	Hepatocytes	Collagenase Type 1: 0.03%
	Mouse	Liver non-parenchymal	Collagenase Type 3: 100 u/ml
	Mouse, male, adult	Hepatocytes	Collagenase Type 4: 0.05%
	Mouse, male	Hepatocytes	Collagenase Type 1: 100 u/ml Elastase: 0.1 u/ml
	Mouse, 7-10 week	Liver endothelial	Collagenase: 0.03%
	Mouse, 10-12 week	Liver sinusoidal endothelial cells	Collagenase Type 1: 0.05% Neutral Protease: 0.025%
	Mouse, 8-10 week	Liver derived stem cells	Collagenase Type 1: 0.1% Neutral Protease: 2.4 u/ml
	Mouse	Liver epithelial progenitor cells	Collagenase Type 4: 0.1% Deoxyribonuclease I: 0.05%
	Mouse	Liver sinusodial endothelial	Collagenase: 0.03%
	Mouse	Hepatocytes	Collagenase Type 1: 0.033%
	Mouse	Hepatocytes	Collagenase Type 1: 0.053%
	Mouse, male, 4-10 week	Hepatocytes	Collagenase Type 2: 100 u/ml
	Mouse	Hepatocytes	Collagenase: 0.05%
	Mouse, 7 week	Kupffer cells	Collagenase Type 4: 0.05%
	Mouse	Nonparenchymal liver	Collagenase Type 1: 0.05%
	Mouse, C3H, 6-8 weeks	Epithelial	Collagenase: 0.10%
	Mouse, 20-30 g	Parenchymal and non-parenchymal	Hyaluronidase: 0.1%
Porcine	Porcine, mini-pig, 13 kg	Hepatocytes	Collagenase Type 4: 0.05% Neutral Protease: 0.84% Deoxyribonuclease I: see reference

Tissue Dissociation Guide

Medium	Reference
DMEM/F12	Weber, A., Groyer-Picard, M. and Dagher, I.: Hepatocyte Transplantation Techniques: Large Animal Models., <i>Methods Mol Biol</i> 481, 83, 2009 (10496)
HEPES buffer	Ulrich, R., Aspar, D., Cramer, C., Kletzien, R., and Ginsberg, L.: Isolation and Culture of Hepatocytes from the Cynomolgus Monkey (<i>Macaca fascicularis</i>), <i>In Vitro Cell Dev Biol</i> 26, 815, 1990 (452)
DMEM	Brundert, M., Heeren, J., Merkel, M., Carambia, A., Herkel, J., Groitl, P., Dobner, T., Ramakrishnan, R., Moore, K. and Rinninger, F.: Scavenger Receptor CD36 Mediates Uptake of High Density Lipoproteins in Mice and by Cultured Cells., <i>J Lipid Res</i> 52, 745, 2011 (10670)
DMEM	Kang, H., Okamoto, K., Kim, Y., Takeda, Y., Bortner, C., Dang, H., Wada, T., Xie, W., Yang, X., Liao, G. and Jetten, A.: Nuclear Orphan Receptor TAK1/TR4-Deficient Mice are Protected Against Obesity-Linked Inflammation, Hepatic Steatosis, and Insulin Resistance., <i>Diabetes</i> 60, 177, 2011 (10673)
Williams E	Holl, D., Kuckenberger, P., Woynecki, T., Egert, A., Becker, A., Huss, S., Stabenow, D., Zimmer, A., Knolle, P., Tolba, R., Fischer, H. and Schorle, H.: Transgenic Overexpression of Tcfap2c/AP-2gamma Results in Liver Failure and Intestinal Dysplasia., <i>PLoS ONE</i> 6, e22034, 2011 (10681)
Williams E	Chung, S., Timmins, J., Duong, M., Degirolamo, C., Rong, S., Sawyer, J., Singaraja, R., Hayden, M., Maeda, N., Rudel, L., Shelness, G. and Parks, J.: Targeted Deletion of Hepatocyte ABCA1 Leads to VLDL Triglyceride Over-Production and LDL Hypercatabolism, <i>J Biol Chem</i> 285, 12197, 2010 (10616)
HBSS	Bosschaerts, T., Guilliams, M., Stijlemans, B., Morias, Y., Engel, D., Tacke, F., Herin, M., De Baetselier, P. and Beschin, A.: Tip-DC Development During Parasitic Infection is Regulated by IL-10 and Requires CCL2/CCR2, IFN-gamma and MyD88 Signaling., <i>PLoS Pathog</i> 6, e1001045, 2010 (10671)
HBSS	Mathijs, K., Kienhuis, A., Brauers, K. J., Jennen, D., Lahoz, A., Kleijnans, J. and van Delft, J.: Assessing the Metabolic Competence of Sandwich-Cultured Mouse Primary Hepatocytes., <i>Drug Metab Dispos</i> 37, 1305, 2009 (10620)
Williams E	Oliva, J., Bardag-Gorce, F., French, B., Li, J., McPhaul, L., Amidi, F., Dedes, J., Habibi, A., Nguyen, S. and French, S.: Fat10 is an Epigenetic Marker for Liver Preneoplasia in a Drug-Primed Mouse Model of Tumorigenesis., <i>Exp Mol Pathol</i> 84, 102, 2008 (10553)
DMEM	Follenzi, A., Benten, D., Novikoff, P., Faulkner, L., Raut, S. and Gupta, S.: Transplanted Endothelial Cells Repopulate the Liver Endothelium and Correct the Phenotype of Hemophilia A Mice., <i>J Clin Invest</i> 118, 935, 2008 (10632)
HEPES	Beldi, G., Wu, Y., Sun, X., Imai, M., Enjyoji, K., Csizmadia, E., Candinas, D., Erb, L. and Robson, S.: Regulated Catalysis of Extracellular Nucleotides by Vascular CD39/ENTPD1 is Required for Liver Regeneration., <i>Gastroenterol</i> 135, 1751, 2008 (10664)
HBSS	Kotton, D., Fabian, A. and Mulligan, R.: A Novel Stem-Cell Population in Adult Liver with Potent Hematopoietic-Reconstitution Activity., <i>Blood</i> 106, 1574, 2005 (10523)
DMEM	Li Wen-Lin, Su Juan, Yao Yu-Cheng, Tao Xin-Rong, Yan Yong-Bi, Yu Hong-Yu, Wang Xin-Min, Li Jian-Xiu, Yang Yong-Ji, Lau Joseph T Y, Hu Yi-Ping: Isolation and characterization of bipotent liver progenitor cells from adult mouse, <i>Stem Cells</i> 24, 322-32, 2006 (10248)
DMEM	Benten Daniel, Follenzi Antonia, Bhargava Kuldeep K, Kumaran Vinay, Palestro Christopher J, Gupta Sanjeev: Hepatic targeting of transplanted liver sinusoidal endothelial cells in intact mice, <i>Hepatology</i> 42, 140-8, 2005 (10259)
Leffert's buffer	Jiang Guoqiang, Li Zhihua, Liu Franklin, Ellsworth Kenneth, Dallas-Yang Qing, Wu Margaret, Ronan John, Esau Christine, Murphy Cain, Szalkowski Deborah, Bergeron Raynald, Doebber Thomas, Zhang Bei: Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearyl-CoA desaturase-1, <i>J Clin Invest</i> 115, 1030-8, 2005 (10266)
DMEM/ F-12	Sazani P., Gemignani F., Kang S., Maier M., Manoharan M., Persmark M., Bortner D., and Kole R.: Systemically Delivered Antisense Oligomers Up-regulate Gene Expression in Mouse Tissues, <i>Nat Biotechnol</i> 20, 1228, 2002 (9884)
HBSS	Lingohr Melissa K, Bull Richard J, Kato-Weinstein Junko, Thrall Brian D: Dichloroacetate stimulates glycogen accumulation in primary hepatocytes through an insulin-independent mechanism, <i>Toxicol Sci</i> 68, 508-15, 2002 (10264)
Williams E	Donato M, Viitala P, Rodriguez-Antona C, Lindfors A, Castell J, Raunio H, Gomez-Lechon M, Pelkonen O: CYP2A5/CYP2A6 expression in mouse and human hepatocytes treated with various in vivo inducers, <i>Drug Metab Dispos</i> 28, 1321-6, 2000 (10267)
HBSS	Angele MK, Knoferl MW, Schwacha MG, Ayala A, Cioffi WG, Bland KI, and Chaudry IH.: Sex steroids regulate pro- and anti-inflammatory cytokine release by macrophages after trauma-hemorrhage, <i>Am J Physiol</i> 277, C35, 1999 (9878)
Hanks	Ling W, Loughheed M, Suzuki H, Buchan A, Kodama T, Steinbrecher UP: Oxidized or acetylated low density lipoproteins are rapidly cleared by the liver in mice with disruption of the scavenger receptor class A type I/II gene, <i>J Clin Invest</i> 100, 244-52, 1997 (10258)
DMEM	Lillehaug, J., Mondal, S., and Heidelberger, C.: Establishment of Epithelial Cell Lines from Mouse Regenerating Liver, <i>In Vitro</i> 15, 910, 1979 (504)
Hank's w/ Insulin, CMF	Crisp, D., and Pogson, C.: Glycolytic and Gluconeogenic Enzyme Activities in Parenchymal and Non-parenchymal Cells from Mouse Liver, <i>Biochem J</i> 126, 1009, 1972 (309)
Williams E	Meng, F., Chen, Z., Han, M., Hu, X., He, X., Liu, Y., He, W., Huang, W., Guo, H. and Zhou, P.: Porcine Hepatocyte Isolation and Reversible Immortalization Mediated by Retroviral Transfer and Site-Specific Recombination., <i>World J Gastroenterol</i> 16, 1660, 2010 (10558)

Tissue Dissociation Guide

Species

Liver (con't)

Cell(s)

Enzyme(s)

	Porcine, Yorkshire, male, 21 day	Hepatocytes	Collagenase Type 1: 0.07%
	Porcine, Chinese mini pig, 6-10 kg	Hepatocytes	Collagenase Type 4: 125 u/ml
	Porcine, one week	Hepatocytes	Collagenase Type 4: 0.05%
	Porcine, male, 2-3 week	Hepatocytes	Collagenase: 0.05%
	Porcine, adult	Hepatocytes	Collagenase: 0.05% Neutral Protease:
	Porcine, male, 6-40 kg	Hepatocytes	Collagenase: 0.8%
Rabbit	Rabbit, New Zealand white	Hepatocytes	Collagenase: 90 u/ml
Rat	Rat, SD, 240-320g	Hepatocytes	HIS kit: per instructions
	Rat, Wistar, female	Hepatic stellate cells	Collagenase Type 1: 0.025-0.1% Pronase: 0.025-0.13%
	Rat, SD	Hepatic stellate cells	Pronase: 0.02% Collagenase: see reference
	Rat, SD, 250-300g	Hepatocytes	HIS kit: per instructions
	Rat, SD	Hepatocytes	Collagenase: 0.05%
	Rat, Lewis, male	Hepatocytes	Collagenase Type 2: 0.1%
	Rat, 200-300 g	Hepatocytes	Collagenase Type 1: 0.067%
	Rat, SD, male, 230-250g	Hepatocytes	Collagenase Type 2: 0.1%
	Rat, Lewis, 150-200g	Hepatocytes	Collagenase Type 2: 0.05%
	Rat, SD, 180-200g	Hepatocytes	Collagenase Type 1: 0.1%
	Rat, Wistar, 220-270g	Hepatocytes	Collagenase Type 1: 200 u/ml
	Rat, Fisher, E14	Hepatocytes	Collagenase Type 1: 0.22%
	Rat, Lewis, female, 150-200 g	Hepatocytes	Collagenase: see references
	Rat	Liver sinusodial endothelial	Collagenase: 0.03%
	Rat, Wistar, 200-300 g	Hepatocytes	Collagenase Type 2: 0.05%
	Rat, adult	Hepatocytes	Collagenase Type 1: 0.1% Pronase: 1% Deoxyribonuclease I: 0.007%

Tissue Dissociation Guide

Medium	Reference
Williams E	Terner, M., Gilmore, W.J., Lou, Y. and Squires, E.J.: The Role of CYP2A and CYP2E1 in the Metabolism of 3-Methylindole in Primary Cultured Porcine Hepatocytes., <i>Drug Metab Dispos</i> 34, 848, 2006 (10559)
Williams E	Li, J, Li, L., Chao, H., Yang, Q., Liu, X., Sheng, J., Yu, H. and Huang, J: Isolation and Short Term Cultivation of Swine Hepatocytes for Bioartificial Liver Support System., <i>Hepatobiliary Pancreat Dis Int Vol.</i> 4, , 249, 2005 (10504)
RPMI 1640	Wang Y., Liu H., Guo H., Wen H., and Liu J.: Primary Hepatocyte Culture in Collagen Gel Mixture and Collagen Sandwich, <i>World J Gastroenterol</i> 10, 699, 2004 (9885)
DMEM	Raman Priya, Donkin Shawn S, Spurlock Michael E: Regulation of hepatic glucose metabolism by leptin in pig and rat primary hepatocyte cultures, <i>Am J Physiol Regul Integr Comp Physiol</i> 286, R206-16, 2004 (10268)
MEM	Zhou X, Liu L, Kano J, Mukaiyama T, and Tokiwa T: Isolation and Cultivation of Porcine Hepatocytes for Extracorporeal Artificial Liver Support System, <i>Chin Med J</i> 114, 946, 2001 (9886)
PBS	Gerlach, J., Brombacher, J., Smith, M., Neuhaus, P.: High Yield Hepatocyte Isolation from Pig Livers for Investigation of Hybrid Liver Support Systems: Influence of Collagenase Concentration and Body Weight, <i>J Surg Res</i> 62 (1), 85, 1996 (1166)
CF EGTA perfusate	Reese, J. and Byard, J.: Isolation And Culture of Adult Hepatocytes from Liver Biopsies, <i>In Vitro</i> 17, 935, 1981 (412)
Waymouth's MB	Pillai, V., and Mehvar, R.: Inhibition of NADPH-Cytochrome P450 Reductase by Tannic Acid in Rat Liver Microsomes and Primary Hepatocytes: Methodological Artifacts and Application to Ischemia-Reperfusion Injury, <i>J Pharm Sci</i> 100, 3495, 2011 (10672)
DMEM	Zvibel, I., Atias, D., Phillips, A., Halpern, Z. and Oren, R.: Thyroid Hormones Induce Activation of Rat Hepatic Stellate Cells Through Increased Expression of p75 Neurotrophin Receptor and Direct Activation of Rho., <i>Lab Invest</i> 90, 674, 2010 (10544)
Medium 199	Handy, J., Saxena, N., Fu, P., Lin, S., Mells, J., Gupta, N. and Anania, F.: Adiponectin Activation of AMPK Disrupts Leptin-Mediated Hepatic Fibrosis via Suppressors of Cytokine Signaling (SOCS-3)., <i>J Cell Biochem</i> 110, 1195, 2010 (10622)
Krebs-Henseleit	Parasrampur, R. and Mehvar, R.: Dose-Dependent Inhibition of Transporter-Mediated Hepatic Uptake and Biliary Excretion of Methotrexate by Cyclosporine A in an Isolated Perfused Rat Liver Model., <i>J Pharm Sci</i> 99, 5060, 2010 (10674)
HBSS	Chung, C., Shugrue, C., Nagar, A., Doll, J., Cornwell, M., Gattu, A., Kolodecik, T., Pandol, S. and Gorelick, F.: Ethanol Exposure Depletes Hepatic Pigment Epithelium-Derived Factor, a Novel Lipid Regulator., <i>Gastroenterol</i> 136, 331, 2009 (10609)
DPBS	Bettinger, C., Kulig, K., Vacanti, J., Langer, R. and Borenstein, J.: Nanofabricated Collagen-Inspired Synthetic Elastomers for Primary Rat Hepatocyte Culture., <i>Tissue Eng Part A Vol.</i> 15, 1321, 2009 (10668)
HBSS	Mula, N., Cubero, F., Codesal, J., de Andres, S., Escudero, C., Garcia-Barrutia, S., Millan, I., Arahuetes, R. and Maganto, P.: Survival of Allogeneic Hepatocytes Transplanted into the Thymus., <i>Cells Tissues Organs</i> 188, 270, 2008 (10371)
HEPES	Doleh, L. and Romani, A.: Biphasic Effect of Extra-Reticular Mg ²⁺ on Hepatic G6P Transport and Hydrolysis., <i>Arch Biochem Biophys</i> 467, 283, 2007 (10351)
Williams E	Smith, M. and Mooney, D.: Hypoxia Leads to Necrotic Hepatocyte Death., <i>J Biomed Mater Res A Vol.</i> 80., 520, 2007 (10543)
(see reference)	Charbonneau, A., Unson, C. and Lavoie, J.: High-Fat Diet-Induced Hepatic Steatosis Reduces Glucagon Receptor Content in Rat Hepatocytes: Potential Interaction with Acute Exercise., <i>J Physiol</i> 579, 255, 2007 (10582)
DMEM	Annaert, P., Turncliff, R., Booth, C., Thakker, D. and Brouwer, K.: P-Glycoprotein-Mediated In Vitro Biliary Excretion in Sandwich-Cultured Rat Hepatocytes., <i>Drug Metab Dispos</i> 29, 1277-83, 2001 (10677)
HBSS	Isabel Zvibel, Miri Bronstein, Einav Hubel, Ella Bar-Lev, Zamir Halpern, Ran Oren: Isolation, characterization and culture of Thy1-positive cells from fetal rat livers, <i>World J Gastroenterol</i> 12, 3841-7, 2006 (10303)
DMEM	Sosef MN, Baust JM, Sugimachi K, Fowler A, Tompkins RG, and Toner M.: Cryopreservation of isolated primary rat hepatocytes: enhanced survival and long-term hepatospecific function, <i>Ann Surg</i> 241, 125, 2005 (10054)
DMEM	Benten Daniel, Follenzi Antonia, Bhargava Kuldeep K, Kumaran Vinay, Palestro Christopher J, Gupta Sanjeev: Hepatic targeting of transplanted liver sinusoidal endothelial cells in intact mice, <i>Hepatol</i> 42, 140-8, 2005 (10259)
Williams E	Putz G, Schmider W, Nitschke R, Kurz G, Blum HE: Synthesis of phospholipid-conjugated bile salts and interaction of bile salt-coated liposomes with cultured hepatocytes, <i>J Lipid Res</i> 46, 2325-38, 2005 (10263)
Williams E	Jensen C., Jauho E., Santoni-Rugiu E., Holmskov U., Teisner B., Tygstrup N., and Bisgaard H.: Trans-activating Ductular (oval) Cells and Their Hepatocytic Progeny are Characterized by a Novel and Distinctive Expression of Delta-like Protein/preadipocyte Factor 1/fetal Antigen 1, <i>Am J Pathol</i> 164(4), 1347, 2004 (9882)

Tissue Dissociation Guide

Species

Liver (con't)

Cell(s)

Enzyme(s)

Rat, Wistar	Hepatocytes	Collagenase Type 4: 0.05%
Rat, SD, male, 200-250 g	Hepatocytes	Collagenase: 0.05%
Rat, SD, male, 230-250 g	Hepatocytes	Collagenase Type 1: 0.1%
Rat, male, 3 week	Hepatocytes	Collagenase Type 4: 0.02%
Rat, SD, male, 450-500 g	Stellate	Protease: 0.02%
Rat, Wistar, male	Hepatocytes	Collagenase Type 2: 120 u/ml
Rat, Lewis, adult	Hepatocytes	Collagenase: 60 u/ml
Rat	Hepatocytes	Collagenase: 0.04% - 0.06%
Rat	Hepatocytes	Collagenase: 0.05%
Rat, SD, male, 200 g	Hepatocytes	Collagenase: 0.05%
Rat, Wistar, adult, male, 100-125 g	Hepatocytes	Collagenase Type 2: 0.05%
Rat, SD, male, 250-350 g	Hepatocytes	Collagenase Type 4: 200 u/ml
Rat, SD, 8 - 12 day	Parenchymal hepatocytes	Collagenase Type 4: 80 u/ml
Rat, SD, male, 250-350 g	Hepatocytes	Collagenase Type 2: 0.05%
Rat, Wistar, male, 200 g	Parenchymal	Collagenase Type 1:
Rat, SD, male, 175-225 g	Hepatocytes	Collagenase:
Rat, SD, neonatal, 8 - 10 days	Hepatocytes	Collagenase Type 4: 0.05%
Rat, Fischer 344, male, 14 months	Hepatocytes	Collagenase: 0.05%
Rat, SD, male, 200-300 g	Hepatocytes	Collagenase: 0.05%
Rat, Wistar, male, 3 month, 200 g	Parenchymal Endothelial Kupffer	Collagenase Type 1: 0.05%
Rat, Wistar, male, 200 - 300 g	Parenchymal Kupffer	Collagenase Type 2: 0.05%
Rat, Wistar, female, 17 days	Hepatocytes	Collagenase: 0.05%
Rat, CD strain, albino, male, 140 -180 g	Hepatocytes and Nonparenchymal	Pronase: 0.1%
Rat, 250-350 g (also guinea-pig & rabbit)	Hepatocytes	Collagenase Type 2: 166 u/ml
Rat, SD, male, 180-200 g	Hepatocytes	Collagenase: 0.05%

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Medium	Reference
RPMI 1640	Wang Y., Liu H., Guo H., Wen H., and Liu J.: Primary Hepatocyte Culture in Collagen Gel Mixture and Collagen Sandwich, <i>World J Gastroenterol</i> 10, 699, 2004 (9885)
DMEM	Raman Priya, Donkin Shawn S, Spurlock Michael E: Regulation of hepatic glucose metabolism by leptin in pig and rat primary hepatocyte cultures, <i>Am J Physiol Regul Integr Comp Physiol</i> 286, R206-16, 2004 (10268)
HBSS	Kuddus, R., Nalesnik, M., Subbotin, V., Rao, A., and Gandhi, C.: Enhanced Synthesis and Reduced Metabolism of Endothelin-1 (ET-1) by Hepatocytes - An Important Mechanism of Increased Endogenous Levels of ET-1 in Liver Cirrhosis, <i>J Hepatol</i> 33, 725, 2000 (1115)
Williams E	Low-Baselli A., Hufnagl K., Parzefall W., Schulte-Hermann R., and Grasl-Kraupp B.: Initiated Rat Hepatocytes in Primary Culture: A Novel Tool to Study Alterations in Growth Control During the First Stage of Carcinogenesis, <i>Carcinogenesis</i> 21, 79, 2000 (10004)
HBSS	Gabriel, A., Kuddus, R., Rao, A., and Gandhi, C.: Down-Regulation of Endothelin Receptors by Transforming Growth Factor B1 in Hepatic Stellate Cells, <i>J Hepatol</i> 30, 440, 1999 (1101)
HBSS	Wolz E, Liechti H, Notter B, Oesterhelt G, Kistler A: Characterization of metabolites of astaxanthin in primary cultures of rat hepatocytes, <i>Drug Metab Dispos</i> 27, 456-62, 1999 (10265)
HEPES	Matsuura T, Gad MZ, Harrison EH, and Ross AC: Lecithin:retinol acyltransferase and retinyl ester hydrolase activities are differentially regulated by retinoids and have distinct distributions between hepatocyte and nonparenchymal cell fractions of rat liver, <i>J Nutr</i> 127, 218, 1997 (10057)
PBS	Alston-Smith, J and Pertoft, H: Isolation of Liver Cells: a System for Obtaining Pure Cells in Monolayer Cultures from a Single Rat Liver, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12B:14.1, 1995 (1275)
DMEM	Matsumoto, K and Nakamura, T: Techniques for the Isolation and Cultivation of Hepatocytes using Collagenase, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12B:16.1, 1995 (1276)
Medium 199	Rana, B., Mischoulon, D., Xie, Y., Bucher, N., and Farmer, S.: Cell-Extracellular Matrix Interactions Can Regulate the Switch between Growth and Differentiation in Rat Hepatocytes: Reciprocal Expression of C/EBP α and Immediate-Early Growth Response Transcription Factors, <i>Mol Cell Biol</i> 14 (9), 5858, 1994 (1210)
Serum-free medium	Liu, J., McKim, J., Liu, Y., and Klaassen, C.: Effects of Butyrate Homologes on Metallothionein Induction in Rat Primary Hepatocyte Cultures, <i>In Vitro Cell Dev Biol</i> 28, 320, 1992 (483)
RPMI 1640	Dixit, V., Arthur, M., and Gitnick, G.: Repeated Transplantation of Microencapsulated Hepatocytes for Sustained Correction of Hyperbilirubinemia in Gunn Rats, <i>Cell Transplant</i> 1, 275, 1992 (681)
HBSS	Davila, J., Reddy, C., Davis, P. and Acosta, D.: Toxicity Assessment of Papaverine Hydrochloride and Papaverine-Derived Metabolites in Primary Cultures of Rat Hepatocytes, <i>In Vitro Cell Dev Biol</i> 26, 515, 1990 (444)
HBSS, CMF	Liu, J., Kershaw, W., and Klaassen, C.: Rat Primary Hepatocyte Cultures are a Good Model for Examining Metallothionein-Induced Tolerance to Cadmium Toxicity, <i>In Vitro Cell Dev Biol</i> 26, 75, 1990 (450)
HEPES, modified	Kindberg, G., Gudmundsen, O., and Berg, T.: The Effect of Vandate on Receptor-mediated Endocytosis of Asialoorosomuroid in Rat Liver Parenchymal Cells, <i>J Biol Chem</i> 265, 8999, 1990 (572)
Kreb's Ringer bicarbonate buffer	Reddy, S., Amick, G., Cooper, R., and Damun, Z.: Insulin Stimulates the Activity of a Protamine in Isolated Rat Hepatocytes, <i>J Biol Chem</i> 265, 7748, 1990 (570)
Hanks' BSS, CF	Davila, J., Lenherr, A., and Acosta, D.: Protective Effect of Flavonoids on Drug-Induced Hepatotoxicity <i>In Vitro</i> , <i>Toxicology</i> 57, 267, 1989 (673)
Ringer's biocarbonate buffer	Nagy, I., Ohno-Iwashita, Y., Ohta, M., Nagy, V., Kitani, K., Ando, S., and Imahori, K.: Effect of Perfringolysin O on the Lateral Diffusion Constant of Membrane Proteins of Hepatocytes as Revealed by Fluorescence Recovery After Photobleaching, <i>Biochim Biophys Acta</i> 939, 551, 1988 (327)
HEPES	Voss, A. and Sprecher, H.: Metabolism of 6,9,12-Octadecatetraenoic and 6,9,12,15-Octadecatetraenoic Acid, <i>Biochim Biophys Acta</i> 958, 153, 1988 (328)
Krebs Henseleit	Kuiper, J., Zijlstra, F., Kamps, J, and vanBerkel, T.: Identification of Prostaglandin D2 as the Major Eicosanoid from Liver Endothelial and Kupffer Cells, <i>Biochim Biophys Acta</i> 959, 143, 1988 (329)
HBSS with CaCl ₂	Cai, H., He, Z., and Ding, Y.: Effects of Monocyte Macrophages Stimulation on Hepatic Lipoprotein Receptors, <i>Biochim Biophys Acta</i> 958, 334, 1988 (331)
HBSS	Cotariu, D., Barr-Nea, L., Papo, N., and Zaidman, J.: Induction of gamma-Glutamyl Transferase by Dexamethasone in Cultured Rat Hepatocytes, <i>Enzyme</i> 40, 212, 1988 (386)
HEPES buffer with calcium	Braun, L., Mead, J., Panzica, M., Mikumo, R., Bell, G., and Fausto, N.: Transforming Growth Factor B mRNA Increases During Liver Regeneration: A Possible Paracrine Mechanism Of Growth Regulation, <i>Proc Natl Acad Sci U S A</i> 85, 1539, 1988 (656)
DMEM	Chang, T., and Chang, C.: Hepatic Uptake of Asialoglycoprotein is Different Among Mammalian Species Due to Different Receptor Distribution, <i>Biochim Biophys Acta</i> 942, 57, 1988 (832)
MEM	Gupta, C., Hattori, A., Betschart, J., Virji, M., and Shinozuka, H.: Modulation of Epidermal Growth Factor Receptors in Rat Hepatocytes by Two Liver-Promoting Regimens, a Choline-Deficient and a Phenobarbital Diet, <i>Cancer Res</i> 48, 1162, 1988 (833)

Tissue Dissociation Guide

Rat, Fisher 344, 150-200 g	Bile ductular epithelial	Collagenase Type 1: 220 u/ml
Rat, Wistar, male, 260-310 g	Hepatocytes	Collagenase Type 1: 0.05%
Rat, SD, male, 350-400 g	Hepatocytes	Collagenase Type 2:
Rat, SD, male, 350 - 450 g	Lipocytes Kupffer Sinusoidal endothelial	Collagenase: 0.015%
Rat, SD, male	Hepatocytes	Collagenase: 100 - 200 µg/g body weight
Rat, Fischer, male, 150-250 g	Hepatocytes	Collagenase Type 1: 125 - 250 u/ml
Rat, SD, female, 100-180 g	Hepatocytes	Collagenase: 0.5%
Rat, Lewis, female, 200-250 g	Hepatocytes, Kupffer, endothelial	Collagenase: 0.05%
Rat, SD, male, 270 - 320 g	Hepatocytes	Collagenase: 0.04%
Rat, Wistar, adult, male, 150-200 g	Hepatocytes	Trypsin: 0.005%
Rat, Fischer, Lewis and SD, male 10-18 months	Epithelial	Trypsin: 0.05%
Rat, Wistar, male, 3 month old	Endothelial Kupffer Parenchymal	Collagenase: 0.05%
Rat, SD, male/female, 250-300 g	Hepatocytes	Collagenase: 100 u/ml
Rat, Wistar, 12 day	Epithelial	Trypsin: 0.05%
Rat, Zucker, obese	Hepatocytes	Collagenase Type 2: 0.30%
Rat, SD	Hepatocytes	Collagenase: 90 u/ml
Rat, Wistar, female, fetus	Hepatocytes	Collagenase: 0.025%
Rat, SD, male, 250-300 g	Parenchymal	Collagenase Type 2: 0.05%
Rat, Wistar, male, 180-210 g	Hepatocytes	Hyaluronidase: 0.02%
Rat, SD, 7-10 day	Liver	Collagenase: 0.05%
Rat, Wistar, male, 200-250 g	Hepatocytes	Hyaluronidase: 460 u/ml
Rat, SD, female, 100-150 g	Hepatocytes	Collagenase Type 1: 0.065%
Rat (also chicken)	Hepatocytes	Collagenase Type 3 & 4:
Rat, SD, male, 200 g	Hepatocytes	Collagenase Type 1: 100 u/ml
Rat (WAG/RIJ), female, 24, 30, and 37 months	Parenchymal	Collagenase Type 1: 0.05% - 0.06%
Rat, Fisher, adult, male	Hepatocytes	Collagenase Type 1: 100 u/ml

Tissue Dissociation Guide

Medium	Reference
Leibovitz L-15	Mathis, G., Walls, S., and Sirica, A.: Biochemical Characteristics of Hyperplastic Rat Bile Ductular Epithelial Cells Cultured "on Top" and "Inside" Different Extracellular Matrix Substitutes, <i>Cancer Res</i> 48, 6145, 1988 (922)
HEPES	McAbee DD and Weigel PH: ATP-dependent inactivation and reactivation of constitutively recycling galactosyl receptors in isolated rat hepatocytes, <i>Biochem</i> 27, 2061, 1988 (1165)
HBSS	Rodriguez de Turco, E., and Spitzer, J.: Kinetics of Diacylglycerol Accumulation in Response to Vasopressin Stimulation in Hepatocytes of Continuously Endotoxaemic Rats, <i>Biochem J</i> 253, 73, 1988 (1216)
DMEM/Ham's F-12	Friedman, S. and Roll, F.: Isolation and Culture of Hepatic Lipocytes, Kupffer Cells, and Sinusoidal Endothelial Cells by Density Gradient Centrifugation with Stractan, <i>Anal Biochem</i> 161, 207, 1987 (301)
Eagle's Eagle's w/HEPES HBSS	Oka, J. and Weigel, P.: Monensin Inhibits Ligand Dissociation Only Transiently and Partially and distinguishes two galactosyl receptor pathways in isolated rat hepatocytes, <i>J Cell Physiol</i> 133, 243, 1987 (595)
MEM (see reference)	Francavilla, A., Ove, P., Polimeno, L., Sciascia, C., Coetzee, M., and Starzi, T.: Epidermal Growth Factor and Proliferation in Rat Hepatocytes in Primary Culture Isolation at Different Times after Partial Hepatectomy, <i>Cancer Res</i> 46, 1318, 1986 (824)
Krebs Ringer bicarbonate buffer	Schwarz, K., Lanier, S., Carter, E., Homcy, C., and Graham, R.: Rapid Reciprocal Changes in Adrenergic Receptors in Intact Isolated Hepatocytes During Primary Cell Culture, <i>Mol Pharmacol</i> 27, 200, 1985 (639)
Gey's BSS	Holstege A, Leser HG, Pausch J, Gerok W: Uridine catabolism in Kupffer cells, endothelial cells, and hepatocytes, <i>Eur J Biochem</i> 149, 169-73, 1985 (10260)
Bicarbonate buffer with calcium added	Brass, E., Garrity, M., and Robertson, R.: Inhibition of Glucagon-Stimulated Hepatic Glycogenolysis by E-Series Prostaglandins, <i>FEBS Lett</i> 169, 293, 1984 (410)
Williams E	Okumura, T. and Saito, K.: Degradation of Prostaglandin E2 in a Primary Culture of Adult Rat Hepatocytes, <i>J Biochem (Tokyo)</i> 96, 429, 1984 (701)
HBSS CMF	Herring, A., Raychaudhuri, R., Kelley, S., and Iybe, P.: Repeated Establishment of Diploid Epithelial Cell Cultures from Normal and Partially Hepatectomized Rats, <i>In Vitro</i> 19, 576, 1983 (528)
HBSS	Nagelkenke, J., Barto, K., and Berkel, T.: In Vivo and in Vitro Uptake and Degradation of Acetylated Low Density Lipoprotein by Rat Liver Endothelial, Kupffer, and Parenchymal Cells, <i>J Biol Chem</i> 258 (20), 12221, 1983 (940)
Krebs Henseleit bicarbonate buffer	Studer, R. and Borle, A.: Differences between Male and Female Rats in the Regulation of Hepatic Glycogenolysis. The Relative Role of Calcium and cAMP in Phosphorylase Activation by Catecholamines, <i>J Biol Chem</i> 257, 7987, 1982 (556)
HBSS, CMF	Malan-Shibley, L., and Iybe, P.: Influence of Cultures on Cell Morphology/Tyrosine Aminotransferase Levels, <i>Exp Cell Res</i> 131, 363, 1981 (391)
Dulbecco-Vogt arginine free Eagle's	Goldstein, A., Palmer, J., and Johnson, P.: Primary Cultures of Fetal Hepatocytes from the Genetically Obese Zucker Rat: Protein Synthesis, <i>In Vitro</i> 17, 651, 1981 (515)
CF EGTA perfusate	Reese, J. and Byard, J.: Isolation And Culture of Adult Hepatocytes from Liver Biopsies, <i>In Vitro</i> 17, 935, 1981 (412)
HEPES buffer	Gugen-Guillouzo, C., Tichonicky, L., Szajnert, M., and Kruh, J.: Changes in Some Chromatin and Cytoplasmic Enzymes of Perinatal Rat Hepatocytes, <i>In Vitro</i> 16, 1, 1980 (505)
Kreb's Henseleit bicarbonate buffer	Yamada, S., Otto, P., Kennedy, D., and Whayne, T.: The Effects of Dexamethasone on Metabolic Activity of Hepatocytes in Primary Monolayer Culture, <i>In Vitro</i> 16, 559, 1980 (508)
Kreb's buffer	De Gerlache, J., Lans, M., Taper, H., and Roberfroid, M.: Separate Isolation of Cells from Nodules and Surrounding Parenchyma of the Same Precancerous Rat Liver: Biochemical and Cytochemical Characterization, <i>Toxicology</i> 18, 225, 1980 (843)
HBSS modified (see reference)	Acosta, D., Anuforo, D., and Smith, R.: , <i>J Tiss Cul Meth</i> 6, 35, 1980 (1268)
Saline	Poli, G., Gravela, E., Albano, E., and Dianzani, M.: Studies on Fatty Liver with Isolated Hepatocytes: II., The Action of Carbon Tetrachloride on Lipid Peroxidation, Protein and Triglyceride Synthesis and Secretion, <i>Exp Mol Pathol</i> 30, 116, 1979 (408)
DMEM (see reference)	Davis, R., Engelhorn, S., Pangburn, S., Weinstein, D., and Steinberg, D.: Very Low Density Lipoprotein Synthesis and Secretion by Cultured Rat Hepatocytes, <i>J Biol Chem</i> 254 (6), 2010, 1979 (820)
HEPES	Weigel, P., Schnaar, R., Kuhlenschmidt, M., Schmall, E., Lee, R., Lee, Y., and Roseman, S.: Adhesion of Hepatocytes to Immobilized Sugars, <i>J Biol Chem</i> 254 (21), 10830, 1979 (1032)
Buffers 1 & 2 (see reference)	Rubin, K., Kjellen, L., and Oslashbrink, B.: Intercellular Adhesion between Juvenile Liver Cells.. A Method to Measure the Formation of Stable Lateral Contacts Between Cells Attached to a Collagen Gel, <i>Exp Cell Res</i> 109, 413, 1977 (387)
HEPES buffer	VanBezodijen, C., Grell, T., and Knook, D.: Effect of Age on Protein Synthesis by Isolated Liver Parenchymal Cells, <i>Mech Ageing Dev</i> 6, 293, 1977 (630)
HBSS (see reference)	Williams, G., Bermudez, E., and Scaramuzzino, D.: Rat Hepatocytes Primary Cell Cultures III. Improved Dissociation and Attachment Techniques and the Enhancement of Survival by Culture Medium, <i>In Vitro</i> 13 (12), 809, 1977 (826)

Tissue Dissociation Guide

Species

Liver (con't)

Cell(s)

Enzyme(s)

Rat , Wistar, male, 250-300 g	Hepatocytes	Collagenase: 0.05%
Rat, Fischer, male, adult, 170-265 g	Hepatocytes	Collagenase: 0.05%-0.10%
Rat, SD, male, 200-250 g	Hepatocytes	Collagenase Type 1: 0.05%
Rat, neonate, 3 day	Hepatocytes	Trypsin: 0.25%
Rat, SD, albino, male	Parenchymal	Hyaluronidase: 0.1%
Rat, embryos, 1-3 days	Hepatocytes	Hyaluronidase: 0.10%
Rat, SD, adult, male, 200-250 g	Parenchymal	Hyaluronidase: 0.10%
Rat, SD, adult, male, 180-300 g	Parenchymal	Collagenase Type 1: 0.05%
Rat, Wistar, 200-250 g	Hepatocytes	Hyaluronidase: 0.10%
Rat, SD, female, 130-160 g	Hepatocytes	Hyaluronidase: 0.08%
Rat, Fisher 344, pregnant (19-21 days gestation), 200-250 g	Hepatocytes	Collagenase: 0.3%
Rat, Wistar, male, 260-310 g	Hepatocytes	Collagenase: 0.01 - 0.08%
Rat, Fisher, 10 day	Epithelial-like	Trypsin: 0.25%
Rat, Wistar, male, 6-8 weeks, 80-160 g	Hepatocytes	Hyaluronidase: 1.0%
Rat, 100-200 g	Hepatocytes	Hyaluronidase: 0.10%
Rat, SD, adult, 200-300 g	Parenchymal	Hyaluronidase: 0.10% Collagenase Type 1: 0.05%

Lung

Species

Cell(s)

Enzyme(s)

Bovine	Bovine	Pulmonary microvessel endothelial	Collagenase Type 2: 1000 u/ml
Guinea-Pig	Guinea-pig, male, 250-300 g	Alveolar type II	Elastase: 40 u/ml
	Guinea-pig, male, 100 g	Single cells	Trypsin: 0.25%
Human	Human	Lung Fibroblasts	Trypsin: 0.05%
	Human, 16-24 week therapeutic abortions	Alveolar type II Fetal	Trypsin: 50 ug/ml
	Human	Epithelial	Trypsin: 0.02%
	Human, 12-16 wk old embryo	Fibroblasts	Trypsin: 0.01%
Mouse	Human fetuses, 80 day (also swine fetuses, 70 day, adult Amer Dutch, 250 day)	Lung	Collagenase: 0.01%
	Mouse	Lung draining	Collagenase Type 4: 0.1-0.125%
	Mouse, female, 6-12 week	Lung mononuclear	Collagenase Type 4: 500 u/ml Deoxyribonuclease I: 0.002%

Tissue Dissociation Guide

Medium	Reference
Hank's solution, CF	Gravela, E., Poli, G., Albano, E., and Dianzani, M.: Studies of Fatty Liver with Isolated Hepatocytes, <i>Exp Mol Pathol</i> 27, 339, 1977 (1209)
Williams E	Laishes, B., and Williams, G.: Conditions Affecting Primary Cell Cultures of Functional Adult Rat Hepatocytes. I.. The Effect of Insulin, <i>In Vitro</i> 12, 521, 1976 (496)
MEM	Witters, L., Alberico, L., and Acruch, J.: Insulin Regulation of Glycogen Synthase in the Isolated Rat Hepatocyte, <i>Biochem Biophys Res Commun</i> 69 (4), 997, 1976 (821)
PBS, CMF	Bausher, J., and Schaeffer, W.: A Diploid Rat Liver Cell Culture. 1. Characterization and Sensitivity to Aflatoxin B1, <i>In Vitro</i> 9, 286, 1974 (540)
Hank's solution, CMF	Bonney, R., Becker, J., Walker, P., and Potter, V.: Primary Monolayer Cultures of Adult Rat Liver Parenchymal Cells Suitable for Study of the Regulation of Enzyme Synthesis, <i>In Vitro</i> 9, 399, 1974 (541)
HBSS	Gerschenson, L., Berliner, J., and Davidson, M.: The Isolation and Culture of Liver Cells, <i>Vol. 32</i> , 733, 1974 (635)
HBSS, CF	Howard, R., Lee, J., and Pesch, L.: The fine structure, potassium content, and respiratory activity of isolated rat liver parenchymal cells prepared by improved enzymatic techniques, <i>J Cell Biol</i> 57, 642, 1973 (586)
Hank's solution, CF	Bissell, D., Hammaker, L., and Meyer, U.: Parenchymal Cells from Adult Rat Liver in Nonproliferating Monolayer Culture. I. Functional Studies, <i>J Cell Biol</i> 59, 722, 1973 (588)
HBSS, CF	Berg, T., Boman, D., and Seglen, P.O.: Induction of Tryptophan Oxygenase in Primary Rat Liver Cell Suspensions by Glucocorticoid Hormone, <i>Exp Cell Res</i> 72, 571, 1972 (404)
HBSS, CF	Johnson, M., Das, N., Butcher, F., and Fain, J.: The Regulation of Gluconeogenesis in Isolated Rat Liver Cells by Glucagon, Insulin, Dibutylryl Cyclic Adenosine Monophosphate, and Fatty Acids, <i>J Biol Chem</i> 247, 3229, 1972 (550)
Modified Eagle's w/ Serum	Leffert, H., and Paul, D.: Studies on Primary Cultures of Differentiated Fetal Liver Cells, <i>J Cell Biol</i> 52, 559, 1972 (585)
HEPES	Seglen, P.: Preparation of Rat Liver Cells, <i>Exp Cell Res</i> 74, 450, 1972 (840)
PBS	Williams, G., Weisburger, E., and Weisburger, J.: Isolation and Long-Term Cell Culture of Epithelial-Like Cells from Rat Liver, <i>Exp Cell Res</i> 69, 106, 1971 (402)
HBSS, CMF	Iype, P.: Cultures from Adult Rat Liver Cells. 1. Establishment of Monolayer Cell-Cultures from Normal Liver, <i>J Cell Physiol</i> 78, 281, 1971 (596)
HBSS, CF	Haug, Y., and Ebner, K.: Induction of Tyrosine Aminotransferase in Isolated Liver Cells, <i>Biochim Biophys Acta</i> 191, 161, 1969 (318)
HBSS, CF	Berry, M., and Friend, D.: High Yield Preparation of Isolated Rat Liver Parenchymal Cells, <i>J Cell Biol</i> 43, 506, 1969 (583)

Lung

Medium	Reference
PBS	Del Vecchio, P., Siflinger-Birnboim, A., Belloni, P., Holleran, L., Lum, H., and Malik, A.: Culture and Characterization of Pulmonary Microvascular Endothelial Cells, <i>In Vitro Cell Dev Biol</i> 28A, 711, 1992 (947)
PBS (see reference)	Sikpi, M., Nair, C., Johns, A., and Das, S.: Metabolic and Ultrastructural Characterization of Guinea-Pig Alveolar Type II Cells Isolated by Centrifugal Elutriation, <i>Biochim Biophys Acta</i> 877, 20, 1986 (324)
CF salt solution	Phillips, H.: Dissociation of Single Cells from Lung or Kidney Tissue with Elastase, <i>In Vitro</i> 8, 101, 1972 (538)
DMEM	Zhu, Y., Skold, C., Liu, X., Wang, H., Kohyama, T., Wen, F., Ertl, R., and Rennard, S.: Fibroblasts and Monocyte Macrophages Contract and Degrade Three-Dimensional Collagen Gels in Extended Co-Culture, <i>Respir Res</i> 2 (5), 295, 2001 (1132)
Ham's F-12, Eagle's MEM	Liley, H., Ertsey, R., Gonzales, L., Odom, M., Hawgood, S., Dobbs, L., and Ballard, P.: Synthesis of Surfactant Components by Cultured Type II Cells From Human Lung, <i>Biochim Biophys Acta</i> 961, 86, 1988 (877)
Medium 199	Lechner, J., Haugen, A., McClendon, I., and Pettis, E.: Clonal Growth of Normal Adult Human Bronchial Epithelial Cells in a Serum-Free Medium, <i>In Vitro</i> 18 (7), 633, 1982 (919)
Eagle's MEM	Kan, M., and Yamane, I.: In Vitro Proliferation and Lifespan of Human Diploid Fibroblasts in Serum-Free BSA-Containing Medium, <i>J Cell Physiol</i> 111, 155, 1982 (975)
HBSS	Hinz, R., and Syverton, J.: Mammalian Cell Cultures for Study of Influenza Virus. I. Preparation of Monolayer Cultures with Collagenase, <i>Proc Soc Exp Biol Med</i> 101, 19, 1959 (662)
HBSS	Rayamajhi, M., Redente, E., Condon, T., Gonzalez-Juarrero, M., Riches, D. and Lenz, L.: Non-Surgical Intratracheal Instillation of Mice with Analysis of Lungs and Lung Draining Lymph Nodes by Flow Cytometry., <i>J Vis Exp</i> 51, 2702, 2011 (10661)
HBSS	Breslow, R., Rao, J., Xing, W., Hong, D., Barrett, N. and Katz, H.: Inhibition of Th2 Adaptive Immune Responses and Pulmonary Inflammation by Leukocyte Ig-Like Receptor B4 on Dendritic Cells., <i>J Immunol</i> 184, 1003, 2010 (10606)

Tissue Dissociation Guide

Species

Lung (con't)

Cell(s)

Enzyme(s)

	Mouse	Lung	Collagenase Type 2: 300 u/ml Deoxyribonuclease I: 0.015%
	Mouse, embryo, day 18	Alveolar and fibroblast	Trypsin: 2.5% Deoxyribonuclease I: 0.2% Collagenase Type 1: 1250 u/ml
	Mouse	Lung	Collagenase Type 4: 0.1% Deoxyribonuclease I: 0.01%
	Mouse	Dendritic	Collagenase Type 1: 0.5%
	Mouse, 18-20 g	Lung	Collagenase Type 3: 0.17%
	Mouse	Lung	Collagenase Type 2: 300 u/ml Deoxyribonuclease I: 0.001%
	Mouse, 8-12 week	Mononuclear cells	Collagenase Type 1: 300 u/ml Deoxyribonuclease I: 50 u/ml
	Mouse, 6-10 week	Murine pulmonary endothelial	Collagenase Type 2: 0.25%
	Mouse	Pulmonary T lymphocytes	Collagenase Type 3: 10 mg/lung Deoxyribonuclease I: 250 ug/lung
	Mouse	Alveolar epithelial cells	Neutral Protease: Deoxyribonuclease I: 0.01%
	Mouse, 6-8 week	Lung and lymph node cells	Collagenase Type 2: 0.1% Deoxyribonuclease I: 0.002%
	Mouse	Lung	Collagenase: 100 u/ml Deoxyribonuclease I: 200 u/ml
	Mouse, 6-8 wk	Lung cells	Collagenase Type 3: 150 u/ml
	Mouse, female, 18-20 g	Murine endothelial cells	Collagenase Type 1: 1 mg/ml
	Mouse, female	Antigen presenting cells	Collagenase: 150 u/ml Deoxyribonuclease I: 30 u/ml
Porcine	Swine fetuses (70 day), adult Amer Dutch 250 day (also human fetuses, 80 day)	Lung	Collagenase: 0.01%
Rabbit	Rabbit, New Zealand, white	Alveolar type II	Trypsin: 0.05%
	Rabbit, New Zealand white, adult, male	Alveolar type II	Trypsin: 0.0025%
	Rabbit, New Zealand, male, 2-3 kg	Clara cells	Protease: 0.1%
	Rabbit, New Zealand, white, male, 1.7 kg	Lung	Pronase: 0.2%
Rat	Rat, SD, 125-150 g	Alveolar type I & II	Elastase: 2.5-8 u/ml Collagenase Type 1: 1.0%
	Rat, SD, 250-300g	Alveolar epithelial	Elastase: 3-4.5 u/ml

Tissue Dissociation Guide

Medium	Reference
RPMI 1640	Ferreira, C., Antunes, F., Leonard, V., Welstead, G., Richardson, C. and Cattaneo, R.: Measles Virus Infection of Alveolar Macrophages and Dendritic Cells Precedes Spread to Lymphatic Organs in Transgenic Mice Expressing Human Signaling Lymphocytic Activation Molecule (SLAM, CD150), <i>J Virol</i> 84, 3033, 2010 (10646)
MEM	Trotter, A., Kipp, M., Schrader, R. and Beyer, C.: Combined Application of 17Beta-Estradiol and Progesterone Enhance Vascular Endothelial Growth Factor and Surfactant Protein Expression in Cultured Embryonic Lung Cells of Mice., <i>Int J Pediatr Vol.</i> 2009, 170491, 2009 (10584)
HBSS	Zhao, J., Zhao, J., Van Rooijen, N. and Perlman, S.: Evasion by Stealth: Inefficient Immune Activation Underlies Poor T Cell Response and Severe Disease in SARS-CoV-Infected Mice., <i>PLoS Pathog</i> 5, e1000636, 2009 (10647)
HBSS	Flano, E., Jewell, N., Durbin, R. and Durbin, J.: Methods Used to Study Respiratory Virus Infection., <i>Curr Protoc Cell Biol Vol. Chapter 26,, , Unit 26.3</i> , 2009 (10648)
HBSS	Ebeling, C., Lam, T., Gordon, J., Hollenberg, M. and Vliagoftis, H.: Proteinase-Activated Receptor-2 Promotes Allergic Sensitization to an Inhaled Antigen through a TNF-Mediated Pathway., <i>J Immunol</i> 179, 2910, 2007 (10492)
Dulbecco's PBS	Finotto, S., Eigenbrod, T., Karwot, R., Boross, I., Doganci, A., Ito, H., Nishimoto, N., Yoshizaki, K., Kishimoto, T., Rose-John, S., Galle, P. and Neurath, M.: Local Blockade of IL-6R Signaling Induces Lung CD4+ T Cell Apoptosis in a Murine Model of Asthma Via Regulatory T Cells., <i>Int Immunol</i> 19, 685, 2007 (10605)
RPMI 1640 medium	Woolard MD, Hodge LM, Jones HP, Schoeb TR, and Simecka JW: The upper and lower respiratory tracts differ in their requirement of IFN-gamma and IL-4 in controlling respiratory mycoplasma infection and disease, <i>J Immunol</i> 172, 6875, 2004 (10014)
HBSS	Andonegui G., Bonder C., Green F., Mullaly S., Zbytniuk L., Raharjo E., and Kubes P.: Endothelium-derived Toll-like Receptor-4 is the Key Molecule in LPS-induced Neutrophil Sequestration into Lungs, <i>J Clin Invest</i> 111, 1011, 2003 (10005)
RPMI medium	Huaux F, Liu T, McGarry B, Ullenbruch M, and Phan SH.: Dual roles of IL-4 in lung injury and fibrosis, <i>J Immunol</i> 170, 2083, 2003 (10009)
DMEM	Paine R 3rd, Wilcoxon SE, Morris SB, Sartori C, Baleeiro CE, Matthay MA, and Christensen PJ: Transgenic overexpression of granulocyte macrophage-colony stimulating factor in the lung prevents hyperoxic lung injury, <i>Am J Pathol</i> 163, 2397, 2003 (10011)
RPMI 1640	Vermaelen KY, Carro-Muino I, Lambrecht BN, and Pauwels RA.: Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes, <i>J Exp Med</i> 193, 51, 2001 (10015)
Krebs-Henseleit Buffer	Freedman SD, Katz MH, Parker EM, Laposata M, Urman MY, and Alvarez JG.: A membrane lipid imbalance plays a role in the phenotypic expression of cystic fibrosis in <i>cftr(-/-)</i> mice, <i>Proc Natl Acad Sci U S A</i> 96, 13995, 1999 (10017)
HBSS	Stampfli MR, Wiley RE, Neigh GS, Gajewska BU, Lei XF, Snider DP, Xing Z, Jordana M: GM-CSF transgene expression in the airway allows aerosolized ovalbumin to induce allergic sensitization in mice, <i>J Clin Invest</i> 102, 1704-14, 1998 (10214)
DMEM	Dong QG, Bernasconi S, Lostaglio S, De Calmanovici RW, Martin-Padura I, Breviario F, Garlanda C, Ramponi S, Mantovani A, and Vecchi A.: A general strategy for isolation of endothelial cells from murine tissues. Characterization of two endothelial cell lines from the murine lung and subcutaneous sponge implants, <i>Arterioscler Thromb Vasc Biol</i> 17, 1599, 1997 (10018)
RPMI 1640	Hamilton-Easton A, and Eichelberger M: Virus-specific antigen presentation by different subsets of cells from lung and mediastinal lymph node tissues of influenza virus-infected mice, <i>J Virol</i> 69, 6359, 1995 (10016)
HBSS	Hinz, R., and Syverton, J.: Mammalian Cell Cultures for Study of Influenza Virus. I. Preparation of Monolayer Cultures with Collagenase, <i>Proc Soc Exp Biol Med</i> 101, 19, 1959 (662)
HBSS	Scott, J.: The Role of Sera, Growth Factors, and Hormones in the <i>In Vitro</i> Production of Disaturated Phosphatidylcholine and Propagation of Undifferentiated Type II Alveolar Cells from the Fetal Rabbit Lung, <i>Exp Lung Res</i> 12, 181, 1987 (879)
Joklik's MEM	Finkelstein, J., Maniscalco, W., and Shapiro, D.: Properties of Freshly Isolated Type II Alveolar Epithelial Cells, <i>Biochim Biophys Acta</i> 762, 398, 1983 (323)
HEPES	Devereux, T., and Fouts, J.: Isolation and Identification of Clara Cells From Rabbit Lung, <i>In Vitro</i> 16 (11), 958, 1980 (878)
Kreb's serum substitute solution, CMF	Gould, M., Clements, J., Jones, A., and Felts, J.: Dispersal of Rabbit Lung into Individual Viable Cells: A New Model for the Study of Lung Metabolism, <i>Science</i> 178, 1209, 1972 (665)
DMEM/F12	Lieber, J., Borok, Z., Li, X., Zhou, B., Sandoval, A., Kim, K. and Crandall, E.: Alveolar Epithelial Type I Cells Express Beta 2-Adrenergic Receptors and G-protein Receptor Kinase 2., <i>J Histochem Cytochem</i> 52, 759, 2004 (10495)
RPMI 1640	Chen J., Chen Z., Narasaraju T., Jin N., and Liu L.: Isolation of Highly Pure Alveolar Epithelial Type I and Type II Cells from Rat Lungs, <i>Lab Invest</i> 84, 727, 2004 (10006)

Tissue Dissociation Guide

Species

Lung (con't)

Cell(s)

Enzyme(s)

Rat, SD, 300-400 g	Pulmonary endothelial cells	Collagenase Type 2: 1000 u/ml
Rat, SD, 200-225 g	Type II alveolar epithelial cells	Elastase: 4.2 u/ml Deoxyribonuclease I: 0.0001%
Rat, SD, male, adult	Alveolar	Elastase: 2.0 - 2.5 u/ml
Rat, Lewis, male, 200-250g	Interstitial lung macrophages	Collagenase Type 1: 100 u/ml Deoxyribonuclease I: 50 u/ml
Rat, male	Alveolar epithelial	Elastase: 40 u/ml
Rat, male, 200-250g	Pulmonary arterial myocytes	Collagenase: 0.15% Papain: 0.15% Elastase: 0.05%
Rat, embryonic day 15	Fetal alveolar epithelial type II	Trypsin: 0.1%
Rat, SD, male, 125-175 g	Alveolar epithelial	Elastase: 2 u/ml
Rat, Wistar	Epithelial	Trypsin: 0.1%
Rat, SD, 8 day	Interstitial	Trypsin: 1.125%
Rat, Wistar, female, virgin	Alveolar epithelial type II	Trypsin: 1%
Rat, Fischer 344, male	Alveolar type II pneumocytes	Elastase: 40 u/ml
Rat, SD, male, 250-400 g	Alveolar type II	Elastase: 40 u/ml
Rat, Wistar, adult, male and pregnant female (known gestation)	Alveolar type II	Trypsin: 0.1%
Rat, SD, male, 150 - 200 g	Pneumocytes type II	Trypsin: 0.30%
Rat, SD, male, 180-200 g	Alveolar type II	Elastase: 4.3 u/ml
Rat, SD, male	Alveolar type I	Trypsin: 0.05%
Rat, fetus, 19 day	Fibroblasts & type II	Trypsin: 0.05%
Rat, SD, male, 150-400 g	Alveolar type II	Elastase: 4 u/ml
Rat, SD, male/female, 180-250 g	Alveolar type II	Trypsin: 0.30%
Rat, Wistar, pathogen free	Alveolar type II	Trypsin: 0.50%
Rat, fetus, 19 days	Alveolar pneumocytes, type II	Trypsin: 0.1 %
Rat, adult	Lung	Collagenase: 0.1%
Rat, SD, male, 100 g	Alveolar type II	Trypsin: 1.0%

Tissue Dissociation Guide

Medium	Reference
DMEM/F-12	King J, Hamil T, Creighton J, Wu S, Bhat P, McDonald F, and Stevens T: Structural and functional characteristics of lung macro- and microvascular endothelial cell phenotypes, <i>Microvasc Res</i> 67, 139, 2004 (10010)
DMEM	Sunil VR, Connor AJ, Guo Y, Laskin JD, and Laskin DL: Activation of type II alveolar epithelial cells during acute endotoxemia, <i>Am J Physiol Lung Cell Mol Physiol</i> 282, L872, 2002 (10013)
DMEM/Ham's F-12 (see reference)	Kemp, P., Kim, K., Borok, Z., and Crandall, E.: Re-evaluating the Na ⁺ Conductance of Adult Rat Alveolar Type II Pneumocytes: Evidence for the Involvement of cGMP-Activated Cation Channels, <i>J Physiol</i> 536 (3), 693, 2001 (1060)
RPMI-1640	Steinmuller C, Franke-Ullmann G, Lohmann-Matthes ML, Emmendorffer A: Local activation of nonspecific defense against a respiratory model infection by application of interferon-gamma: comparison between rat alveolar and interstitial lung macrophages, <i>Am J Respir Cell Mol Biol</i> 22, 481-90, 2000 (10220)
DMEM	Planus, E., Galiacy, S., Matthay, M., Laurent, V., Gavrilovic, J., Murphy, G., Clerici, C., Isabey, D., Lafuma, C., and d'Ortho, M.: Role of Collagenase in Mediating in Vitro Alveolar Epithelial Wound Repair, <i>J Cell Sci</i> 112, 243, 1999 (9828)
PBS	Bakhramov, A, Evans, M and Kozlowski, R: Differential effects of hypoxia on the intracellular Ca ²⁺ concentration of myocytes isolated from different regions of the rat pulmonary arterial tree, <i>Exp Physiol</i> 83, 337-47, 1998 (10102)
DMEM	Fraslon-Vanhulle, C, Bourbon, J and Batenburg, J: Culture of Fetal Alveolar Epithelial Type II Cells, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 2</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 13A:2.1, 1995 (1278)
EBSS	Kim, K., Suh, D., Lubman, R., Danto, S., Borok, Z., and Crandall, E.: Studies on the Mechanisms of Active Ion Fluxes Across Alveolar Epithelial Cell Monolayers, <i>J Tiss Cul Meth</i> 14, 187, 1992 (896)
HBSS	Jassal, D., Han, R., Caniggia, I., Post, M., and Tanswell, A.: Growth of Distal Fetal Rat Lung Epithelial Cells in a Defined Serum-Free Medium, <i>In Vitro Cell Dev Biol</i> 27A, 625, 1991 (471)
HEPES buffer	Berk, J., Franzblau, C., and Goldstein, R.: Recombinant Interleukin-1beta Inhibits Elastin Formation by a Neonatal Rat Lung Fibroblast Subtype, <i>J Biol Chem</i> 266, 3192, 1991 (574)
Eagle's MEM	Fraslon, C., Rolland, G., Bourbon, J., Rieurtort, M., and Valenza, C.: Culture of Fetal Alveolar Epithelial Type II Cells in Serum-Free Medium, <i>In Vitro Cell Dev Biol</i> 27A, 843, 1991 (927)
HEPES buffer (see reference)	Mangum, J., Everitt, J., Bonner, J., Moore, L., and Brody, A.: Co-Culture of Primary Pulmonary Cells to Model Alveolar Injury and Translocation of Proteins, <i>In Vitro Cell Dev Biol</i> 26, 1135, 1990 (428)
Phosphate-buffered medium (see reference)	Ma, J., LaCagnin, L., Bowman, L., and Miles, P.: Carbon Tetrachloride Inhibits Synthesis of Pulmonary Surfactant Disaturated Phosphatidylcholines and ATP Production in Alveolar Type II Cells, <i>Biochim Biophys Acta</i> 1003, 136, 1989 (314)
RPMI 1640	Batenburg, J., Otto-Verberne, C., Have-Opbroek, A., and Klazinga, W.: Isolation of Alveolar Type II Cells from Fetal Rat Lung by Differential Adherence in Monolayer Culture, <i>Biochim Biophys Acta</i> 960, 441, 1988 (332)
BSS	Brown, L. and Longmore, W.: Altered Phospholipid Secretion in Type II Pneumocytes Isolated from Streptozotocin-diabetic Rats, <i>Biochim Biophys Acta</i> 878, 258, 1986 (325)
HEPES (see reference)	Dobbs, L., Gonzalez R., and Williams, M.: An Improved Method for Isolating Type II Cells in High Yield and Purity, <i>Am Rev Respir Dis</i> 134, 141, 1986 (700)
DMEM	Weller, N., and Karnovsky, M.: Isolation of Pulmonary Alveolar Type I Cells From Adult Rats, <i>Am J Pathol</i> 124, 448, 1986 (990)
HBSS: serum free MEM, CMF	Post, M., Torday, J., and Smith, B.: Alveolar Type II Cells Isolated from Fetal Rat Lung Organotypic Cultures Synthesize and Secrete Surfactant-Associated Phospholipids and Respond to Fibroblast-Pneumonocyte Factor, <i>Exp Lung Res</i> 7, 53, 1984 (407)
Auto-Pow Eagle's modified MEM	Goodman, B., Fleischer, R., and Crandall, E.: Evidence for Active Na ⁺ Transport by Cultured Monolayers of Pulmonary Alveolar Epithelial Cells, <i>Am J Physiol</i> 245, C78, 1983 (292)
BSS	Mason, R., Williams, M., Greenleaf, R., and Clements, J.: Isolation and Properties of Type II Alveolar Cells from Rat Lung, <i>Am Rev Respir Dis</i> 115, 1015, 1977 (697)
Earle's MEM	King, R.: Metabolic Fate of the Apoproteins of Pulmonary Surfactant, <i>Am Rev Respir Dis</i> 115, 73, 1977 (699)
HBSS, CMF	Douglas, W., and Teel, R.: An Organotypic in Vitro Model System for Studying Pulmonary Surfactant Production by Type II Alveolar Pneumonocytes, <i>Am Rev Respir Dis</i> 113, 17, 1976 (698)
Moscona saline, CMF	Douglas, W., and Kaighn, M.: Clonal Isolation of Differentiated Rat Lung Cells, <i>In Vitro</i> 10, 230, 1974 (493)
Joklik's medium	Kikkawa, Y., and Yoneda, K.: Type II Epithelial Cell of the Lung. I. Method of Isolation, <i>Lab Invest</i> 30, 76, 1974 (623)

Tissue Dissociation Guide

Lymph nodes			
Species		Cell(s)	Enzyme(s)
Mouse	Mouse	Follicular dendritic	Collagenase Type 4: 0.25% Deoxyribonuclease I: 0.5%
	Mouse	Lung draining	Collagenase Type 4: 0.1-0.125%
Mammary			
Species		Cell(s)	Enzyme(s)
Bovine	Bovine, heifer, 200kg	Mammary epithelial	Collagenase Type 2: 1% Hyaluronidase: 1% Deoxyribonuclease I: 0.03%
	Bovine	Epithelial	Hyaluronidase: 0.005%
	Bovine	Epithelial	Deoxyribonuclease I: 0.04%
	Bovine, young, lactating, female	Mammary	Collagenase: 0.30 %
	Bovine, dairy, purebred, (also rat, Holtzmann, albino, white)	Secretory Mammary gland	Collagenase: 0.02 - 0.03%
Goat	Goat, lactating, 1 month	Mammary gland	Collagenase: 0.02 - 0.03%
Guinea-Pig	Guinea-pig, pregnant, 4-10 day	Mammary gland	Trypsin NF 1:250: 0.25%
Human	Human	Mammary epithelial cells	Collagenase Type 1: 0.1%
	Human, normal biopsy	Fibroblasts	Collagenase Type 3: 900 u/ml
	Human	Epithelial Fibroblasts	Collagenase Type 1: 450 IU/ml
	Human	Myofibroblasts	Collagenase: 900 IU/ml
	Human, female, 15-61 yrs old	Epithelial	Hyaluronidase: 150 IU/ml
	Human	Epithelial	Collagenase: 2.0%
	Human	Tumor, breast	Neuraminidase: 0.8 u/ml
	Human	Epithelial	Hyaluronidase: 100 u/ml
Mouse	Mouse, 11 week	Epithelial	Collagenase Type 3: 25 u/ml Hyaluronidase: 0.1% Protease XIV: 0.05% Deoxyribonuclease I: 0.04%
	Mouse, 12 week	Mammary epithelial	Collagenase: 0.3% Hyaluronidase: 100 u/ml Trypsin: 0.25% Neutral Protease: 0.5% Deoxyribonuclease I: 0.01%
	Mouse	Mammary epithelial stem	Collagenase Type 3: 0.1% Hyaluronidase: 0.1% Pronase: 1.25% Deoxyribonuclease I: 0.2%
	Mouse, BALB/C	Epithelium	Collagenase: 250 u/ml
	Mouse, BALB/c, virgin, female, 4 month	Adipocytes	Trypsin: 50 µg/ml

Tissue Dissociation Guide

Lymph nodes

Medium	Reference
HBSS	Kapasi ZF, Qin D, Kerr WG, Kosco-Vilbois MH, Shultz LD, Tew JG, Szakal AK: Follicular dendritic cell (FDC) precursors in primary lymphoid tissues, <i>J Immunol</i> 160, 1078-84, 1998 (10270)
HBSS	Rayamajhi, M., Redente, E., Condon, T., Gonzalez-Juarrero, M., Riches, D. and Lenz, L.: Non-Surgical Intratracheal Instillation of Mice with Analysis of Lungs and Lung Draining Lymph Nodes by Flow Cytometry., <i>J Vis Exp</i> 51, 2702, 2011 (10661)

Mammary

Medium	Reference
M-199	Weber M., Purup S., Vestergaard M., Ellis S., Scndergard-Andersen J., Akers R., and Sejrnsen K.: Contribution of Insulin-like Growth Factor (IGF)-I and IGF-binding Protein-3 to Mitogenic Activity in Bovine Mammary Extracts and Serum, <i>J Endocrinol</i> 161, 365, 1999 (10000)
HBSS	Gibson, C., Vega, J., Baumrucker, C., Oakley, C., and Welsch, C.: Establishment And Characterization Of Bovine Mammary Epithelial Cell Lines, <i>In Vitro Cell Dev Biol</i> 27, 585, 1991 (469)
HBSS/Medium 199	Baumrucker, C., Deemer, K., Walsh, R., Riss, T., and Akers, R.: Primary Culture of Bovine Mammary Acini on a Collagen Matrix, <i>Tissue Cell</i> 20 (4), 541, 1988 (874)
HBSS or EBSS	Anderson, C., and Larson, B.: Comparative Maintenance of Function in Dispersed Cell and Organ Cultures, <i>Exp Cell Res</i> 61, 24, 1970 (399)
HBSS or EBSS	Schingoethe, D., Hageman, E., and Larson, B.: Essential Amino Acids for Milk Protein Synthesis in the <i>In Vitro</i> Secretory Cell and Stimulation by Elevated Levels, <i>Biochim Biophys Acta</i> 148, 469, 1967 (316)
HBSS or EBSS	Blanco, A., Rife, U., and Larson, B.: Lactate Dehydrogenase Isozymes during Dedifferentiation in Cultures of Mammary Secretory Cells, <i>Nature</i> 214, 1331, 1967 (640)
Dulbecco phosphate	Turba, F., and Hilpert, N.: Secretion and Resorption of Proteins by Isolated Mammary Gland Cells. German, <i>Biochem Z</i> 334, 501, 1961 (1282)
DMEM	Huss, F. and Kratz, G.: Mammary Epithelial Cell and Adipocyte Co-Culture in a 3-D Matrix: The First Step Towards Tissue-Engineered Human Breast Tissue., <i>Cells Tissues Organs</i> 169, 361-7, 2001 (10690)
DMEM/F-12	Ronnov-Jessen L., Villadsen R., Edwards J., and Petersen O.: Differential Expression of a Chloride Intracellular Channel Gene, CLIC4, in Transforming Growth Factor-beta1-mediated Conversion of Fibroblasts to Myofibroblasts, <i>Am J Pathol</i> 161, 471, 2002 (9998)
DMEM/Ham's F-12	Ogmondottir, H., Petursdottir, I., Gudmundsdottir, I., Amundadottir, L., Ronnov-Jessen, L., and Petersen, O.: Effects of Lymphocytes and Fibroblasts on the Growth of Human Mammary Carcinoma Cells Studied in Short-Term Primary Cultures, <i>In Vitro Cell Dev Biol</i> 29A, 936, 1993 (871)
DME - F12	Ronnov-Jessen, L., VanDeurs, B., Nielsen, M., and Petersen, O.W.: Identification, Paracrine Generation, and Possible Function of Human Breast Carcinoma Myofibroblasts in Culture, <i>In Vitro Cell Dev Biol</i> 28, 273, 1992 (482)
DMEM	Berthon, P., Pancino, G., Cremoux, P., Roseto, A., Gespach, C., and Calvo, F.: Characterization of Normal Breast Epithelial Cells in Primary Cultures: Differentiation and Growth Factor Receptors Studies, <i>J Tiss Cul Meth</i> 28A, 716, 1992 (904)
DMEM/Ham's F-12	Emerman, J. and Wilkinson, D.: Routine Culturing of Normal, Dysplastic and Malignant Human Mammary Epithelial Cells from Small Tissue Samples, <i>In Vitro Cell Dev Biol</i> 26, 1186, 1990 (429)
HBSS	Leung, C., and Shiu, R.: Morphological and Proliferative Characteristics of Human Breast Tumor Cells Cultured on Plastic and in Collagen Matrix, <i>In Vitro</i> 18, 476, 1981 (521)
DMEM/Ham's F-12	Stampfer, M., Hallowes, R., and Hackett, A.: Growth of Normal Human Mammary Cells in Culture, <i>In Vitro</i> 16 (5), 415, 1980 (856)
DMEM/F12	Mueller, S., Clark, J., Myers, P. and Korach, K.: Mammary Gland Development in Adult Mice Requires Epithelial and Stromal Estrogen Receptor Alpha., <i>Endocrinology</i> 143, 2357, 2002 (10369)
(see reference)	Taddei Ilaria, Deugnier Marie-Ange, Faraldo Marisa M, Petit Valerie, Bouvard Daniel, Medina Daniel, Fssler Reinhard, Thiery Jean Paul, Glukhova Marina: Beta1 integrin deletion from the basal compartment of the mammary epithelium affects stem cells, <i>Nat Cell Biol</i> 10, 716-22, 2008 (10320)
DMEM	Boulanger CA, Smith GH: Reducing mammary cancer risk through premature stem cell senescence, <i>Oncogene</i> 20, 2264-72, 2001 (10225)
HBSS	Kanazawa, T., and Hosick, H.: Transformed Growth Phenotype of Mouse Mammary Epithelium in Primary Culture Induced by Specific Fetal Mesenchymes, <i>J Cell Physiol</i> 153, 381, 1992 (961)
DMEM	Beck, J., Hosick, H., and Watkins, B.: Growth of Epithelium From a Preneoplastic Mammary Outgrowth on Response to Mammary Adipose Tissue, <i>In Vitro Cell Dev Biol</i> 25 (5), 409, 1989 (964)

Tissue Dissociation Guide

Species

Mammary (con't)

Cell(s)

Enzyme(s)

	Mouse BALB/cCrgl, mature	Epithelial	Pronase: 0.01%
	Mouse, BALB/c, female, 6-8 week	Epithelial	Collagenase Type 3: 0.1%
	Mouse C3H/HeN, female	Epithelial	Deoxyribonuclease I: 0.0001%
	Mouse, BALB/c/Crgl Me, female, pregnant	Epithelial	Pepsin: 0.1% and 0.05%
	Mouse, female	Epithelial	Deoxyribonuclease I: 0.1%
	Mouse NMuMG, female, 2 months	Epithelial	Collagenase Type 3: 0.1%
	Mouse, BALB/cCr1, female	Epithelial	Collagenase Type 2: 0.2%
	Mouse BALB/cC3H	Mammary tumors Epithelial	Collagenase: 1.0%
	Mouse, pregnant 8-12 day	Mammary	(see reference):
	Mouse BALB/cCrgl, virgin, female, 4-8 month	Epithelial Mammary gland	Collagenase Type 3: 0.1%
	Mouse BALB/c, pregnant, 60-80 days	Mammary	Hyaluronidase: 0.1%
	Mouse, BALB/cfC3H/Crgl, virgin, female	Nodule-transformed	Hyaluronidase: 0.1%
	Mouse, BALB/cCrgl, female also guinea-pig	Swiss 3T3	Collagenase Type 3: 0.2%
	Mouse, (C3H/Crgl or BALB/cCrgl), 8-10 day pregnant	Epithelial	Collagenase Type 3: 0.12%
	Mouse, lactating	Parenchymal	Collagenase Type 1: 0.3%
	Mouse, female, early pregnancy <10 days	Epithelial	Collagenase: 0.1%
	Mouse, CBA, virgin, 9/10 wk	Mammary	Hyaluronidase: 0.1%
	Mouse, lactating, 14-18 day	Parenchymal	Collagenase: 0.33%
	Mouse, lactating, 14 day	Mammary	Trypsin NF 1:250: 0.25%
	Mouse, pregnant, 14-17 day	Mammary	Collagenase: 0.05% - 0.1%
	Mouse, adult, 1-10 days pregnant	Epithelial Mammary	Collagenase: 0.02%
Rat	Rat	Mammary epithelial	Collagenase Type 3: 0.35%
	Rat, female, 50 day	Mammary epithelial	Collagenase Type 3: 0.15%
	Rat, female	Mammary gland epithelial	Collagenase Type 3: 0.35%
	Rat, SD	Mammary fibroblasts	Collagenase Type 3: 0.2% Neutral Protease: 0.2% Deoxyribonuclease I: 0.01%
	Rat, SD, 50 day	Mammary epithelial	Collagenase Type 3: 0.2% Neutral Protease: 0.2%

Tissue Dissociation Guide

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Medium	Reference
Hepes buffered medium 199	Bandyopadhyay, G., Imagawa, W., Wallace, D., and Nandi, S.: Proliferative effects of insulin and epidermal growth factor on mouse mammary epithelial cells in primary culture, <i>J Biol Chem</i> 263, 7567, 1988 (563)
DMEM	Ehmann, U., Peterson, W., and Misfeldt, D.: To Grow Mouse Mammary Epithelial Cells in Culture, <i>J Cell Biol</i> 98, 1026, 1984 (926)
Medium 199	Taketani, Y., and Oka, T.: EGF Stimulates Cell Proliferation and Inhibits Functional Differentiation of Mouse Mammalian Dipithelial Cells in Culture, <i>Endocrinology</i> 113, 871, 1983 (380)
HBSS with 0.2% EDTA, CMF	Riser, M., Huff, B., and Medina, D.: Pepsin Can Be Used To Subculture Viable Mammary Epithelial Cells, <i>In Vitro</i> 19, 730, 1983 (532)
DMEM	Jones, W., and Hallows, R.: Isolation of the Epithelial Subcomponents of the Mouse Mammary Gland for Tissue-Level Culture Studies, <i>J Tiss Cul Meth</i> 8 (1), 17, 1983 (873)
DMEM	Ehmann, U., and Misfeldt, D.: Mouse Mammary Cells in D-Valine Medium, <i>In Vitro</i> 18, 407, 1982 (519)
HBSS/DMEM	Asch, B., Burstein, N., Vidrich, A., and Sun, T.: Identification of Mouse Mammary Epithelial Cells by Immunofluorescence With Rabbit and Guinea-Pig Antikeratin Antisera, <i>Proc Natl Acad Sci U S A</i> 78, 5643, 1981 (892)
HBSS	Yang, J., Guzman, R., Richards, J., and Nandi, S.: Primary Cultures of Mouse Mammary Tumor Epithelial Cells Embedded in Collagen Gels, <i>In Vitro</i> 16, 502, 1980 (507)
HBSS	Yang J, Richards J, Guzman R, Imagawa W, and Nandi S.: Sustained growth in primary culture of normal mammary epithelial cells embedded in collagen gels, <i>Proc Natl Acad Sci U S A</i> 77, 2088, 1980 (10019)
HBSS	White, M., Hu, A., Hamamoto, S. and Nandi: In vitro analysis of proliferating epithelial cell populations from the mouse mammary gland: fibroblast-free growth and serial passage, <i>In Vitro</i> 14, 271, 1978 (498)
CMF medium	Ceriani, R., Peterson, J., and Abraham, S.: Removal of Cell Surface Material by Enzymes Used to Dissociate Mammary Gland Cells, <i>In Vitro</i> 14, 887, 1978 (499)
Medium 199	DeOme, K., Miyamoto, M., Osborn, R., Guzman, R., and Lum, K.: Detection of Inaparent Nodule-transformed Cells in the Mammary Gland Tissues of Virgn Female BALB/cfC3H Mice, <i>Cancer Res</i> 38, 2103, 1978 (872)
PBS CMF	Asch, B., and Medina, D.: Concanavalin A-Induced Agglutinability of Normal, Preneoplastic, and Neoplastic Mouse Mammary Cells, <i>J Natl Cancer Inst</i> 61 (6), 1423, 1978 (1012)
HBSS	Emerman, J., Enami, J., Pitelka, D., and Nandi, S.: Hormonal Effects on Intracellular and Secreted Casein in Cultures of Mouse Mammary Epithelial Cells on Floating Collagen Membranes, <i>Proc Natl Acad Sci U S A</i> 74 (10), 4466, 1977 (930)
Kreb's Ringer bicarbonate buffer	Kerkof, P., and Abraham, S.: Preparation of Adipose Cell-Free Suspensions of Mammary Gland Parenchymal Cells from Lactating Mice, <i>Methods Enzymol</i> 69, 693, 1976 (696)
Eagle's MEM	Moore, D., and Lasfargues, E.: Method for the Continuous Cultivation of Mammary Epithelium, <i>In Vitro</i> 7, 21, 1971 (537)
BSS, CMF	Prop, F., and Wiepjes, G.: Improved Method for Preparation of Single-cell Suspensions from Mammary Glands of Adult Virgin Mouse, <i>Exp Cell Res</i> 61, 451, 1970 (400)
Kreb's buffer	Pitelka, D., Kerkof, P., Gagne, H., Smith, S., and Abraham, S.: Characteristics of Cells Dissociated from Mouse Mammary Glands. I. Method of Separation and Morphology of Parenchymal Cells from Lactating Glands, <i>Exp Cell Res</i> 57, 43, 1969 (398)
HBSS	Kopelovich, L., Abraham, S., McGrath, H., DeOme, K., Chaikoff, I.: Metabolic Characteristics of a Naturally Occurring Preneoplastic Tissue. I. Glycolytic Enzyme Activators of Hyperplastic Alveolar Nodule Outgrowths and Adenocarcinomas of Mouse Mammary Gland, <i>Cancer Res</i> 26, 1534, 1966 (352)
HBSS	Daniel, C., and DeOme, K.: Growth of Mouse Mammary Glands <i>In Vivo</i> After Monolayer Culture, <i>Science</i> 149, 634, 1965 (663)
Simm's	Lasfargues, E.: Cultivation and Behavior <i>In Vitro</i> of the Normal Mammary Epithelium of the Adult Mouse. II. Observations on the Secretory Activity, <i>Exp Cell Res</i> 13, 553, 1957 (390)
HBSS	Mei, N., McDaniel, L., Dobrovolsky, V., Guo, X., Shaddock, J., Mittelstaedt, R., Azuma, M., Shelton, S., McGarrity, L., Doerge, D. and Heflich, R.: The Genotoxicity of Acrylamide and Glycidamide in Big Blue Rats., <i>Toxicol Sci</i> 115, 412, 2010 (10638)
DMEM/F12	Maffini M., Soto A., Calabro J., Ucci A., and Sonnenschein C.: The Stroma as a Crucial Target in Rat Mammary Gland Carcinogenesis, <i>J Cell Sci</i> 117, 1495, 2004 (10001)
(see reference)	Djuric Z., Lewis S., Lu M., Mayhugh M., Naegeli L., Tang N., and Hart R.: Effect of Varying Caloric Restriction Levels on Female Rat Growth and 5-hydroxymethyl-2'-deoxyuridine in DNA, <i>Toxicol Sci</i> 66, 125, 2002 (9957)
DMEM/F-12	Brake P., Zhang L., and Jefcoate C.: Aryl Hydrocarbon Receptor Regulation of Cytochrome P4501B1 in Rat Mammary Fibroblasts: Evidence for Transcriptional Repression by Glucocorticoids, <i>Mol Pharmacol</i> 54, 825, 1998 (9999)
DMEM/F12	Varela L., Darcy K., and Ip M.: The Epidermal Growth Factor Receptor is not Required for Tumor Necrosis Factor-alpha Action in Normal Mammary Epithelial Cells, <i>Endocrinology</i> 138, 3891, 1997 (10002)

Tissue Dissociation Guide

Species

Mammary (con't)

Cell(s)

Enzyme(s)

Rat (also human)	Epithelial	Collagenase Type 1: 0.4%
Rat, SD, female, 60-90 day	Epithelial	Collagenase Type 3: 0.5%
Rat, Lewis, female, 90 days	Epithelial Mammary	Collagenase: 0.05%
Rat, SD, female, 55 day	Epithelial	Neutral Protease: 3 u/ml
Rat, 50-60 days	Epithelial	Neutral Protease: 0.2%
Rat, Fisher 344, virgin, 80-100 day	Epithelial	Hyaluronidase: 0.1%
Rat, LEW, virgin, female, 45-50 day	Epithelial	Collagenase: 0.1%
Rat, inbred LEW, female, 50-60 day old	Epithelial	Collagenase Type 3: 0.1%
Rat, Wistar, 13-18 day postpartum	Acini	Collagenase: 0.05%
Rat, SD, 15-20 day postpartum	Mammary	Collagenase: 0.2%
Rat, virgin	Mammary	Collagenase: 0.35%

Miscellaneous

Species

Cell(s)

Enzyme(s)

Human	Human	Synoviocytes	Collagenase: 0.2%
	Human	Carotid artery plaque macrophage	Collagenase Type 4: 450 u/ml Deoxyribonuclease I: 500 u/ml Soybean Trypsin Inhibitor: 0.1%
	Human	Periapical granuloma	CLSIPA: 0.25%
Insect	Insect, <i>Lepidoptera</i>	Lepidopteran	Collagenase Type 3: 0.35% Hyaluronidase: 0.01%
Mouse	Mouse	Spleen, bone marrow endothelial	Collagenase Type 4: 0.3-1.0% Deoxyribonuclease I: 20 u/ml
	Mouse, 6-8 week	Bone marrow mesenchymal stem	Collagenase Type 1: 0.25%
	Mouse	Bone marrow	Collagenase Type 1: 0.15% Neutral Protease: 0.15%
	Mouse, 25-30 g	Tracheal inflammatory cells	Collagenase Type 4: 0.1% Deoxyribonuclease I: 50 u/ml Soybean Trypsin Inhibitor: 0.1%
Rabbit	Rabbit, New Zealand, 8-10 week	Tenocytes and tendon stem cells	Collagenase Type 1: 0.3% Neutral Protease: 0.4%
Rat	Rat, Wistar, 7-9 week	Gingival mitochondria	Collagenase Type 1: 0.115-0.130 %

Tissue Dissociation Guide

Mammary (con't)

Medium	Reference
DMEM	Soriano, J., Pepper, M., Nakamura, T., Orci, L., and Montesano, R.: Hepatocyte Growth Factor Stimulates Extensive Development of Branching Duct-like Structures by Cloned Mammary Gland Epithelial Cells, <i>J Cell Sci</i> 108, 413-430, 1995 (740)
EBSS	Laduca, J., and Sinha, D.: In Vitro Carcinogenesis of Mammary Epithelial Cells by N-Nitroso-N-Methylurea Using a Collagen Gel Matrix Culture, <i>In Vitro Cell Dev Biol</i> 29A, 789, 1993 (895)
Medium 199	Lin, T., Hom, Y.K., Richards, J. and Nandi, S.: Effects of Antioxidants and Reduced Oxygen Tension on Rat Mammary Epithelial Cells in Culture, <i>In Vitro Cell Dev Biol</i> 27A, 191, 1991 (458)
Medium 199	Ehmann, U., Osborn, R., Guzman, R., and Fajardo, L.: Cultured Proliferating Rat Mammary Epithelial Cells, <i>In Vitro Cell Dev Biol</i> 27, 749, 1991 (477)
EBSS	Hahm, H.A., Ip, M.M.: Primary Culture of Normal Rat Mammary Epithelial Cells Within a Basement Matrix. 1. Regulation of Proliferation by Hormones and Growth Factors, <i>In Vitro Cell Dev Biol</i> 26, 791, 1990 (451)
Medium 199	McGrath, M., Palmer, S., and Nandi, S.: Differential Response of Normal Rat Mammary Epithelial Cells to Mammogenic Hormones and EGF, <i>J Cell Physiol</i> 125, 182, 1985 (924)
Medium 199	Ethler, S.: Primary Culture and Serial Passage of Normal and Carcinogen-Treated Rat Mammary Epithelial Cells In Vitro, <i>J Natl Cancer Inst</i> 74 (6), 1307, 1985 (1017)
Medium 199	Richards, J., and Nandi, S.: Primary Culture of Rat Mammary Epithelial Cells. I. Effect of Plating Density, Hormones, and Serum on DNA Synthesis, <i>J Natl Cancer Inst</i> 61 (3), 765, 1978 (1018)
HBSS	Katz, J., Wals, P. and Van de Velde, R: Lipogenesis by Acini from Mammary Gland of Lactating Rats, <i>J Biol Chem</i> 249, 7348, 1974 (551)
Kreb's Ringer bicarbonate buffer	Martin, R., and Baldwin, R.: Effects of Insulin on Isolated Rat Mammary Cell Metabolism: Glucose Utilization and Metabolite Patterns, <i>Endocrinology</i> 89, 1263, 1971 (384)
Medium 199	Moon, R., Janns, D., and Young, S.: Preparation of Fat Cell-"Free" Rat Mammary Gland, <i>J Histochem Cytochem</i> 17 (3), 182, 1969 (1177)

Miscellaneous

Medium	Reference
DMEM/F12	Chen V, Croft D, Purkis P, Kramer IM: Co-culture of synovial fibroblasts and differentiated U937 cells is sufficient for high interleukin-6 but not interleukin-1beta or tumour necrosis factor-alpha release., <i>Br J Rheumatol Vol. 37</i> , 148-56, 1998 (10360)
HBSS	Patino WillmarD, Kang Ju-Gyeong, Matoba Satoaki, Mian OmarY, Gochuico BernadetteR, Hwang PaulM: Atherosclerotic plaque macrophage transcriptional regulators are expressed in blood and modulated by tristetraprolin, <i>Circ Res</i> 98, 1282-9, 2006 (10336)
RPMI-1640	Stern MH, Dreizen S, Mackler BF, Levy BM: Isolation and characterization of inflammatory cells from the human periapical granuloma, <i>J Dent Res</i> 61, 1408-12, 1982 (10292)
Dulbecco PBS	Goodwin, R and McCawley, P: Initiating Attached Cell Lines From the Lepidoptera (Insecta), <i>Meth Cell Sci</i> 3, 567, 1977 (10675)
PBS	Shi, C., Jia, T., Mendez-Ferrer, S., Hohl, T., Serbina, N., Lipuma, L., Leiner, I., Li, M., Frenette, P. and Pamer, E.: Bone Marrow Mesenchymal Stem and Progenitor Cells Induce Monocyte Emigration in Response to Circulating Toll-Like Receptor Ligands., <i>Immunity</i> 34, 590, 2011 (10641)
RPMI 1640	Xu, S., De Becker, A., Van Camp, B., Vanderkerken, K. and Van Riet, I.: An Improved Harvest and In Vitro Expansion Protocol for Murine Bone Marrow-Derived Mesenchymal Stem Cells., <i>J Biomed Biotechnol Vol. 2010</i> , 105940, 2010 (10617)
PBS	Bertoncello, I. and Williams, B.: Hematopoietic Stem Cell Characterization by Hoechst 33342 and Rhodamine 123 Staining., <i>Methods Mol Biol</i> 263, 181, 2004 (10528)
RPMI 1640	Minamoto Kanji, Pinsky DavidJ: Recipient iNOS but not eNOS deficiency reduces luminal narrowing in tracheal allografts, <i>J Exp Med</i> 196, 1321-33, 2002 (10299)
DMEM	Zhang, J. and Wang, J.: Characterization of Differential Properties of Rabbit Tendon Stem Cells and Tenocytes., <i>BMC Musculoskelet Disord</i> 11, 10, 2010 (10639)
HBSS	Kaneko, N., Rikimaru, T., Fujimura, T., Mori, S., Hidaka, S. and Kaya, H.: Preparation of Rat Gingival Mitochondria with an Improved Isolation Method., <i>Int J Dent Vol. 2010</i> , 275103, 2010 (10603)

Tissue Dissociation Guide

TISSUE TABLES

Species	Miscellaneous (con't)	Cell(s)	Enzyme(s)
	Rat, 8-10 week	Synovial cells	Collagenase Type 1: 250 u/ml
Muscle			
Species		Cell(s)	Enzyme(s)
Bovine	Bovine	Smooth muscle	Trypsin: 0.25%
	Bovine	Vascular smooth muscle	Elastase Type 3: 50 u/ml
	Bovine	Smooth muscle, fibroblasts	Trypsin: 0.055%
Canine	Dog	Smooth muscle	Elastase: 50 u/ml
	Dog, beagle, adult	Smooth muscle Vascular	Elastase: 34 u/ml
	Dog	Artery Carotid	Elastase: 80 u/ml
Chicken	Chicken, 1-2 day	Gizzard and aorta smooth muscle	Collagenase Type 1: 0.15%
	Chick	Smooth muscle	Trypsin: 0.05% - 0.1%
	Chick, white leghorn, 12 day	Muscle	Trypsin: 0.25%
	Chick, white leghorn, embryos, 11 day	Muscle	Trypsin: 0.05%
	Chick embryo	Thyroid Muscle Heart	Collagenase: 0.25%
	Chick, embryonic	Muscle	Trypsin: 0.1%
Feline	Chick embryonic	Various tissues (heart, liver, skeletal, cardiac)	Trypsin: various grades
	Cat, adult mongrel, either sex, 2.5-4.0 kg	Cerebral arteries	Elastase: 50 u/ml
	Cat, mongrel, adult, 2-4 kg	Myocytes	Collagenase: 0.12%
Fish	Dogfish, <i>Squalus acanthias</i> , 2-6 Kg (also guinea-pig, rabbit, frog)	Myocytes, heart and stomach	Protease XIV: 0.028%
Frog	Frog, <i>Xenopus laevis</i> , embryos stage 17 & 19	Muscle	Collagenase: 0.10%
	Frog	Myocytes	Trypsin: 0.1%
	Frog, <i>Rana pipiens</i> (50-100 g) (also guinea-pig, rabbit, dogfish, <i>Squalus acanthias</i>)	Myocytes, heart and stomach	Protease XIV: 0.028%
Guinea-Pig	Frog, <i>Xenopus laevis</i>	Muscle	Trypsin: 0.5%
	Guinea-pig	Bladder smooth muscle	Collagenase Type 2: 0.1-0.2%
	Guinea-pig, 200-380 g	Capillaries Myocytes	Collagenase Type 2: 0.15%
	Guinea pig, 2-4 wk old, male, female	Smooth muscle Gallbladder	Papain: 0.1%
	Guinea-pig, adult, 250-350 g	Smooth muscle Gallbladder	Papain: 0.1%
	Guinea-pig, Dunkin-Hartley, female	Myocytes	Protease:
	Guinea-pig (also rat, rabbit)	Smooth muscle	Trypsin: 0.1%

Tissue Dissociation Guide

Medium	Reference
RPMI 1640	Moghaddami, M., Cleland, L. and Mayrhofer, G.: MHC II+ CD45+ Cells from Synovium-Rich Tissues of Normal Rats: Phenotype, Comparison with Macrophage and Dendritic Cell Lineages and Differentiation into Mature Dendritic Cells In Vitro., <i>Int Immunol</i> 17, 1103, 2005 (10581)
Muscle	
Medium	Reference
DMEM	Absher, M., Woodcock-Mitchell, J., Mitchell, J., Baldor, L., Low, R., and Warshaw, D.: Characterization of Vascular Smooth Muscle Cell Phenotype in Long-Term Culture, <i>In Vitro Cell Dev Biol</i> 25 (2), 183, 1989 (862)
PSS	Warshaw, D., Szarek, J., Hubbard, M., and Evans, J.: Pharmacology and Force Development of Single Freshly Isolated Bovine Carotid Artery Smooth Muscle Cells, <i>Circ Res</i> 58, 399, 1986 (865)
DMEM	Davies, P. and Kerr, C.: Modification of LDL Metabolism by Growth Factors in Cultured Vascular Cells and Human Skin Fibroblasts, <i>Biochim Biophys Acta</i> 712, 26, 1982 (322)
PSS	Subramanian, M., Madden, J., and Harder, D.: A Method for the Isolation of Cells from Arteries of Various Sizes, <i>J Tiss Cul Meth</i> 13, 13, 1991 (1240)
Tyrode's solution w/ calcium	Wilde, D., and Lee, K.: Outward Potassium Currents in Freshly Isolated Smooth Muscle Cell of Dog Coronary Arteries, <i>Circ Res</i> 65, 1718, 1989 (368)
PSS	Dobrin, P., and Canfield, T.: Elastase, Collagenase, and the Biaxial Elastic Properties of Dog Carotid Artery, <i>Am J Physiol Heart Circ Physiol</i> 247 (16), H124, 1984 (1236)
HBSS	Dirksen W., Vlastic F., and Fisher S.: A Myosin Phosphatase Targeting Subunit Isoform Transition Defines a Smooth Muscle Developmental Phenotypic Switch, <i>Am J Physiol/Cell</i> 278(3), C589, 2000 (9837)
HBSS	Chamley-Campbell, J., Campbell, G., and Ross, R.: The Smooth Muscle in Cell Culture, <i>Physiol Res</i> 59, 1, 1979 (648)
Puck's saline A	Bullaro, J., and Brookman, D.: Comparison of Skeletal Muscle Monolayer Cultures Initiated With Cells Dissociated by the Vortex and Trypsin Methods, <i>In Vitro</i> 12, 564, 1976 (497)
Saline G	Tepperman, K., Morris, G., Essien, F., and Heywood, S.M.: A Mechanical Dissociation Method For Preparation of Muscle Cell Cultures, <i>J Cell Physiol</i> 86, 561, 1975 (597)
Tyrode's saline, potassium free	Hilfer, S., and Brown, J.: Collagenase. Its Effectiveness as a Dispersing Agent for Embryonic Chick Thyroid and Heart, <i>Exp Cell Res</i> 65, 246, 1971 (401)
CMF HBSS	Hilfer, S.: Collagenase Treatment of Chick Heart and Thyroid, <i>Tissue Cult Methods & Applications</i> , Kruse, P., and Patterson, M., 246, 1971 (1283)
CMF Tyrode's solution	Rinaldini, L.: An Improved Method for the Isolation and Quantitative Cultivation of Embryonic Cells, <i>Exp Cell Res</i> 16, 477, 1959 (394)
Puck's solution	Madden, J., Vadula, M., and Kurup, V.: Effects of Hypoxia and Other Vasoactive Agents on Pulmonary and Cerebral Artery Smooth Muscle Cells, <i>Am J Physiol</i> 263, L384, 1992 (778)
Kreb's Henseleit, CF	Follmer, C.H., Ten Eick, R.E., and Yeh, J.Z.: Sodium Current Kinetics in Cat Atrial Myocytes, <i>J Physiol</i> 384, 169, 1987 (724)
Solution C (see reference)	Mitra, R. and Morad, M.: A Uniform Enzymatic Method for Dissociation of Myocytes from Hearts and Stomachs of Vertebrates, <i>Am J Physiol</i> 249, H1056, 1985 (294)
Steinberg's solution	Stollberg, J. and Fraser, S.: Acetylcholine Receptors and Con A-Binding Sites on <i>Xenopus</i> Muscle Cells, <i>J Cell Biol</i> 107, 1397, 1988 (579)
CF Ringer	Shepherd, N. and Kavalier, F.: Direct Control of Contraction Force of Single Frog Atrial Cells by Extracellular Ions, <i>Am J Physiol</i> 251, C653, 1986 (296)
Solution C (see reference)	Mitra, R. and Morad, M.: A Uniform Enzymatic Method for Dissociation of Myocytes from Hearts and Stomachs of Vertebrates, <i>Am J Physiol</i> 249, H1056, 1985 (294)
L15 medium (see reference)	Anderson, M.J., Cohen, M.W., and Zorychta, E.: Effects of Innervation on the Distribution of Acetylcholine Receptors on Cultured Muscle Cells, <i>J Physiol</i> 268, 731, 1977 (722)
Krebs-Ringer bicarbonate	Shieh CC, Feng J, Buckner SA, Brioni JD, Coghlan MJ, Sullivan JP, Gopalakrishnan M: Functional implication of spare ATP-sensitive K(+) channels in bladder smooth muscle cells, <i>J Pharmacol Exp Ther</i> 296, 669-75, 2001 (10238)
CF solution	Schnitzler, M., Derst, C., Daut, J., and Preisig-Muller, R.: ATP-Sensitive Potassium Channels in Capillaries Isolated From Guinea-Pig Heart, <i>J Physiol</i> 525 (2), 307, 2000 (744)
Krebs solution	Firth, T., Mawe, G., and Nelson, M.: Pharmacology and Modulation of K _{ATP} Channels by Protein Kinase C and Phosphatates in Gallbladder Smooth Muscle, <i>Am J Physiol Cell Physiol</i> 278, C1031, 2000 (1131)
NaCl, sodium glutamate, MgCl, KCl, glucose, Kreb's, and HEPES	Jennings, L., Xu, Q., Firth, T., Nelson, M., and Mawe, G.: Cholesterol Inhibits Spontaneous Action Potentials and Calcium Currents in Guinea Pig Gallbladder Smooth Muscle, <i>Am J Physiol</i> 277, G1017, 1999 (1114)
DMEM	Ryder, K., Bryant, S., and Hart, G.: Membrane Current Changes in Left Ventricular Myocytes Isolated From Guinea-Pigs After Abdominal Aortic Coarctation, <i>Cardiovasc Res</i> 27, 1278, 1993 (970)
Potassium buffer solution	Hu, S., and Kim, H.: Activation of K ⁺ Channel in Vascular Smooth Muscle by Cytochrome P-450 Metabolites of Arachidonic Acid, <i>FASEB J</i> 6, A383, 1992 (409)

Tissue Dissociation Guide

Species	Muscle (con't)	Cell(s)	Enzyme(s)	
Hamster	Guinea-pig, 200-300 g	Smooth muscle Mesenteric artery	Collagenase: 0.3%	
	Guinea-pig, 200-400 g (also rabbit, frog, dogfish)	Myocytes, heart and stomach	Protease XIV: 0.028%	
	Guinea-pig, prepubertal	Smooth muscle Aortic	Trypsin: 0.05%	
	Hamster, male, 60-70 day	Satellite	Trypsin: 0.25%	
Human	Human, male	Myogenic	Collagenase Type 4: 0.1% Neutral Protease: 2.4 u/ml	
	Human	Muscle derived multiprogenitor cells	Collagenase Type 2: 0.05%	
	Human	Endothelial and vascular smooth muscle	Collagenase Type 1: 0.2%	
	Human	Urinary tract smooth muscle	Collagenase Type 4: 100 u/ml	
	Human, female	Smooth muscle Myometrial	Deoxyribonuclease I: 0.015% and 0.007%	
	Human, female	Smooth muscle Myometrial	Deoxyribonuclease I: 0.12%	
	Human, fetal (also bovine)	Smooth muscle, fibroblasts	Trypsin: 0.055%	
	Human	Smooth muscle	Trypsin: 0.25%	
	Human (also rat, guinea-pig, chick, monkey)	Smooth muscle	Trypsin: 0.05% - 0.1%	
	Lizard	Lizard (<i>Anolis carolinensis</i>)	Myoblasts, tail	Collagenase: 0.2%
		Monkey	Monkey (<i>Macaca nemestrina</i>)	Smooth muscle
	Rhesus monkey, 1 year (also human, rabbits)		Smooth muscle, saphenous vein	Elastase: 0.05%
Mouse	Mouse	Myoblast	Collagenase Type 2: 0.2%	
	Mouse, neonatal	Skeletal muscle myotubes	NCIS kit: per instructions	
	Mouse, male, 6-14 week	Precursor cells	Collagenase Type 2: 0.5%	
	Mouse, 1-25 month	Myocytes, endothelial	Neutral Protease: 1.2 u/ml Collagenase Type 4: 0.2%	
	Mouse, 15-20 day	Intersitial cells of Cajal	Collagenase Type 2: 0.13%	
	Mouse, 6-8 week	Skeletal muscle progenitor	Collagenase Type 2: 0.2%	
	Mouse, neonatal	Myocytes	Collagenase Type 1: 0.5%	
	Mouse, 6-8 week	Myocytes	Collagenase Type 2: 0.2% Trypsin: 0.25%	
	Ovine	Sheep, adult and neonatal	Tracheal smooth muscle cells	Papain: 0.2% Deoxyribonuclease I: 0.1%

Tissue Dissociation Guide

Medium	Reference	Muscle (con't)
CF solution	Ohya, Y. and Sperelakis, N.: ATP Regulation of the Slow Calcium Channels in Vascular Smooth Muscle Cells, <i>Circ Res</i> 64, 145, 1989 (366)	
Solution C (see reference)	Mitra, R. and Morad, M.: A Uniform Enzymatic Method for Dissociation of Myocytes from Hearts and Stomachs of Vertebrates, <i>Am J Physiol</i> 249, H1056, 1985 (294)	
Dulbecco-Vogt modification of Eagle's	Ross, R.: The Smooth Muscle Cell . II. Growth of Smooth Muscle in Culture and Formation of Elastic Fibers, <i>J Cell Biol</i> 50, 172, 1971 (584)	
DMEM	Nakamura, T., Iwata, Y., Sampaolesi, M., Hanada, H., Saito, N., Artman, M., Coetzee, W., and Shigekawa, M.: Stretch-Activated Cation Channels in Skeletal Muscle Myotubes From Sarcoglycan-Deficient Hamsters, <i>Am J Physiol Cell Physiol</i> 281, C690, 2001 (747)	
HBSS	Stadler, G., Chen, J., Wagner, K., Robin, J., Shay, J., Emerson, C. and Wright, W.: Establishment of Clonal Myogenic Cell Lines from Severely Affected Dystrophic Muscles - CDK4 Maintains the Myogenic Population., <i>Skelet Muscle</i> 1, 12, 2011 (10667)	
DMEM	Nesti, L., Jackson, W., Shanti, R., Koehler, S., Aragon, A., Bailey, J., Sracic, M., Freedman, B., Giuliani, J. and Tuan, R.: Differentiation Potential of Multipotent Progenitor Cells Derived from War-Traumatized Muscle Tissue., <i>J Bone Joint Surg Am Vol.</i> 90, 2390, 2008 (10490)	
HBSS	Moss, S., Bates, M., Parrino, P. and Woods, T.C.: Isolation of Endothelial Cells and Vascular Smooth Muscle Cells from Internal Mammary Artery Tissue., <i>Ochsner J</i> 7, 133, 2007 (10636)	
DMEM	Kimuli, M., Eardley, I. and Southgate, J.: In Vitro Assessment of Decellularized Porcine Dermis as a Matrix for Urinary Tract Reconstruction., <i>BJU Int Vol.</i> 94, 859, 2004 (10570)	
HBSS	Richardson, M., Taylor, D., Casey, M., MacDonald, P., and Stull, J.: Biochemical Markers of Contraction in Human Myometrial Smooth Muscle Cells in Culture, <i>In Vitro Cell Dev Biol</i> 23, 21, 1987 (420)	
HBSS	Casey, M., MacDonald, P., Mitchell, M., and Snyder, J.: Maintenance and Characterization of Human Myometrial Smooth Muscle Cells in Monolayer Culture, <i>In Vitro</i> 20, 396, 1984 (533)	
DMEM	Davies, P. and Kerr, C.: Modification of LDL Metabolism by Growth Factors in Cultured Vascular Cells and Human Skin Fibroblasts, <i>Biochim Biophys Acta</i> 712, 26, 1982 (322)	
DMEM	Eskin, S., Sybers, H., Lester, J., Navarro, L., Gotto, A., and DeBakey, M.: Human Smooth Muscle Cells Cultured From Atherosclerotic Plaques and Uninvolved Vessel Wall, <i>In Vitro</i> 17 (8), 713, 1981 (864)	
HBSS	Chamley-Campbell, J., Campbell, G., and Ross, R.: The Smooth Muscle in Cell Culture, <i>Physiol Res</i> 59, 1, 1979 (648)	
GM III (see reference)	Cox, P., and Simpson, Jr., S.: A Microphotometric Study of Myogenic Lizard Cells Grown in Vitro, <i>Dev Biol</i> 23, 433, 1970 (369)	
Dulbecco-Vogt	Chait, A., Ross, R., Albers, J., and Bierman, E.: Platelet-Derived Growth Factor Stimulates Activity of LDL Receptors, <i>Proc Natl Acad Sci U S A</i> 77, 4084, 1980 (654)	
BSS	Chamley, J., Campbell, G., McConnell, J., and Groschel-Stewart, U.: Comparison of Vascular Smooth Muscle Cells from Adult Human, Monkey and Rabbit in Primary Culture and in Subculture, <i>Cell Tissue Res</i> 177, 503, 1977 (354)	
DMEM	Shi, H., Boadu, E., Mercan, F., Le, A., Roth Flach, R., Zhang, L., Tyner, K., Olwin, B. and Bennett, A.: MAP Kinase Phosphatase-1 Deficiency Impairs Skeletal Muscle Regeneration and Exacerbates Muscular Dystrophy., <i>FASEB J</i> 24, 2985, 2010 (10662)	
L-15	Johnson, B., Scheuer, T. and Catterall, W.: Convergent Regulation of Skeletal Muscle Ca ²⁺ Channels by Dystrophin, the Actin Cytoskeleton, and cAMP-Dependent Protein Kinase., <i>Proc Natl Acad Sci U S A</i> 102, 4191, 2005 (10542)	
DMEM/F12	Winitzky, S., Gopal, T., Hassanzadeh, S., Takahashi, H., Gryder, D., Rogawski, M., Takeda, K., Yu, Z., Xu, Y. and Epstein, N.: Adult Murine Skeletal Muscle Contains Cells That Can Differentiate into Beating Cardiomyocytes In Vitro., <i>PLoS Biol Vol.</i> 3, e87, 2005 (10613)	
PBS	Ieronimakis Nicholas, Balasundaram Gayathri, Reyes Morayma: Direct isolation, culture and transplant of mouse skeletal muscle derived endothelial cells with angiogenic potential, <i>PLoS ONE</i> 3, e0001753, 2008 (10313)	
M199	Li CX, Liu BH, Tong WD, Zhang LY, and Jiang YP: Dissociation, culture and morphologic changes of interstitial cells of Cajal in vitro, <i>World J Gastroenterol</i> 11, 2838, 2005 (10007)	
DMEM	Majka S., Jackson K., Kienstra K., Majesky M., Goodell M., and Hirschi K.: Distinct Progenitor Populations in Skeletal Muscle Are Bone Marrow Derived and Exhibit Different Cell Fates During Vascular Regeneration, <i>J Clin Invest</i> 111, 71, 2003 (9843)	
DMEM	Fukada S., Miyagoe-Suzuki Y., Tsukihara H., Yuasa K., Higuchi S., Ono S., Tsujikawa K., Takeda S., and Yamamoto H.: Muscle Regeneration by Reconstitution with Bone Marrow or Fetal Liver Cells from Green Fluorescent Protein-gene Transgenic Mice, <i>J Cell Sci</i> 115, 1285, 2002 (9866)	
HBSS	McKinney-Freeman SL, Jackson KA, Camargo FD, Ferrari G, Mavilio F, Goodell MA.: Muscle-derived hematopoietic stem cells are hematopoietic in origin, <i>Proc Natl Acad Sci U S A</i> 99, 1341, 2002 (10032)	
MOPS-PSS	Driska S., Laudadio R., Wolfson M., and Shaffer T.: A Method for Isolating Adult and Neonatal Airway Smooth Muscle Cells and Measuring Shortening Velocity, <i>J Appl Physiol</i> 86(1), 427, 1999 (9841)	

Tissue Dissociation Guide

Species	Muscle (con't)	Cell(s)	Enzyme(s)	
Porcine	Porcine, male, 25-40 kg	Coronary myocytes	Collagenase Type 2: 294 u/ml Elastase: 6.5 u/ml Deoxyribonuclease I: 0.04% Soybean Trypsin Inhibitor: 0.1%	
	Porcine, adult, 35-45 kg	Arterial smooth muscle	Collagenase Type 2: 294 u/ml Elastase: 6.5 u/ml Deoxyribonuclease I: 0.4 mg/ml Soybean Trypsin Inhibitor: 1 mg/ml	
	Porcine, Yorkshire, 30kg	Coronary smooth muscle	Collagenase Type 2: 150 u/ml Elastase: 0.05%	
	Porcine	Bladder smooth muscle	Collagenase Type 2: 0.1-0.2%	
	Porcine, juvenile, 30kg	Coronary smooth muscle cells	Collagenase: 0.3% Elastase: 0.05%	
	Porcine	Smooth muscle, aorta	Collagenase: 0.3%	
	Porcine	Smooth muscle Aorta	Trypsin: 0.05%	
	Porcine	Smooth muscle Aortic medial tissue	Collagenase: 0.30%	
Quail	Quail, embryo, 10 day	Myoblasts	Collagenase Type 2: 0.1%	
Rabbit	Rabbit, New Zealand White	Aortic smooth muscle	Collagenase Type 2: 300 u/ml Elastase: 5 u/ml	
	Rabbit, New Zealand, male, 2.5-3.5 kg	Enterocytes	Trypsin: 0.1%	
	Rabbit (also rat, guinea-pig)	Smooth muscle	Trypsin: 0.1%	
	Rabbit, adult, 1-2 kg	Smooth muscle	Elastase: 0.17 - 0.25%	
	Rabbit, New Zealand white, 1500 g	Smooth muscle, aortic	Trypsin: 0.038%	
	Rabbit, adult, 1-2 kg	Smooth muscle, ear artery	Trypsin: 0.1%	
	Rabbit, 0.5-1 kg (also guinea-pig, frog, dogfish)	Myocytes, heart and stomach	Protease XIV: 0.028%	
	Rabbit, white New Zealand, adult, male, 2 kg	Smooth muscle, aorta	Trypsin: 0.1%	
	Rabbits, New Zealand white albino, 5-6 months (also human, Rhesus monkey, rabbit)	Smooth muscle, saphenous vein	Elastase: 0.05%	
	Rabbit, chinchilla, 5-8 month, virgin, female, 2-3 kg	Smooth muscle, aorta	Hyaluronidase: 800 u/ml	
	Rabbit, New England, albino	Thoracic aorta	Elastase: 0.008%	
	Rat	Rat, fetal 17-18 day	Myotubes	Trypsin: 0.05%
		Rat, Lewis, neonatal	Myoblasts	Collagenase Type 2: 1.0% Neutral Protease: 2.4 u/ml
		Rat, SD, adult and neonatal w/in 1 day of birth	Myooids	Neutral Protease: 4 u/ml
Rat, SD, 3 month		Smooth muscle cells	Collagenase Type 2: 0.2% Elastase: 0.04% Soybean Trypsin Inhibitor: 0.1%	

Tissue Dissociation Guide

Medium	Reference
low calcium physiological saline	Korzick, D., Laughlin, M., and Bowles, D.: Alterations in PKC signaling underlie enhanced myogenic tone in exercise-trained porcine coronary resistance arteries, <i>J Appl Physiol</i> 96, 1425-32, 2004 (10127)
MEM	Wamhoff BR, Dixon JL, Sturek M: Atorvastatin treatment prevents alterations in coronary smooth muscle nuclear Ca ²⁺ signaling in diabetic dyslipidemia, <i>J Vasc Res</i> 39, 208, 2002 (10030)
HBSS	Sirous ZN, Fleming JB, Khalil RA: Endothelin-1 enhances eicosanoids-induced coronary smooth muscle contraction by activating specific protein kinase C isoforms, <i>Hypertension</i> 37, 497-504, 2001 (10119)
Krebs-Ringer bicarbonate	Shieh CC, Feng J, Buckner SA, Brioni JD, Coghlan MJ, Sullivan JP, Gopalakrishnan M: Functional implication of spare ATP-sensitive K(+) channels in bladder smooth muscle cells, <i>J Pharmacol Exp Ther</i> 296, 669-75, 2001 (10238)
HBSS	Huckle WR, Drag MD, Acker WR, Powers M, McFall RC, Holder DJ, Fujita T, Stabiliito II, Kim D, Ondeyka DL, Mantlo NB, Chang RS, Reilly CF, Schwartz RS, Greenlee WJ, Johnson RG: Effects of subtype-selective and balanced angiotensin II receptor antagonists in a porcine coronary artery model of vascular restenosis, <i>Circulation</i> 93, 1009-19, 1996 (10131)
DMEM	Xiong, Yimin, Xu, Shangzhe, and Slakey, Linda L: Modulation of Response to Adenosine in Vascular Smooth Muscle Cells Cultured in Defined Medium, <i>In Vitro Cell Dev Biol</i> 27, 355, 1991 (463)
EDTA 0.02%	Breton, M., Berrou, E., Deudon, E. and Picard, J.: Changes in Proteoglycans of Cultured Pig Aortic Smooth Muscle Cells During Subculture, <i>In Vitro Cell Dev Biol</i> 26, 157, 1990 (431)
DMEM	Fehr, T., Dickinson, E., Goldman, S. and Slakey, L.: Cyclic AMP Efflux is Regulated by Occupancy of The Adenosine Receptor in Pig Aortic Smooth Muscle Cells, <i>J Biol Chem</i> 265, 10974, 1990 (566)
Puck's solution	Konigsberg, I.: Skeletal Myoblasts in Culture, <i>Methods Enzymol LVIII</i> , 511, 1979 (638)
F10 Ham's	Croons Valerie, Martinet Wim, Herman Arnold G, Timmermans Jean-Pierre, De Meyer Guido R Y: Selective clearance of macrophages in atherosclerotic plaques by the protein synthesis inhibitor cycloheximide, <i>J Pharmacol Exp Ther</i> 320, 986-93, 2007 (10348)
RPMI 1640 w/ 1% fetal bovine serum PBS	Santos, M., Nguyen, B., Thompson, J.: Factors Affecting in Vitro Growth of Harvested Enterocytes, <i>Cell Transplant</i> 1, 299, 1992 (358)
Potassium buffer solution	Hu, S., and Kim, H.: Activation of K ⁺ Channel in Vascular Smooth Muscle by Cytochrome P-450 Metabolites of Arachidonic Acid, <i>FASEB J</i> 6, A383, 1992 (409)
Saline	Benham, C., Bolton, T., Byrne, N., and Large, W.: Action of Extremely Applied Adenosine Triphosphate O Single Smooth Muscle Cells Dispersed From Rabbit Ear Artery, <i>J Physiol</i> 387, 473, 1987 (863)
MEM	Knodle, S., Anderson, S., and Papaioannou, S.: Large Scale Preparation of Rabbit Aortic Smooth Muscle Cells For Use in Calcium Uptake Studies, <i>In Vitro Cell Dev Biol</i> 22, 23, 1986 (416)
CF solution (see reference)	Benham, C.D., Bolton, T.B.: Spontaneous Transient Outward Currents in Single Visceral and Vascular Smooth Muscle Cells of the Rabbit, <i>J Physiol</i> 381, 385, 1986 (720)
Solution C (see reference)	Mitra, R. and Morad, M.: A Uniform Enzymatic Method for Dissociation of Myocytes from Hearts and Stomachs of Vertebrates, <i>Am J Physiol</i> 249, H1056, 1985 (294)
Krebs Ringer HEPES solution	Ives, H., Schultz, G., Galardy, R., and Jamieson, J.: Preparation of Functional Smooth Muscle Cells from the Rabbit Aorta, <i>J Exp Med</i> 148, 1400, 1978 (603)
BSS	Chamley, J., Campbell, G., McConnell, J., and Groschel-Stewart, U.: Comparison of Vascular Smooth Muscle Cells from Adult Human, Monkey and Rabbit in Primary Culture and in Subculture, <i>Cell Tissue Res</i> 177, 503, 1977 (354)
HBSS	Peters, T., Muller, M., and deDuve, C.: Lysosomes of the Arterial Wall. I. Isolation and Subcellular Fractionation of Cells from Normal Rabbit Aorta, <i>J Exp Med</i> 136, 1117, 1972 (601)
Kreb's Ringer (see reference)	Day, A., Phil, D., and Newman, H.: Synthesis of Phospholipid by Foam Cells Isolated from Rabbit Atherosclerotic Lesions, <i>Circ Res XIX</i> , 122, 1966 (777)
	Das, M., Rumsey, J., Bhargava, N., Stancescu, M. and Hickman, J.: Skeletal Muscle Tissue Engineering: A Maturation Model Promoting Long-Term Survival of Myotubes, Structural Development of the Excitation-Contraction Coupling Apparatus and Neonatal Myosin Heavy Chain Expression., <i>Biomaterials</i> 30, 5392, 2009 (10654)
Ham's F-10	Kim, J., Hadlock, T., Cheney, M., Varvares, M. and Marler, J.: Muscle Tissue Engineering for Partial Glossectomy Defects., <i>Arch Facial Plast Surg</i> 5, 403, 2003 (10637)
Ham's F-12	Dennis, R., Kosnik II, P., Gilbert, M., and Faulkner, J.: Excitability and Contractility of Skeletal Muscle Engineered from Primary Cultures and Cell Lines, <i>Am J Physiol Cell Physiol</i> 280, C288, 2001 (1111)
M-199	Su E., Stevenson S., Rollence M., Marshall-Neff J., and Liao G.: A Genetically Modified Adenoviral Vector Exhibits Enhanced Gene Transfer of Human Smooth Muscle Cells, <i>J Vasc Res</i> 38(5), 471, 2001 (9860)

Tissue Dissociation Guide

Species

Muscle (con't)

Cell(s)

Enzyme(s)

Rat, SD, 250 g	Arterial smooth muscle	Papain: 0.03% Collagenase: 0.1%
Rat, Wistar, adult, male	Smooth & skeletal muscle Cardiac myocytes	Protease: 0.01%
Rat, SD, male, 250-350 g	Vascular smooth muscle	Collagenase Type 2: 0.1% Elastase: 0.0125%
Rat, SD, male, 150-175 g	Smooth muscle, endothelial	Trypsin: 0.04%
Rat, Wistar Kyoto, 10-15 weeks	Smooth muscle, mesenteric artery	Trypsin: 0.05%
Rat (also rabbit, guinea-pig)	Smooth muscle	Trypsin: 0.1%
Rat, SD, 19 days	Smooth muscle, myometrial	Trypsin: 150 µg/ml
Rat, SHRs and WKY, male, 10 - 14 weeks	Smooth muscle, tail arteries	Papain: 0.1%
Rat, 1-3 day	Smooth muscle, aortic	Elastase: 0.0125%
Rat	Muscle, mesenteric arteries	Trypsin: 0.05%
Rat, SHR, WKY. either sex, 12-19 day, 3 month, and retired breeders	Endothelial, aortic	Elastase: 0.05%
Rat, male, 150-250 g	Endothelial, aortic	Trypsin:
Rat, Wistar, female, 10 weeks	Myocytes	Collagenase: 0.1%
Rat, SD, male, 200 - 250 g	Smooth muscle, thoracic aorta	Trypsin: 0.0375%
Rat, SD, 225-250g	Mesenteric artery smooth muscle cells	Elastase: .0125% Soybean Trypsin Inhibitor: 0.025% Collagenase Type 1: 0.1%
Rat, 3-4 day	Myocardial	Trypsin NF 1:250: 0.125%
Rat, 200 g	Muscle	Trypsin: 0.05%
Rat, Wistar, 3-10 day	Heart	Trypsin NF 1:250: 250: 0.1%

Neural

Species

Cell(s)

Enzyme(s)

Bovine	Bovine (also rat)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%
	Bovine	Microvascular endothelial	Collagenase/Dispase: 0.1%
Chicken	Calf (also lamb)	Oligodendroglia Neural	Trypsin: 0.1%
	Chicken, White Leghorn, embryos, 17-21 day	Neurons	Papain: 40 u/ml
	Chick, embryo, 10-14 day	Flat, retina	Trypsin: 0.1%
	Chick, White Leghorn, embryo (also rat, SD, embryo)	Spinal cord	Trypsin: 0.05%
	Chick	Dorsal root ganglion neurons Spinal cord	Trypsin: 0.1%
	Chick, White Leghorn embryo, 8 day	Neurons, ganglia	Trypsin: 0.25%
Chicken, White Leghorn, fertile eggs	Ciliary ganglion neurons	Trypsin: 0.25%	

Tissue Dissociation Guide

Medium	Reference
(see reference)	Jaggar JH: Intravascular pressure regulates local and global Ca(2+) signaling in cerebral artery smooth muscle cells, <i>Am J Physiol Cell Physiol</i> 281, C439-48, 2001 (10325)
PSS	Wellman, G., Barrett-Jolley, R., Koppel, H. Everitt, D., and Quayle, J.: Inhibition of Vascular K _{ATP} Channels by U-37883A: A Comparison with Cardiac and Skeletal Muscle, <i>Br J Pharmacol</i> 128, 909, 1999 (1065)
DMEM	Hrometz, S., Edelmann, S., McCune, D., Olges, J., Hadley, R., Perez, D., and Piascik, M.: Expression of Multiple Alpha1-Adrenoceptors on Vascular Smooth Muscle: Correlation with the Regulation of Contraction, <i>J Pharmacol Exp Ther</i> 290(1), 452, 1999 (9867)
MEM	Redmond, E., Cahill, P., and Sitzmann, J.: Perfused Transcapillary Smooth Muscle and Endothelial Cell Co-Culture-A Novel <i>In Vitro</i> Model, <i>In Vitro Cell Dev Biol Anim</i> 31, 601, 1995 (1234)
MEM	McGuire, P., Walker-Caprioglio, H., Little, S., and McGuffee, L.: Isolation and Culture of Rat Superior Mesenteric Artery Smooth Muscle Cells, <i>In Vitro Cell Dev Biol</i> 29, 135, 1993 (491)
Potassium buffer solution	Hu, S., and Kim, H.: Activation of K ⁺ Channel in Vascular Smooth Muscle by Cytochrome P-450 Metabolites of Arachidonic Acid, <i>FASEB J</i> 6, A383, 1992 (409)
HBSS or PSS, CMF	Loch-Caruso, R., Pahl, M., and Juberg, D.: Rat Myometrial Smooth Muscle Cells Show High Levels of Gap Junctional Communication Under a Variety of Culture Conditions, <i>In Vitro Cell Dev Biol</i> 28, 97, 1992 (489)
HEPES buffer (see reference)	Bolzon, B. and Cheung, D.: Isolation and Characterization of Single Vascular Smooth Muscle Cells From Spontaneously Hypertensive Rats, <i>Hypertension</i> 14, 137, 1989 (694)
DMEM	Barone, L., Wolfe, L., Faris, B., and Franzblau, C.: Elastin mRNA Levels and Insoluble Elastin Accumulation in Neonatal Rat, <i>Biochem</i> 27, 3175, 1988 (313)
HEPES KG solution (see reference)	Bean, B., Sturek, M., Puga, A., and Hermsmeyer, K.: Calcium Channels in Muscle Cells Isolated From Rat Mesenteric Arteries: Modulation by Dihydropyridine Drugs, <i>Circ Res</i> 59, 229, 1986 (364)
Waymouth's culture medium	Gordon, D., Mohai, L., and Schwartz, S.: Induction of Polyploidy in Cultures of Neonatal Rat Aortic Smooth Muscle Cells, <i>Circ Res</i> 59, 633, 1986 (866)
RPMI 1640	Cole, O., Fan, T., and Lewis, G.: Isolation, Characterization, Growth and Culture of Endothelial Cells From the Rat Aorta, <i>Cell Biol Int Rep</i> 10 (6), 399, 1986 (884)
HBSS	Boulanger-Saunier, C., Kattenburg, D., and Stoclet, J.: Cyclic AMP-dependent Phosphorylation of a 16kDa Protein in a Plasma Membrane-enriched Fraction of Rat Aortic Myocytes, <i>FEBS Lett</i> 193, 283, 1985 (411)
Eagle's MEM with calcium	Brock, T., Alexander, R., Ekstein, L., Atkinson, W., and Gimbrone, M.: Angiotensin Increases Cytosolic Free Calcium in Cultured Vascular Smooth Muscle Cells, <i>Hypertension</i> 7, 105, 1985 (693)
HBSS	Gunther S, Alexander RW, Atkinson WJ, and Gimbrone MA Jr.: Functional angiotensin II receptors in cultured vascular smooth muscle cells, <i>J Cell Biol</i> 92, 289, 1982 (10058)
HBSS CMF	Kasten, F.: Rat Myocardial Cells <i>In Vitro</i> : Mitosis and Differentiated Properties, <i>In Vitro</i> 8, 128, 1972 (539)
Kreb's Henseleit bicarbonate buffer	Kono, T.: Roles of Collagenases and Other Proteolytic Enzymes in the Dispersal of Animal Tissues, <i>Biochim Biophys Acta</i> 178, 397, 1969 (317)
Saline A (see reference)	Harary, I., and Farley, B.: <i>In Vitro</i> Studies on Single Beating Rat Heart Cells, <i>Exp Cell Res</i> 29, 451, 1963 (395)

Neural

Medium	Reference
25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, <i>In Vitro Cell Dev Biol</i> 26, 335, 1990 (438)
MEM	Bowman, P., Betz, A., and Goldstein, G.: Primary Culture of Microvascular Endothelial Cells From Bovine Retina, <i>In Vitro</i> 18 (7), 626, 1982 (945)
(see reference)	Poduslo, S., Miller, K., and McKhann, G.: Metabolic Properties of Maintained Oligodendroglia Purified from Brain, <i>J Biol Chem</i> 253, 1592, 1978 (552)
HEPES	Raman, I., and Trussell, L.: The Kinetics of the Response to Glutamate and Kainate in Neurons of the Avian and Cochlear Nucleus, <i>Neuron</i> 9, 173, 1992 (692)
Tyrode's solution, CMF	Moyer, M., Bullrich, F., and Sheffield, J.: Emergence of Flat Cells From Glia in Stationary Cultures of Embryonic Chick Neural Retina, <i>In Vitro Cell Dev Biol</i> 26, 1073, 1990 (427)
Phosphate buffer (see reference)	Schnaar, R., and Schaffner, A.: Separation of cell types from embryonic chicken and rat spinal cord: characterization of motoneuron-enriched fractions, <i>J Neurosci</i> 1, 204, 1981 (610)
Puck's saline, CMF	Choi, D., and Fischbach, G.: GABA Conductance of Chick Spinal Cord and Dorsal Root Ganglion Neurons in Cell Culture, <i>J Neurophysiol</i> 45, 605, 1981 (717)
HBSS, CMF	Bottenstein, J., Skaper, S., Varon, S., and Sato, G.: Selective Survival of Neurons from Chick Embryo Sensory Ganglionic Dissociates Utilizing Serum-Free Supplemented Medium, <i>Exp Cell Res</i> 125, 183, 1980 (388)
Eagle's MEM	Tuttle, J., Suszkiw, J., and Ard, M.: Long-Term Survival and Development of Dissociated Parasympathetic Neurons in Culture, <i>Brain Res</i> 183, 161, 1980 (994)

Tissue Dissociation Guide

Species	Neural (con't)	Cell(s)	Enzyme(s)	
Fish	Chicken, embryos, 9-10 days old	Dorsal root ganglia neurons	Collagenase: 0.01%	
	Chick embryos	Ganglion chains, sympathetic ganglia	Trypsin: 0.25%	
	Black ghose knige fish, adult (<i>Apteronotus albifrons</i>)	Neurons, spinal cord	Trypsin: 0.4%	
Frog	<i>Xenopus</i> , embryonic	Neuron	Collagenase Type 1: 0.1%	
Guinea-Pig	Guinea-pig, newborn	Neuron, enteric	Trypsin: 0.125%	
Hamster	Hamster, male	Vomer nasal organ neurons	Collagenase Type 1: 0.02% Trypsin: 0.02%	
Human	Human	Neural, various	Papain: 12 u/ml Trypsin: see reference Collagenase/Dispase: see reference	
	Human, 9 month	Neurons	Collagenase Type 4: 1.33% Papain: 0.07 u/ml Neutral Protease: 1 mg/ml	
	Human, 26 week	Neural progenitor cells	Papain: 2.5 u/ml Deoxyribonuclease I: 250 u/ml Neutral Protease: 1 u/ml	
	Human, adult	Ventricular epithelial	Papain: 11.4 u/ml Deoxyribonuclease I: 10 u/ml	
	Human, 5-65 years	Retinal pigment epithelial (RPE)	Trypsin: 0.25%	
	Human	Dorsal root ganglion neurons	Collagenase Type 1: 0.2% Neutral Protease: 0.5%	
	Insect	<i>Drosophila</i>	Dendrites	Collagenase: 0.05% Neutral Protease: 0.2%
		<i>Gryllus Bimaculatus</i>	Giant interneurons	Collagenase: 0.05% Neutral Protease: 0.2%
Mouse	Mouse, embryo, 14 day	Spinal cord neurons	Papain: 0.05% Deoxyribonuclease I: 0.004%	
	Mouse, 1-2 day	Oligodendrocytes, dorsal root ganglia	Papain: 0.15% Deoxyribonuclease I: 0.006%	
	Mouse	Hippocampal and retinal neurons	Papain: 1% Deoxyribonuclease I: 5 u/ml	
	Mouse, adult	Spinal microganglia	Papain: 0.2%	
	Mouse, neonatal	DRG neurons	Collagenase: 0.2% Trypsin: 0.05%	
	Mouse, 6-8 week	Sensory neurons, DRG	Papain: 20 u/ml Collagenase Type 2: 0.4% Neutral Protease: 0.46%	
	Mouse, male	Trigeminal sensory neurons	Papain: 20 u/ml	
	Mouse, embryonic	Dopaminergic neurons	Trypsin: 0.1% Deoxyribonuclease I: 0.02%	
	Mouse, 6 mo	Neurons, neurospheres	Papain: 0.2%	

Tissue Dissociation Guide

Medium	Reference	Neural (con't)
Eagle's MEM	Mudge, A., Leeman, S., and Fischbach, G.: Enkephalin Inhibits Release of Substance P From Sensory Neurons in Culture and Decreases Action Potential Duration, <i>Proc Natl Acad Sci U S A</i> 76 (1), 526, 1979 (995)	
Krebs Phosphosaline	McCarthy, K., and Partlow, L.: Preparation of Pure Neuronal and Non-Neuronal Cultures From Embryonic Chick Sympathetic Ganglia: A New Method Based on Both Differential Cell Adhesiveness and the Formation of Homotypic Neuronal Aggregates, <i>Brain Res</i> 114, 391, 1976 (345)	
PBS, CMF	Anderson, M.J.: Differences in Growth of Neurons from Normal and Regenerated Teleost Spinal Cord in vitro, <i>In Vitro Cell Dev Biol</i> 29A, 145, 1993 (492)	
Steinberg's solution	Takahashi, T., Nakajima, Y., Hirose, K., Nakajima, S., and Onodera, K.: Structure and Physiology of Developing Neuromuscular Synapses in Culture, <i>J Neurosci</i> 7, 473, 1987 (619)	
Medium 199	Jessen, K, McConnell, J., Purves, R., Burnstock, G., and Chamley-Campbell, J.: Tissue Culture of Mammalian Enteric Neurons, <i>Brain Res</i> 152, 573, 1978 (347)	
PBS	Liman ER: Regulation by voltage and adenine nucleotides of a Ca ²⁺ -activated cation channel from hamster vomeronasal sensory neurons, <i>J Physiol</i> 548, 777, 2003 (10044)	
(see reference)	Panchision David M, Chen Hui-Ling, Pistollato Francesca, Papini Daniela, Ni Hsiao-Tzu, Hawley Teresa S: Optimized flow cytometric analysis of central nervous system tissue reveals novel functional relationships among cells expressing CD133, CD15, and CD24, <i>Stem Cells</i> 25, 1560-70, 2007 (10297)	
DMEM/F12	Dietrich J, Lacagnina M, Gass D, Richfield E, Mayer-Proschel M, Noble M, Torres C, Proschel C.: EIF2B5 mutations compromise GFAP+ astrocyte generation in vanishing white matter leukodystrophy, <i>Nat Med</i> 11, 277, 2005 (10046)	
DMEM/F-12	Fuja TJ, Schwartz PH, Darcy D, Bryant PJ.: Asymmetric localization of LGN but not AGS3, two homologs of Drosophila pins, in dividing human neural progenitor cells, <i>J Neurosci Res</i> 75, 782, 2004 (10045)	
DMEM/F12	Roy NS, Benraiss A, Wang S, Fraser RA, Goodman R, Couldwell WT, Nedergaard M, Kawaguchi A, Okano H, Goldman SA: Promoter-targeted selection and isolation of neural progenitor cells from the adult human ventricular zone, <i>J Neurosci Res</i> 59, 321, 2000 (10038)	
HBSS	Von Recum, H., Okano, T., Kim, S, and Bernstein, P.: Maintenance of Retinoid Metabolism in Human Retinal Pigment Epithelium Cell Culture, <i>Exp Eye Res</i> 69, 97, 1999 (1185)	
DMEM/F-12	Dib-Hajj SD, Tyrrell L, Cummins TR, Black JA, Wood PM, Waxman SG.: Two tetrodotoxin-resistant sodium channels in human dorsal root ganglion neurons, <i>FEBS Lett</i> 462, 117, 1999 (10042)	
HBSS	Sanchez-Soriano, N., Bottenberg, W., Fiala, A., Haessler, U., Kerassoviti, A., Knust, E., Lohr, R. and Prokop, A.: Are Dendrites in Drosophila Homologous to Vertebrate Dendrites?, <i>Dev Biol</i> 288, 126, 2005 (10367)	
Leibovitz's L15	Kloppenburg, P. and Horner, M.: Voltage-Activated Currents in Identified Giant Interneurons Isolated from Adult Crickets <i>Gryllus Bimaculatus</i> , <i>J Exp Biol</i> 201 (Pt 17), 2529, 1998 (10366)	
PBS/DMEM	Pollari, E., Savchenko, E., Jaronen, M., Kanninen, K., Malm, T., Wojciechowski, S., Antoniemi, T., Goldsteins, G., Giniatullina, R., Giniatullin, R., Koistinaho, J. and Magga, J.: Granulocyte Colony Stimulating Factor Attenuates Inflammation in a Mouse Model of Amyotrophic Lateral Sclerosis., <i>J Neuroinflammation Vol. 8</i> , , 74, 2011 (10576)	
DMEM	O'Meara, R., Ryan, S., Colognato, H. and Kothary, R.: Derivation of Enriched Oligodendrocyte Cultures and Oligodendrocyte/Neuron Myelinating Co-Cultures from Post-Natal Murine Tissues., <i>J Vis Exp</i> 54, 3324, 2011 (10650)	
HBSS	Brown, J., Gianino, S. and Gutmann, D.: Defective cAMP Generation Underlies the Sensitivity of CNS Neurons to Neurofibromatosis-1 Heterozygosity, <i>J Neurosci</i> 30, 5579, 2010 (10545)	
Hibernate A	Yip, P., Kaan, T., Fenesan, D. and Malcangio, M.: Rapid Isolation and Culture of Primary Microglia from Adult Mouse Spinal Cord., <i>J Neurosci Methods</i> 183, 223-37, 2009 (10574)	
Ham's F12	Pedrola, L., Espert, A., Valdes-Sanchez, T., Sanchez-Piris, M., Sirkowski, E., Scherer, S., Farinas, I., and Palau, F.: Cell Expression of GDAP1 in the Nervous System and Pathogenesis of Charcot-Marie-Tooth Type 4A Disease., <i>J Cell Mol Med Vol. 12</i> , 679, 2008 (10508)	
HBSS	Malin, S., Davis, B. and Molliver, D.: Production of Dissociated Sensory Neuron Cultures and Considerations for their use in Studying Neuronal Function and Plasticity, <i>Nat Protoc</i> 2, 152, 2007 (10623)	
HEPES buffered saline	Roberts, L., MacDonald, C. and Mark, C.: Anandamide is a Partial Agonist at Native Vanilloid Receptors in Acutely Isolated Mouse Trigeminal Sensory Neurons., <i>Br J Pharmacol</i> 137, 421, 2002 (10625)	
DMEM	Radad Khaled, Gille Gabriele, Rausch Wolf-Dieter: Dopaminergic neurons are preferentially sensitive to long-term rotenone toxicity in primary cell culture, <i>Toxicol In Vitro</i> 22, 68-74, 2008 (10347)	
Hibernate	Brewer Gregory J, Torricelli John R: Isolation and culture of adult neurons and neurospheres, <i>Nat Protoc</i> 2, 1490-8, 2007 (10095)	

Tissue Dissociation Guide

Species

Neural (con't)

Cell(s)

Enzyme(s)

	Mouse	Neural, various	Papain: 12 u/ml Trypsin: see reference Collagenase/Dispase: see reference
	Mouse, C57BL/6, 1 year	Neurons	Papain: 0.2%
	Mouse, adult	Brain and spinal cord cells	Trypsin: 0.25%
	Mouse, neonatal	Neurons	PDS kit:
	Mouse, C57BL	Cerebellar granule cell precursors	Papain: 16.5 u/ml Deoxyribonuclease I: 0.008%
	Mouse, 1-3 day	Neurons, ganglia	Papain: 20 u/ml Deoxyribonuclease I: 100 u/ml Collagenase: 0.3% Trypsin: 0.05%
	Mouse, postnatal-day-1-old (P1)	Neurons	Trypsin: 0.25%
	Mouse embryos	Neurons, DRG and SCG	Trypsin: 0.25%
	mouse, 65 days	Neurons, neuronal precursors	Trypsin: 0.1% Deoxyribonuclease I: 0.001%
	Mouse, CD-1, neonate	Neurons, dorsal root ganglion	Trypsin: 0.25%
	Mouse, fetal	Precursor	Trypsin: 0.5%
	Mouse (SWR or CF1), 1-3 months	Papillae, taste receptor	Pronase E: 0.15%
	Mouse, neonatal (also chick)	PNS test neurons	Trypsin: 0.08%
	Mouse (BALB/c), adult	Neurons, spinal cord	Collagenase Type 3: 0.25%
	Mouse, 0-30 day	Neural	Trypsin NF 1:250: 50 0.25%
Porcine	Porcine, adult, 60-100 kg	Superior cervical ganglia	Papain: 2 u/ml Collagenase: 0.12% Neutral Protease: 0.48%
Quail	Quail	Neural crest	Trypsin: 0.05%
Rat	Rat, SD, 200-300 g	Dorsal root ganglia, fibroblast	Collagenase: 0.125%
	Rat, Fisher, 7-21 month	Hippocampal neurons	Papain: 0.2%
	Rat, SD, 7 day	Cerebellar granule neurons	PDS kit: per instructions
	Rat, SD, embryonic day 18	Hippocampal neurons	Papain: 20 u/ml
	Rat, Wistar, 7 day	Superior cervical ganglion	Collagenase: 0.05%
	Rat, SD, neonatal	Astrocytes	PDS kit: per instructions
	Rat, SD, male, 5-8 week	Trigeminal neurons	Papain: 20 u/ml Collagenase: 0.3%

Tissue Dissociation Guide

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Medium	Reference
(see reference)	Panchision David M, Chen Hui-Ling, Pistollato Francesca, Papini Daniela, Ni Hsiao-Tzu, Hawley Teresa S: Optimized flow cytometric analysis of central nervous system tissue reveals novel functional relationships among cells expressing CD133, CD15, and CD24, <i>Stem Cells</i> 25, 1560-70, 2007 (10297)
DMEM	Eide, L, and McMurray, C: Culture of Adult Mouse Neurons, <i>Biotechniques</i> 38(1), 99-104, 2005 (9787)
PBS	Gonzalez John M, Bergmann Cornelia C, Fuss Babette, Hinton David R, Kangas Cindy, Macklin Wendy B, Stohman Stephen A: Expression of a dominant negative IFN-gammareceptor on mouse oligodendrocytes, <i>Glia</i> 51, 22-34, 2005 (10111)
EBSS	Gill JC, Moenter SM, Tsai PS: Developmental regulation of gonadotropin-releasing hormone neurons by fibroblast growth factor signaling, <i>Endocrinology</i> 145, 3830, 2004 (10021)
Dulbecco's PBS	Okano-Uchida T, Himi T, Komiya Y, and Ishizaki Y: Cerebellar granule cell precursors can differentiate into astroglial cells, <i>Proc Natl Acad Sci U S A</i> 101, 1211, 2004 (10061)
HBSS	Savchenko V, Sung U, Blakely RD.: Cell surface trafficking of the antidepressant-sensitive norepinephrine transporter revealed with an ectodomain antibody, <i>Mol Cell Neurosci</i> 24, 1131, 2003 (10040)
NGF-containing medium	Deshmukh, M., Kuida, K., and Johnson Jr., E.: Caspase Inhibition Extends the Commitment to Neuronal Death Beyond Cytochrome c Release to the Point of Mitochondrial Depolarization, <i>J Cell Biol</i> 150 (1), 131, 2000 (1121)
L-15 medium	Lee, K., Davies, A., and Jaenisch, R.: P75-Efficient Embryonic Dorsal Root Sensory and Neonatal Sympathetic Neurons Display a Decreased Sensitivity to NGF, <i>Development</i> 120, 1027, 1994 (1084)
DMEM	Richards L., Kilpatrick T., and Bartlett P.: De Novo Generation of Neuronal Cells from the Adult Mouse Brain, <i>Proc Natl Acad Sci U S A</i> 89, 8591, 1992 (9807)
HBSS	Quinn, S. and De Boni, U.: Enhanced Neuronal Regeneration by Retinoic Acid of Murine Dorsal Root Ganglia and of Fetal Murine and human Spinal Cord in vitro, <i>In Vitro Cell Dev Biol</i> 27, 55, 1991 (468)
PBS	Kitani, H., Shiurba, R., Sakakura, T., Tomooka, Y.: Isolation and Characterization Of Mouse Neural Precursor Cells in Primary Culture, <i>In Vitro Cell Dev Biol</i> 27, 615, 1991 (470)
Carbonate-Phosphate buffer (see reference)	Spielman, A., Mody, I., Brand, J., Whitney, G., MacDonald, J., and Salter, M.: A Method for Isolating and Patch-Clamping Single Mammalian Taste Receptor Cells, <i>Brain Res</i> 503, 326, 1989 (350)
Eagle's Basal Medium (see reference)	Varon, S., Skaper, S., Barbin, G., Selak, I., and Manthorpe, M.: Low Molecular Weight Agents Support Survival of Cultured Neurons From the Central Nervous System, <i>J Neurosci</i> 4 (3), 654, 1984 (1000)
Hank's BSS, CMF	Eagleson, K. and Bennett, M.: Survival of Purified Motor Neurons <i>In Vitro</i> : Effects of Skeletal Muscle-Conditioned Medium, <i>Neurosci Lett</i> 38, 187, 1983 (645)
BSS	Shrier, B., Wilson, S., and Nirenberg, M.: Cultured Cell Systems and Methods for Neurobiology, Vol. 32, 765, 1974 (637)
HBSS	Si ML, Lee TJ.: Presynaptic alpha7-nicotinic acetylcholine receptors mediate nicotine-induced nitric oxidergic neurogenic vasodilation in porcine basilar arteries, <i>J Pharmacol Exp Ther</i> 298, 122, 2001 (10052)
MEM, HBSS	Sieber-Blum, M., and Cohen, A.: Clonal Analysis of Quail Neural Crest Cells: They are Pluripotent and Differentiate in Vitro in the Absence of Noncrest Cells, <i>Dev Biol</i> 80, 96, 1980 (371)
DMEM	East, E., de Oliveira, D., Golding, J. and Phillips, J.: Alignment of Astrocytes Increases Neuronal Growth in Three-Dimensional Collagen Gels and is Maintained Following Plastic Compression to Form a Spinal Cord Repair Conduit., <i>Tissue Eng Part A Vol. 16</i> , 3173, 2010 (10634)
Hibernate A	Chen, N., Newcomb, J., Garbuzova-Davis, S., Davis Sanberg, C., Sanberg, P. and Willing, A.: Human Umbilical Cord Blood Cells Have Trophic Effects on Young and Aging Hippocampal Neurons in Vitro., <i>Aging Dis</i> 1, 173, 2010 (10663)
PBS	Tanaka, S., Shaikh, I., Chiocca, E. and Saeki, Y.: The Gs-Linked Receptor GPR3 Inhibits the Proliferation of Cerebellar Granule Cells During Postnatal Development., <i>PLoS ONE</i> 4, e5922, 2009 (10487)
Neurobasal/B27	Liu, Y., Yohrling, G., Wang, Y., Hutchinson, T., Brenneman, D., Flores, C., Zhao, B.: Carisbamate, a Novel Neuromodulator, Inhibits Voltage-Gated Sodium Channels and Action Potential Firing of Rat Hippocampal Neurons., <i>Epilepsy Res Vol. 83</i> , 66, 2009 (10550)
L-15	Sakisaka, T., Yamamoto, Y., Mochida, S., Nakamura, M., Nishikawa, K., Ishizaki, H., Okamoto-Tanaka, M., Miyoshi, J., Fujiyoshi, Y., Manabe, T. and Takai, Y.: Dual Inhibition of SNARE Complex Formation by Tomosyn Ensures Controlled Neurotransmitter Release., <i>J Cell Biol</i> 183, 323, 2008 (10547)
DMEM	Lacroix-Fralish, M., Tawfik, V., Nutile-McMenemy, N., Harris, B. and Deleo, J.: Differential Regulation of Neuregulin 1 Expression by Progesterone in Astrocytes and Neurons., <i>Neuron Glia Biol Vol. 2</i> , 227, 2006 (10679)
CMF Hanks	Connor, M, Naves, L. and McCleskey, E.: Contrasting Phenotypes of Putative Proprioceptive and Nociceptive Trigeminal Neurons Innervating Jaw Muscle in Rat., <i>Mol Pain Vol. 1</i> , 31, 2005 (10624)

Tissue Dissociation Guide

Species

Neural (con't)

Cell(s)

Enzyme(s)

Rat, 1-6 day	DRG neurons	Papain: 20 u/ml Collagenase Type 1: 150 u/ml Neutral Protease: 0.8%
Rat, embryonic	Hypothalamic neurons and glia	Papain: 0.5 u/ml
Rat, Wistar, 4 day	Hippocampal neurons	PDS kit:
Rat, 2 month	Spinal cord progenitor cells Hybrid toxins	PDS kit: see reference
Rat, SD, E19	Dorsal root ganglia	PDS kit:
Rat, Wistar, E15	Vomeronal receptor neurons	Collagenase/Dispase: 0.1% Papain: 0.5 u/ml
Rat, SD, 1-2 day	Cortical astrocytes	Papain: see reference
Rat, SD, 0-2 day	Superior cervical ganglion	Collagenase Type 4: 20 u/ml Trypsin: 0.25%
Rat, fetal	Brainstem and cortical neurons	PDS kit:
Rat, SD, male 150-220 g	Dorsal root ganglion neurons	Collagenase Type 4: 0.125% Trypsin: 0.05%
Rat, 15 week	Neurons	Papain: 0.2%
Rat, SD, male, 270-330 g	Spinal progenitor cells	PDS kit: with modifications
Rat, Fisher, 8-9 week	Adult progenitor	Papain: 2.5 u/ml Deoxyribonuclease I: 250 u/ml Neutral Protease: 1 u/ml
Rat	Neurons, hippocampal	Papain: 15 - 20 u/ml
Rat, newborn, 7 days old	Neurons	Trypsin: 0.25%
Rat, embryonic 18-19 day	Hippocampal neurons	PDS kit: see reference
Rat, embryonic, day 18	Cortical	Papain:
Rat, Wistar, postnatal, P1-3 days	Neurons, hippocampal	Papain: 20 u/ml
Rat, SD, female, pregnant	Sciatic nerves	Trypsin: 0.025%
Rat (also mice)	Spinal cord	Trypsin: 0.133%
Rat, (Long Evans), 2-5 day old	Neurons, hippocampal	Papain: 20 u/ml
Rat, Wistar and SD, newborn, 0-21 days, either sex	Myenteric ganglia	Trypsin: 0.05%
Rat, pups, 24-48 h old	Neurons, hippocampal	Papain: 20 u/ml

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HBSS	Robertson, S., Rae, M., Rowan, E. and Kennedy, C.: Characterization of a P2X-Purinoceptor in Cultured Neurones of the Rat Dorsal Root Ganglia., <i>Br J Pharmacol</i> 118, 951, 1996 (10618)
DMEM	Yokosuka Makoto, Ohtani-Kaneko Ritsuko, Yamashita Kayoko, Muraoka Daisuke, Kuroda Yoichiro, Watanabe Chiho: Estrogen and environmental estrogenic chemicals exert developmental effects on rat hypothalamic neurons and glias, <i>Toxicol In Vitro</i> 22, 1-9, 2008 (10346)
Neurobasal A	Obradovic Darja, Gronemeyer Hinrich, Lutz Beat, Rein Theo: Cross-talk of vitamin D and glucocorticoids in hippocampal cells, <i>J Neurochem</i> 96, 500-9, 2006 (10108)
Neurobasal A	Mothe, A., Kulbatski, I., Van Bendegem, R., Lee, L., Kobayashi, E., Keating, A., and Tator, C.: Analysis of Green Fluorescent Protein Expression in Transgenic Rats for Tracking Transplanted Neural Stem/ Progenitor Cells, <i>J Histochem Cytochem</i> 53(10), 1215, 2005 (1031)
MEM/Ham's F12	Gavva NR, Tamir R, Qu Y, Klionsky L, Zhang TJ, Immke D, Wang J, Zhu D, Vanderah TW, Porreca F, Doherty EM, Norman MH, Wild KD, Bannon AW, Louis JC, Treanor JJ.: AMG 9810 [(E)-3-(4-t-butylphenyl)-N-(2,3-dihydrobenzo[b][1,4] dioxin-6-yl)acrylamide], a novel vanilloid receptor 1 (TRPV1) antagonist with antihyperalgesic properties, <i>J Pharmacol Exp Ther</i> 313, 474, 2005 (10025)
DMEM/F12	Moriya-Ito K, Osada T, Ishimatsu Y, Muramoto K, Kobayashi T, Ichikawa M: Maturation of vomeronasal receptor neurons in vitro by coculture with accessory olfactory bulb neurons, <i>Chem Senses</i> 30, 111, 2005 (10036)
DMEM	Floyd Candace L, Gorin Fredric A, Lyeth Bruce G: Mechanical strain injury increases intracellular sodium and reverses Na ⁺ /Ca ²⁺ exchange in cortical astrocytes, <i>Glia</i> 51, 35-46, 2005 (10113)
DMEM	Pedraza Carlos E, Podlesniy Petar, Vidal Noemi#, Arevalo Juan Carlos, Lee Ramee, Hempstead Barbara, Ferrer Isidre, Iglesias Montse, Espinet Carme: Pro-NGF isolated from the human brain affected by Alzheimer's disease induces neuronal apoptosis mediated by p75NTR, <i>Am J Pathol</i> 166, 533-43, 2005 (10296)
DMEM	Lovshin JA, Huang Q, Seaberg R, Brubaker PL, Drucker DJ: Extrahypothalamic expression of the glucagon-like peptide-2 receptor is coupled to reduction of glutamate-induced cell death in cultured hippocampal cells, <i>Endocrinology</i> 145, 3495, 2004 (10037)
DMEM/Ham's F12	Hu Hong-Zhen, Gu Qihai, Wang Chunbo, Colton Craig K, Tang Jisen, Kinoshita-Kawada Mariko, Lee Lu-Yuan, Wood Jackie D, Zhu Michael X: 2-aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3, <i>J Biol Chem</i> 279, 35741-8, 2004 (10255)
Hibernate A	Evans J, Sumners C, Moore J, Huentelman MJ, Deng J, Gelband CH, and Shaw G: Characterization of mitotic neurons derived from adult rat hypothalamus and brain stem, <i>J Neurophysiol</i> 87, 1076, 2002 (10059)
Neurobasal medium	Lin CR, Wu PC, Shih HC, Cheng JT, Lu CY, Chou AK, Yang LC.: Intrathecal spinal progenitor cell transplantation for the treatment of neuropathic pain, <i>Cell Transplant</i> 11, 17, 2002 (10027)
DMEM/F-12	Lie DC, Dziejczapolski G, Willhoite AR, Kaspar BK, Shults CW, Gage FH.: The adult substantia nigra contains progenitor cells with neurogenic potential, <i>J Neurosci</i> 22, 6639, 2002 (10039)
Eagle's MEM (see reference)	Liu, Q., Kawai, H., and Berg, D.: B-Amyloid Peptide Blocks the Response of $\alpha 7$ -Containing Nicotinic Receptors on Hippocampal Neurons, <i>PNAS</i> 98 (8), 4734, 2001 (1094)
MEM10	Acosta, C., Fabrega, A., Masco, D., and Lopez, H.: A Sensory Neuron Subpopulation with Unique Sequential Survival Dependence on Nerve Growth Factor and Basic Fibroblast Growth Factor during Development, <i>J Neurosci</i> 21 (22), 8873, 2001 (1136)
DMEM	Mabuchi T, Kitagawa K, Kuwabara K, Takasawa K, Ohtsuki T, Xia Z, Storm D, Yanagihara T, Hori M, Matsumoto M: Phosphorylation of cAMP response element-binding protein in hippocampal neurons as a protective response after exposure to glutamate in vitro and ischemia in vivo, <i>J Neurosci</i> 21, 9204-13, 2001 (10122)
Neurobasal medium and DMEM	O'Connor, S., Andreadis, J., Shaffer, K., Ma, W., Pancrazio, J., and Stenger, D.: Immobilization of Neural Cells in Three-Dimensional Matrices for Biosensor Applications, <i>Biosensors & Bioelectronics</i> 14, 871, 2000 (1091)
EBSS	Neuhoff, H., Roeper, J., Schweizer, M.: Activity-Dependent Formation of Perforated Synapses in Cultured Hippocampal Neurons, <i>Eur J Neurosci</i> 11, 4241, 1999 (1096)
L-15 medium (see reference)	Morrison, S., White, P., Zock, C., and Anderson, D.: Prospective Identification, Isolation by Flow Cytometry, and <i>In Vivo</i> Self-Renewal of Multipotent Mammalian Neural Crest Stem Cells, <i>Cell</i> 96, 737, 1999 (1099)
HBSS and PIPES	Johansson, C., Momma, S., Clarke, D., Risling, M., Lendahl, U., and Frisen, J: Identification of a Neural Stem Cell in the Adult Mammalian Central Nervous System, <i>Cell</i> 96, 25, 1999 (1100)
EBSS	Wilding, T., and Huettner, J.: Activation and Desensitization of Hippocampal Kainate Receptors, <i>J Neurosci</i> 17 (8), 2713, 1997 (1092)
MEM-HEPES	Schafer, K., Saffrey, M., Burnstock, G., and Mastres-Ventura, P.: A New Method for the Isolation of Myenteric Plexus from the Newborn Rat Gastrointestinal Tract, <i>Brain Res Proto</i> 1, 109, 1997 (1093)
Harvest buffer	Hall, R., and Soderling, T.: Differential Surface Expression and Phosphorylation of the N-Methyl-D-Aspartate Receptor NR1 and NR2 in Cultured Hippocampal Neurons, <i>J Biol Chem</i> 272 (7), 4135, 1997 (1095)

Tissue Dissociation Guide

Species

Neural (con't)

Cell(s)

Enzyme(s)

Rat, SD, adult, 250-300g, P8	Neurons, DRG	Neutral Protease: 0.5%
Rat, 1-4 day old	Neurons, hippocampal	Papain: 20 u/ml
Rat, SD, Fisher	Hippocampal neurons	Papain: 0.2%
Rat, E18	Hippocampal neurons	Papain: 20 u/ml Deoxyribonuclease I: 0.01%
Rat, pups, 14 day old	Neurons, sympathetic	Neutral Protease: 0.24%
Rats, SD, 11-14 day old, 29-32 g	Basal forebrain neurons	Trypsin: 0.125%
Rat, Wistar-Hanover, 7-12 day	Postnatal dopamine neurons	Trypsin: 0.035%
Rat, SD, 18-day-old, fetus	Hippocampal	Trypsin: 0.2%
Rat	Stem, neural crest	Collagenase: 0.075%
Rat, SD, pregnant, Charles River	Pyramidal neurons Nonpyramidal neurons	Trypsin: 0.027%
Rat, SD, embryos, 19-21 days gestation	Schwann, dorsal root ganglia	Trypsin: 0.25%
Rat (also bovine)	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%
Rat, SD, 8 day old pups	Cerebellar neurons	Trypsin: 0.025%
Rat, fetuses, 18 day old	Hippocampal neurons	Trypsin: 0.2%
Rat, postnatal	Septal neurons	Papain: 0.05%
Rat, Long Evans, 1-15 days	Neurons, visual cortex	Papain: 20 u/ml
Rat, SD, female, 4 month old	CNS cells	Trypsin: 0.25%
Rat, embryo, 15 day	Dorsal horn neurons Spinal	Trypsin: 0.025%
Rat, fetus, 18-20 day	Hippocampal neurons	Trypsin: 0.25%
Rat, postnatal	Ganglion, retina	Papain: 12.5 u/ml
Rat, SD, pups	Retina	Trypsin: 0.25%
Rat, W/FU, 5-8 day	Neurons and glial	Trypsin: 0.25%
Rat, fetus	Neurons, sympathetic	Trypsin: 0.25%
Rat, Wistar/Furth, newborn (also bovine)	Schwann	Trypsin: 0.25%
Rat, embryo	Neurons, cortical	Trypsin: 0.027%
Rat, newborn	Neurons, sympathetic	Collagenase Type 1: 0.01%
Rat, neonatal	Neurons, superior cervical ganglia	Trypsin: 0.1%
Ovine Lamb (also calf)	Oligodendroglia Neural	Trypsin: 0.1%

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Medium	Reference
L-15 w/ CO ₂	Davies, S., Fitch, M., Memberg, S., Hall, A, Raisman, G., and Silver, J.: Regeneration of Adult Axons in White Matter Tracts of the Central Nervous System, <i>Nature</i> 390, 680, 1997 (1098)
MEM	Twitchell, W., Brown, S., and Mackie, K.: Cannabinoids Inhibit N- and P/Q-Type Calcium Channels in Cultured Rat Hippocampal Neurons, <i>J Neurophysiol</i> 78, 43, 1997 (1127)
HibernateA/B27	Brewer, G.J.: Isolation and culture of adult rat hippocampal neurons of any age, <i>J Neurosci Methods</i> 71, 143, 1997 (10067)
MEM	Liu QY, Schaffner AE, Li YX, Dunlap V, Barker JL: Upregulation of GABAA current by astrocytes in cultured embryonic rat hippocampal neurons, <i>J Neurosci</i> 16, 2912-23, 1996 (10123)
HBSS	McFarlane, S., and Cooper, E.: Extrinsic Factors Influence the Expression of Voltage-Gated K Currents on Neonatal Rat Sympathetic Neurons, <i>J Neurosci</i> 13 (6), 2591, 1993 (774)
Gey's BSS	Allen, T., Sim, J., and Brown, D.: The Whole-Cell Calcium Current in Acutely Dissociated Magnocellular Cholinergic Basal Forebrain Neurons of the Rat, <i>J Physiol</i> 460, 91, 1993 (999)
(see reference)	Rayport, S., Sulzer, D., Shi, W., Sawasdikosol, S., Monaco, J., Batson, D., and Rajendran, G.: Identified Postnatal Mesolimbic Dopamine Neurons in Culture: Morphology and Electrophysiology, <i>J Neurosci</i> 12 (11), 4264, 1992 (1002)
HBSS	Cheng, B., and Mattson, M.: IGF-I and IGF-II Protect Cultured Hippocampal and Septal Neurons Against Calcium-Mediated Hypoglycemic Damage, <i>J Neurosci</i> 12 (4), 1558, 1992 (1201)
Ringer's solution	Stemple, D., and Anderson, D.: Isolation of a Stem Cell for Neurons and Glia from the Mammalian Neural Crest, <i>Cell</i> 71, 973, 1992 (1297)
HEPES	Buchhalter, J., and Dichter, M.: Electrophysiological Comparison of Pyramidal and Stellate Nonpyramidal Neurons in Dissociated Cell Culture of Rat Hippocampus, <i>Brain Res Bull</i> 26, 333, 1991 (1005)
HBSS, CMF	Mithen, F., Reiker, M., and Birchem, R.: Effects of Ethanol on Rat Schwann Cell Proliferation and Myelination in Culture, <i>In Vitro Cell Dev Biol</i> 26, 129, 1990 (430)
25mM HEPES buffered Locke's solution, CMF	Trifaro, J., Tang, R., and Novas, M.: Monolayer Co-Culture of Rat Heart Cells and Bovine Adrenal Chromaffin Paraneurons, <i>In Vitro Cell Dev Biol</i> 26, 335, 1990 (438)
Eagle's MEM	Novelli, A., Reilly, J., Lysko, P., and Henneberry, R.: Glutamate Becomes Neurotoxic Via the N-methyl-D-aspartate Receptor When Intracellular Energy Levels are Reduced, <i>Brain Res</i> 451, 205, 1988 (859)
Eagle's MEM	Mattson, M., and Kater, S.: Isolated Hippocampal Neurons in Cryopreserved Long-Term Cultures: Development of Neuroarchitecture and Sensitivity to Nmda, <i>Int J Dev Neurosci</i> 6 (5), 439, 1988 (998)
PBS, CMF	Hatanaka, H., Tsukui, H., Nihonmatsu, I.: Septal Cholinergic Neurons From Postnatal Rat Can Survive In The Dissociate Culture Conditions In The Presence Of Nerve Growth Factor, <i>Neurosci Lett</i> 79, 85, 1987 (646)
BSS (see reference)	Huettnner, J., and Baughman, R.: Primary Culture of Identified Neurons From the Visual Cortex of Postnatal Rats, <i>J Neurosci</i> 6, 3044, 1986 (617)
EBSS	Wood, P., and Bunge, R.: Evidence That Axons are Mitogenic for Oligodendrocytes Isolated From Adult Animals, <i>Nature</i> 320, 756, 1986 (781)
Ham's F-12	Jahr, C. and Jessell, T.: Synaptic Transmission between Dorsal Root Ganglion and Dorsal Horn Neurons in Culture: Antagonism of Monosynaptic Excitatory Postsynaptic Potentials and Glutamate Excitation by Kynurenate, <i>J Neurosci</i> 5, 2281, 1985 (614)
HBSS, CMF	Bartlett, W. and Banker, G.: An Electron Microscopic Study of the Development of Axons and Dendrites by Hippocampal Neurons in Culture. I. Cells Which Develop Without Intercellular Contacts, <i>J Neurosci</i> 4, 1944, 1984 (613)
HBSS w/5 mM HEPES	Leifer, D., Lipton, S., Barnstable, C., and Masland, R.: Monoclonal Antibody to Thy-1 Enhances Regeneration of Processes by Rat Retinal Ganglion Cells in Culture, <i>Science</i> 224, 303, 1984 (667)
Ham's F-12	Sarthy PV, Curtis BM, and Catterall WA.: Retrograde Labeling, Enrichment, and Characterization of Retinal Ganglion Cells from the Neonatal Rat, <i>J Neurosci</i> 3 (12), 2532, 1983 (1199)
MEM (see reference)	Raff, M., Fields, K., Hakomori, S., Mirsky, R., Pruss, R., and Winter, J.: Cell-Type-Specific Markers for Distinguishing and Studying Neurons And the Major Classes of Glial Cells in Culture, <i>Brain Res</i> 174, 283, 1979 (348)
L-15 or HBSS, CMF	Wakshull, E., Johnson, M., Burton, H.: Postnatal Rat Sympathetic Neurons In Culture. 1. A Comparison With Embryonic Neurons, <i>J Neurophysiol</i> 42, 1410, 1979 (716)
DMEM	Brockes, J., Fields, K., and Raff, M.: Studies on Cultured Rat Schwann Cells. I. Establishment of Purified Populations From Cultures of Peripheral Nerve, <i>Brain Res</i> 165, 105, 1979 (991)
MEM	Dichter, M.: Rat Cortical Neurons in Cell Culture: Culture Methods, Cell Morphology, Electrophysiology, and Synapse Formation, <i>Brain Res</i> 149, 279, 1978 (346)
Hank's solution, CF	Reichardt, L., Patterson, P.: Neurotransmitter Synthesis and Uptake by Isolated Sympathetic Neurons in Microcultures, <i>Nature</i> 270, 147, 1977 (642)
Basal L-15 medium	Mains, R., and Patterson, P.: Primary Cultures of Dissociated Sympathetic Neurons I. Establishment of Long-Term Growth in Culture and Studies of Differentiated Properties, <i>J Cell Biol</i> 59, 329, 1973 (587)
(see reference)	Poduslo, S., Miller, K., and McKhann, G.: Metabolic Properties of Maintained Oligodendroglia Purified from Brain, <i>J Biol Chem</i> 253, 1592, 1978 (552)

Tissue Dissociation Guide

TISSUE TABLES

Species	Neural (con't)	Cell(s)	Enzyme(s)
Salamander	Salamander, 18-25 cm	Retina	Papain: 14 u/ml
	Salamander (<i>A. tigrinum</i>)	Photoreceptors, retina	Papain: 0.05%
Shellfish	<i>Aplysia californica</i>	Neurons	Neutral Protease: 1.0%
	Snails (<i>Helisoma trivolis</i>), albino, adult	Buccal ganglia; SLT muscle	Trypsin: 0.2%
	Pond snail (<i>Helisoma</i>) albino, adult	Somata, buccal ganglia	Trypsin: 0.2%
	<i>Helisoma trivolis</i>	Buccal ganglia	Trypsin: 0.2%
	Mollusc, juvenile 1-2 gm or adult 50-100 gm	Neurons LUQ cells	Protease: 1%
	<i>Aplysia californica</i>	LUQ cells RUQ cells	Protease: 1%
	Turtle	Turtle (<i>Pseudemys scripta elegans</i>)	Retinal

Pancreas

Species		Cell(s)	Enzyme(s)
Bovine	Bovine	Duct epithelial	Collagenase: 0.1%
	Bovine (<i>Bos taurus</i>)	Ductal	Neutral Protease: 0.05%
	Bovine	Platelets	Trypsin:
Canine	Canine	Buccal ganglia; SLT muscle	Trypsin: 0.2%
Fish	Fish, <i>Osphronemus gourami</i> , 3-month-old, 0.5 mg	Islets	Collagenase: 0.12 - 0.46 u/ml
Guinea-Pig	Guinea-pig	Acinar	Collagenase Type 3: 60 u/ml
	Guinea-pig, Hartley, albino, male, 350-400 g	Acinar	Soybean Trypsin Inhibitor: 0.01%
	Guinea-pig	Exocrine	Hyaluronidase: 0.15% - 0.2%
Hamster	Hamster	Islets	Collagenase Type 4: 1.3% - 2.0%
Human	Human	Pancreatic cancer stem cells	Collagenase Type 4: 200 u/ml
	Human, 25-55 yrs old (also porcine)	Islets	Collagenase: 0.4%
	Human	Islets	Collagenase Type 4: 0.8%
	Human	Islets	Collagenase (1 or 4): 0.60%
	Human, infant, age 1 day-1 year	Islets	Collagenase: 170-210 u/ml
Monkey	Monkey, 3-5 Kg	Islets	Hyaluronidase: 0.05%
Mouse	Mouse, 10 week	Islets	Collagenase: 0.2%
	Mouse, adult, male	Ancinar	CLSPA: see reference Soybean Trypsin Inhibitor: 0.001%
	Mouse, 3-4 week, 20-24 g	Islets	Collagenase Type 4: 0.2%

Tissue Dissociation Guide

Medium	Reference
Saline	Townes-Anderson, E., MacLeish, P., and Raviola, E.: Rod Cells Dissociated from Mature Salamander Retina: Ultrastructure and Uptake of Horseradish Peroxidase, <i>J Cell Biol</i> 100, 175, 1985 (1200)
(see reference)	Bader, C., MacLeish, P., and Schwartz, E.: Responses to Light of Solitary Rod Photoreceptors Isolated From Tiger Salamander Retina, <i>Proc Natl Acad Sci U S A</i> 75, 3507, 1978 (652)
L-15-ASW	Lee, A., Decourt, B. and Suter, D.: Neuronal Cell Cultures from Aplysia for High-Resolution Imaging of Growth Cones., <i>J Vis Exp</i> 12, 662, 2008 (10491)
DMEM	Zoran, M., Doyle, R. and Haydon, P.: Target Contact Regulates the Calcium Responsiveness of the Secretory Machinery During Synaptogenesis, <i>Neuron</i> 6, 145, 1991 (691)
Antibiotic saline, Leibowitz 50%	Haydon, P.: The Formation of Chemical Synapses Between Cell-Cultured Neuronal Somata, <i>J Neurosci</i> 8, 1032, 1988 (620)
L-15 medium	Cohan, C., Haydon, P., and Kater, S.: Single Channel Activity Differs in Growing and Nongrowing Growth Cones of Isolated Identified Neurons of Helisoma, <i>J Neurosci Res</i> 13, 285, 1985 (609)
L15 medium	Schacher, S., and Proshansky, E.: Neurite Regeneration by Aplysia Neurons in Dissociated Cell Culture: Modulation by Aplysia Hemolymph and the Presence of the Initial Axonal Segment, <i>J Neurosci</i> 3 (12), 2403, 1983 (980)
L15 medium	Camardo, J., Proshansky, E., and Schacher, S.: Identified Aplysia Neurons Form Specific Chemical Synapses in Culture, <i>J Neurosci</i> 3 (12), 2614, 1983 (1044)
Kreb's Ringer	Lam, D.: Biosynthesis of Acetylcholine in Turtle Photoreceptors, <i>Proc Natl Acad Sci U S A</i> 69, 1987, 1972 (649)

Pancreas

Medium	Reference
HEPES	Cotton, C., and Al-Nakkash, L.: Isolation and Culture of Bovine Pancreatic Duct Epithelial cells, <i>Am J Physiol</i> 272, G1328, 1997 (1184)
EBSS	Sato, T., Sato, M., Hudson, E., and Jones, R.: Characterization of Bovine Pancreatic Ductal Cells Isolated by a Perfusion-Digestion Technique, <i>In Vitro</i> 19, 651, 1983 (529)
(see reference)	Stiles, G., and Lefkowitz, R.: Hormone-Sensitive Adenylate Cyclase, <i>J Biol Chem</i> 257 (11), 6287, 1982 (1053)
DMEM	Zoran, M., Doyle, R. and Haydon, P.: Target Contact Regulates the Calcium Responsiveness of the Secretory Machinery During Synaptogenesis, <i>Neuron</i> 6, 145, 1991 (691)
RPMI 1640	Schrezenmeir, J., Laue, C., Sternheim, E., Wolbert, K., Darquy, S., Chicheportiche, D., Kirchgessner, J., and Reach, G.: Long-Term Function of Single-Cell Preparations of Piscine Principal Islets in Hollow Fibers, <i>Transplant Proc</i> 24 (6), 2941, 1992 (1221)
Kreb's Ringer	Schultz, G., Sarras, Jr, M., Gunther, G., Hull, B., Alicea, H., Gorelick, F., and Jamieson, J.: Guinea Pig Pancreatic Acini Prepared with Purified collagenase, <i>Exp Cell Res</i> 130, 49, 1980 (1152)
Kreb's Ringer	Gardner, J., Conlon, T., Klaeveman, H., Adams, T., and Ondetti, M.: Action of Cholecystokinin and Cholinergic Agents on Calcium Transport in Isolated Pancreatic Acinar Cells, <i>J Clin Invest</i> 56, 366, 1975 (599)
Kreb's Ringer	Amsterdam, J., and Jamieson, J.: Structural and Functional Characterization of Isolated Pancreatic Exocrine Cells, <i>Proc Natl Acad Sci U S A</i> 69 (10), 3028, 1972 (1151)
HBSS	Feldman, J., and Chapman, B.: Preparation of Islets of Langerhans from Rabbits and Hamsters by the Collagenase Digestion Technique, <i>Acta Diabetol</i> 12, 208, 1975 (686)
Medium 199	Li, C., Heidt, D., Dalerba, P., Burant, C., Zhang, L., Adsay, V., Wicha, M., Clarke, M. and Simeone, D.: Identification of Pancreatic Cancer Stem Cells., <i>Cancer Res</i> 67, 1030, 2007 (10514)
HBSS	Contractor, H., Johnson, P., Chadwick, D., Robertson, G., and London, N.: The Effect of UW Solution and Its Components on the Collagenase Digestion of Human and Porcine Pancreas, <i>Cell Transplant</i> 4 (6), 615, 1995 (762)
HBSS	Izumi, R., Konishi, K., Ueno, K., Shimizu, K., Hirose, H., Takahashi, N., and Miyazaki, I.: Isolation of Human Pancreatic Islets from Cryopreserved Pancreas, <i>Transplant Proc</i> XVII, 383, 1985 (689)
HBSS	Gray, D., McShane, P., Grant, A., and Morris, P.: A Method for Isolation of Islets of Langerhans from the Human Pancreas, <i>Diabetes</i> 33, 1055, 1984 (690)
HBSS	Sutherland, D., Matas, A., Steffes, M., and Najarian, J.: Infant Human Pancreas: A Potential Source of Islet Tissue for Transplantation, <i>Diabetes</i> 25 (12), 1123, 1976 (810)
HBSS	Scharp, D., Murphy, J., Newton, W., Ballinger, W., and Lacy, P.: Application of an Improved Isolation Technique for Islet Transplantation in Primates and Rats, <i>Transplant Proc</i> 7, 739, 1975 (688)
RPMI 1640	Kobayashi, T., Yamaguchi, T., Hamanaka, S., Kato-Itoh, M., Yamazaki, Y., Ibata, M., Sato, H., Lee, Y., Usui, J., Knisely, A., Hirabayashi, M. and Nakauchi, H.: Generation of Rat Pancreas in Mouse by Interspecific Blastocyst Injection of Pluripotent Stem Cells., <i>Cell</i> 142, 787, 2010 (10591)
DMEM	Ji, B., Gaiser, S., Chen, X., Ernst, S. and Logsdon, C.: Intracellular Trypsin Induces Pancreatic Acinar Cell Death but not NF-KappaB Activation., <i>J Biol Chem</i> 284, 17488, 2009 (10510)
RPMI 1540	Huang, H., Xie, Q., Kang, M., Zhang, B., Zhang, H., Chen, J., Zhai, C., Yang, D., Jiang, B. and Wu, Y.: Labeling Transplanted Mice Islet with Polyvinylpyrrolidone Coated Superparamagnetic Iron Oxide Nanoparticles for In Vivo Detection By Magnetic Resonance Imaging., <i>Nanotechnology Vol. 20</i> , 365101, 2009 (10513)

Tissue Dissociation Guide

Species

Pancreas (con't)

Cell(s)

Enzyme(s)

	Mouse	Islets	Collagenase: 1,000 u/ml	
	Mouse	Islets	Collagenase: 0.03-0.08%	
	Mouse, 6-10 month	Pancreatic ductal	CLSPA: 50 u/ml Hyaluronidase: 400 u/ml Soybean Trypsin Inhibitor: 0.02%	
	Mouse, adult	Acinar	CLSPA: 200 u/ml	
	Mouse	Acinar cells and acini	Collagenase Type 1: see reference CLSPA: see reference	
	Mouse, albino, 25 g	Acinar	CLSPA: see reference	
	Mouse, 7-10 week	Islets	Collagenase: see reference	
	Mouse	Pancreatic islet	Collagenase Type 4: 0.2%	
	Mouse	Islets	Collagenase Type 4: 0.2%	
	Mouse, BALB/c, 6-8 week-old, either sex	Acinar	Collagenase: 0.1%	
	Mouse	Islets	Collagenase Type 2: 0.2%	
	Mouse	Pancreatic islets	Collagenase Type 4: 0.4%	
	Mouse	Duct	Papain: 25 u/ml	
	Mouse	Acinar	Collagenase: 100 u/ml	
	Mouse (C57BL/6J-ob/ob), 9-12 wks, male	Islets	Hyaluronidase: 0.5%	
	Mouse, male, 18-24 g	Acinar	CLSPA: 70-90 u/ml Soybean Trypsin Inhibitor: 0.01%	
Porcine	Porcine, juvenile	Islets	Collagenase: see reference	
	Porcine, 3 month, 15-20 kg	Acinar	Collagenase Type 3: 200 u/ml	
	Porcine, 1-3 year, 1.5-2.0 kg, either sex	Islets	Collagenase: 0.25%	
	Porcine, female	Islets	Collagenase: 0.1%	
	Porcine	Islets	Collagenase: 0.1%-0.2%	
	Porcine, <2 year, 200-250 kg	Islets	Collagenase: 0.1%	
	Porcine, 10-36 months, 200-300 kg	Islets	Collagenase: 0.2%	
	Porcine	Acinar	Collagenase: 100 u/ml	
	Rabbit	Rabbit, New Zealand white, Male/Female, 2-3 Kg	Acinar	Hyaluronidase: 0.2%
		Rabbit, also hamster	Islets	Collagenase Type 4: 1.3% - 2.0%

Tissue Dissociation Guide

Medium	Reference
RPMI 1640	Li, D., Yuan, Y., Tu, H., Liang, Q. and Dai, L.: A Protocol for Islet Isolation from Mouse Pancreas., <i>Nat Protoc</i> 4, 1649, 2009 (10643)
RPMI 1640	Szot, G., Koudria, P. and Bluestone, J.: Murine Pancreatic Islet Isolation., <i>J Vis Exp</i> 7, 255, 2007 (10649)
DMEM	Wang, Y., Soyombo, A., Shcheynikov, N., Zeng, W., Dorwart, M., Marino, C., Thomas, P. and Muallem, S.: Slc26a6 Regulates CFTR Activity In Vivo to Determine Pancreatic Duct HCO ₃ ⁻ Secretion: Relevance to Cystic Fibrosis., <i>EMBO J</i> 25, 5049, 2006 (10607)
(see reference)	Voronina, S., Barrow, S., Gerasimenko, O., Petersen, O. and Tepikin, A.: Effects of Secretagogues and Bile Acids on Mitochondrial Membrane Potential of Pancreatic Acinar Cells: Comparison of Different Modes of Evaluating Delta Psi., <i>J Biol Chem</i> 279, 27327, 2004 (10568)
(see reference)	Toivola, D., Ku, N., Ghorri, N., Lowe, A., Michie, S. and Omary, M.: Effects of Keratin Filament Disruption on Exocrine Pancreas-Stimulated Secretion and Susceptibility to Injury., <i>Exp Cell Res</i> 255, 156, 2000 (10569)
(see reference)	Fogarty, K., Kidd, J., Tuft, R. and Thorn, P.: A Bimodal Pattern of InsP(3)-Evoked Elementary Ca(2+) Signals in Pancreatic Acinar Cells., <i>Biophys J</i> 78, 2298, 2000 (10630)
DMEM	Yesil, P, Michel, M., Chwalek, K., Pedack, S., Jany, C., Ludwig, B., Bornstein, S. and Lammert, E.: A New Collagenase Blend Increases the Number of Islets Isolated from Mouse Pancreas., <i>Islets Vol. 1</i> , 185, (10614)
HBSS	Haefliger Jacques-Antoine, Tawadros Thomas, Meylan Laure, Gurun SabineLe, Roehrich Marc-Etienne, Martin David, Thorens Bernard, Waeber Gerard: The scaffold protein IB1/JIP-1 is a critical mediator of cytokine-induced apoptosis in pancreatic beta cells, <i>J Cell Sci</i> 116, 1463-9, 2003 (10151)
HBSS	Wu Yulian, Han Bing, Luo Hongyu, Roduit Raphael, Salcedo TheodoraW, Moore PaulA, Zhang Jun, Wu Jiangping: DcR3/TR6 effectively prevents islet primary nonfunction after transplantation, <i>Diabetes</i> 52, 2279-86, 2003 (10288)
Waymouth's MB	Kurup, S., and Bhonde, R.: Analysis and Optimization of Nutritional Set-up for Murine Pancreatic Acinar Cells, <i>JOP</i> 3 (1), 8, 2002 (1070)
CF Medium	Koster, J., Marshall, B., Ensor, N., Corbett, J., and Nichols, C.: Targeted Overactivity of Cell K _{ATP} Channels Induces Profound Neonatal Diabetes, <i>Cell</i> 100, 645, 2000 (1126)
Gey's BSS	Strowski M, Parmar R, Blake A, Schaeffer J: Somatostatin inhibits insulin and glucagon secretion via two receptors subtypes: an in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice, <i>Endocrinology</i> 141, 111-7, 2000 (10276)
DMEM /F-12	Githens, S: Pancreatic Duct Epithelial Cells, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12B:12.1, 1995 (1274)
HEPES	Jauch, P., Peterson, O., and Lauger, P.: Electrogenic Properties of the Na-Alanine Cotransporter in Acinar Cells, <i>J Membr Biol</i> 94, 99, 1986 (605)
Kreb's Ringer bicarbonate buffer	Dalpe-Scott, M., Heick, H., and Begin-Heick, N.: Secretion in the Obese (ob/ob) Mouse. The Effect of Oxytetracycline on Insulin Release, <i>Diabetes</i> 32, 932, 1983 (687)
Krebs-Henseleit	Burnham DB, Williams JA: Effects of carbachol, cholecystokinin, and insulin on protein phosphorylation in isolated pancreatic acini, <i>J Biol Chem</i> 257, 10523-8, 1982 (10135)
Univ of Wisconsin solution	Van der Burg Michael P M, Graham John M: Iodixanol Density Gradient Preparation in University of Wisconsin solution for porcine islet purification, <i>ScientificWorldJournal</i> 3, 1154-9, 2003 (10286)
RPMI 1640	Zhao, X., Han, J., and Tang, C.: Primary Culture of Porcine Pancreatic Acinar Cells, <i>JOP</i> 2 (2), 78, 2001 (768)
HBSS	Korbitt, G., Elliott, J., Ao, Z., Smith, D., Warnock, G., and Rajotte, R.: Large Scale Isolation, Growth, and Function of Porcine Neonatal Islet Cells, <i>J Clin Invest</i> 97 (9), 2119, 1996 (761)
HBSS	Brandhorst, D., Brandhorst, H., Hering, B., Federlin, K., and Bretzel, R.: Islet Isolation from the Pancreas of Large Mammals and Humans: 10 Years of Experience, <i>Exp Clin Endocrinol</i> 103, 3, 1995 (760)
HBSS	Johnson, P., van Suylichem, P., Roberts, D., Vos-Scheperkeuter, G., White, S., van Schilfgaarde, R., London, N.: Design of a Simple, <i>in vitro</i> Method for Evaluation of the Efficiency of Crude <i>Clostridium histolyticum</i> Collagenase and its Components for Porcine Islet Isolation, <i>Xenotransplantation</i> 2, 165, 1995 (729)
HBSS	Heiser, A., Ulrichs, K., and Muller-Ruchholtz, W.: Isolation of Porcine Pancreatic Islets: Low Trypsin Activity During the Isolation Procedure Guarantees Reproducible High Islet Yields, <i>J Clin Lab Anal</i> 8, 407, 1994 (754)
HBSS	Ricordi, C., Soggi, C., Davalli, A., Staudacher, C., Baro, P., Vertova, A., Sassi, I., Gavazzi, F., Pozza, G., and Di Carlo, V.: Isolation of the Elusive Pig Islet, <i>Surgery</i> 107 (6), 688, 1990 (806)
Saline	Iwatsuki, N., and Peterson, O.: Action of Tetraethylammonium on Calcium-Activated Potassium Channels in Pig Pancreatic Acinar Cells Studied by Patch-Clamp Single-Channel and Whole-Cell Current Recording, <i>J Membr Biol</i> 86, 139, 1985 (604)
Kreb's Ringer bicarbonate buffer	Renckens, B., Schrijen, J., Swarts, H., DePont, J., and Bonting, S.: Role of Calcium in Exocrine Pancreatic Secretion. IV. Calcium Movements in Isolated Acinar Cells of Rabbit Pancreas, <i>Biochim Biophys Acta</i> 544, 338, 1978 (321)
HBSS	Feldman, J., and Chapman, B.: Preparation of Islets of Langerhans from Rabbits and Hamsters by the Collagenase Digestion Technique, <i>Acta Diabetol</i> 12, 208, 1975 (686)

Tissue Dissociation Guide

Species

Pancreas (con't)

Cell(s)

Enzyme(s)

Species	Pancreas (con't)	Cell(s)	Enzyme(s)
Rat	Rat, Wistar, 300g	Islets	Collagenase Type 4: 0.2%
	Rat, male, 7-11 week	Islets	Collagenase Type 4: see reference
	Rat, Wistar, male, 250-400g	Pancreatic islets	Collagenase: 0.75%
	Rat, 250-350 g	Pancreatic islet	Collagenase Type 4: 0.2%
	Rat	Pancreatic acini	CLSPA: 30 u/ml Collagenase Type 4: 30 u/ml Soybean Trypsin Inhibitor: 0.01%
	Rat, SD, male	Ancinar	CLSPA: see reference Soybean Trypsin Inhibitor: 0.01%
	Rat	Islets	Collagenase: 126 - 196 u/ml
	Rat	Islets	Collagenase: 0.5 - 0.9%
	Rat	Duct	Papain: 25 u/ml
	Rat, SD, male, 40 - 100 g	Acinar	Hyaluronidase: 462 u/ml
	Rat, SD, male, 100 - 150 g	Parotid acinar	Trypsin: 0.001%
	Rat, Wistar, male	Parotid acinar	Trypsin: 0.02%
	Rat, S-Wistar, male, 230 - 270 g (also mouse, 6 - 8 wk old)	Islets	Collagenase: 0.1% - 0.2%
	Rat, SD, both sexes, 6-20 wks old	Interlobular ducts	Papain: 25 u/ml
	Rat, SD, male, 150 - 200 g	Acinar	Soybean Trypsin Inhibitor: 0.01%
	Rat, Wistar, male, 250 - 350 g	Acinar, parotid	Hyaluronidase: 0.015%
	Rat, SD, male, 50 - 125 g	Acinar Exorbital lacrimal, parotid, pancreas	Trypsin: 0.01%
	Rat, Fischer-344, either sex, 120-150 g	Epithelial	Trypsin: 0.1%
	Rat, SD, male, 42 - 48 day, 175 - 200 g	Acinar, submandibular gland	Hyaluronidase: 0.1 %
	Rat, Dark Agouti and Lewis rat donors, adult	Islets	Collagenase Type 1: 0.3%
	Rat, SD, male, 50-75 g	Acinar	Hyaluronidase: 0.1%
	Rat, SD, 125 - 350 g	Duct	Trypsin: 0.01%
	Rat	Exocrine	Hyaluronidase: 0.9%
	Rat, Wistar, male	Islets	Collagenase Type 4: 1%
	Rat, SD, male, 250 - 350 g (also mouse, white, Swiss, male, 20 - 24 g)	Acinar	Hyaluronidase: 0.18%
	Rat, Wistar, male, 200 - 300 g	Islets	Collagenase Type 4: 0.5%
	Rat	Exocrine	Hyaluronidase: 0.15%

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Medium	Reference
CMRL 1066	Verga Falzacappa, C., Mangialardo, C., Raffa, S., Mancuso, A., Piergrossi, P., Moriggi, G., Piro, S., Stigliano, A., Torrì, M., Brunetti, E., Toscano, V. and Misiti, S.: The Thyroid Hormone T3 Improves Function and Survival of Rat Pancreatic Islets During In Vitro Culture., <i>Islets Vol. 2</i> , 96, 2010 (10615)
RPMI 1640	Getty-Kaushik, L., Richard, A., Deeney, J., Shirihai, O. and Corkey, B.: The CB1 Antagonist Rimonabant Decreases Insulin Hypersecretion in Rat Pancreatic Islets, <i>Obesity 17</i> , 1856, 2009 (10531)
RPMI 1640	Tian XH, Xue WJ, Ding XM, Pang XL, Teng Y, Tian PX, and Feng XS: Small intestinal submucosa improves islet survival and function during in vitro culture, <i>World J Gastroenterol 11</i> , 7378, 2005 (10008)
HBSS	Haefliger Jacques-Antoine, Tawadros Thomas, Meylan Laure, Gurun SabineLe, Roehrich Marc-Etienne, Martin David, Thorens Bernard, Waeber Gerard: The scaffold protein IB1/JIP-1 is a critical mediator of cytokine-induced apoptosis in pancreatic beta cells, <i>J Cell Sci 116</i> , 1463-9, 2003 (10151)
M199	Blinman TA, Gukovsky I, Mouria M, Zaninovic V, Livingston E, Pandol SJ, Gukovskaya AS: Activation of pancreatic acinar cells on isolation from tissue: cytokine upregulation via p38 MAP kinase, <i>Am J Physiol Cell Physiol 279</i> , C1993-2003, 2000 (10133)
DMEM	Ji B, Kopin AS, Logsdon CD: Species differences between rat and mouse CCKA receptors determine the divergent acinar cell response to the cholecystokinin analog JMV-180, <i>J Biol Chem 275</i> , 19115-20, 2000 (10157)
HBSS	Verspohl, E., and Wienecke, A.: The Role of Protein Kinase C in the Desensitization of Rat Pancreatic Islets to Cholinergic stimulation, <i>J Endocrinol 159</i> , 287, 1998 (1073)
HBSS	Takaki, R and Ono J: Culture of Pancreatic Islet Cells, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12B:11.1, 1995 (1112)
DMEM /F-12	Githens, S: Pancreatic Duct Epithelial Cells, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12B:12.1, 1995 (1274)
Ham's F12	Hirschi, K., Kenny, S., Justice, J., Brannon, P.: Effects of Secretin And Caerulein On Enzymes Of Cultured Pancreatic Acinar Cells, <i>In Vitro Cell Dev Biol 27</i> , 660, 1991 (472)
F12 medium	Yeh, C., Mertz, P., Oliver, C., Baum, B., and Kousvelari, E.: Cellular Characteristics of Long-Term Cultured Rat Parotid Acinar Cells, <i>In Vitro Cell Dev Biol 27</i> , 707, 1991 (473)
Solution B (see reference)	Foskett, J., Roifman, C. and Wong, D.: Activation of Calcium Oscillations by Thapsigargin in Parotid Acinar Cells, <i>J Biol Chem 266</i> , 2778, 1991 (573)
HBSS	Ohzato, H., Gotoh, M., Monden, M., Dono, K., Kanai, T., and Mori, T.: Improvement in the Islet Yield From a Cold-Preserved Pancreas by Pancreatic Ductal Collagenase Distention at the Time of Harvesting, <i>Transplantation 51</i> , 566, 1991 (798)
DMEM/Ham's F-12	Githens, S., Schexnayder, J., Desai, K., and Patke, C.: Rat Pancreatic Interlobular Duct Epithelium: Isolation and Culture in Collagen Gel, <i>In Vitro Cell Dev Biol 25</i> (8), 679, 1989 (790)
HEPES	Menozi, D., Sato, S., Jensen, R., and Gardner, J.: Cyclic GMP Does Not Inhibit Protein Kinase C-Mediated Enzyme Secretion in Rat Pancreatic Acini, <i>J Biol Chem 264</i> , 995, 1989 (565)
Earle's MEM	Melvin, J., Kawaguchi, M., Baum, B., and Turner, R.: A Muscarinic Agonist-Stimulated Chloride Efflux Pathway is Associated With Fluid Secretion in Rat Parotid Acinar Cells, <i>Biochem Biophys Res Commun 145</i> , 754, 1987 (308)
HBSS, CMF	Oliver, C., Waters, J., Tolbert, C., and Kleinman, H.: Growth of Exocrine Acinar Cells on a Reconstituted Basement Membrane Gel, <i>In Vitro Cell Dev Biol 23</i> , 465, 1987 (421)
Ham's F-12/HBSS (see reference)	Tsao, M., and Duguid, W.: Establishment of Propagable Epithelial Cell Lines From Normal Adult Rat Pancreas, <i>Exp Cell Res 168</i> , 365, 1987 (793)
HBSS, CF	Quissell, D., Redman, R., and Mark, M.: Short-Term Primary Culture of Acinar-Intercalated Duct Complexes From Rat Submandibular Glands, <i>In Vitro Cell Dev Biol 22</i> , 469, 1986 (419)
Hank's solution	Sutton, R., Peters, M., McShane, P., Gray, D., and Morris, P.: An Improved Method for the Isolation of Islets of Langerhans From the Adult Rat Pancreas, <i>Transplant Proc XVII</i> (6), 1819, 1986 (789)
HBSS (see reference)	Brannon, P., Orrison, B., and Kretchmer, N.: Primary Cultures of Rat Pancreatic Acinar Cells in Serum-Free Medium, <i>In Vitro Cell Dev Biol 21</i> (1), 6, 1985 (788)
HBSS	Githens, S., Holmquist, D., Whelan, J., and Ruby, J.: Ducts of the Rat Pancreas in Agarose Matrix Culture, <i>In Vitro 16</i> , 797, 1980 (510)
Kreb's Ringer	Schulz, I., Heil, K., Kribben, A., Sachs, G., and Haase, W.: Isolation and Functional Characterization of Cells From the Exocrine Pancreas, <i>Biology of Normal and Cancerous Exocrine Pancreatic Cells</i> , 1980 (1155)
Medium 199	Katada, T., and Ui, M.: Enhanced Insulin Secretion and Cyclic Amp Accumulation in Pancreatic Islets Due to Activation of Native Calcium Ionophores, <i>J Biol Chem 254</i> (2), 469, 1979 (791)
Kreb's Henseleit bicarbonate buffer	Williams, J., Korc, M., and Dormer, R.: Action of Secretagogues on a New Preparation of Functionally Intact, Isolated Pancreatic Acini, <i>Am J Physiol 235</i> , 517, 1978 (288)
HBSS	Shibata, A., Ludvigsen, C., Naber, S., McKaneil M., and Lacy, P.: Standardization of a Digestion-Filtration Method for Isolation of Islets, <i>Diabetes 25</i> , 667, 1976 (677)
Krebs	Kondo, S., and Schulz, I.: Calcium Ion Uptake in Isolated Pancreas Cells Induced by Secretagogues, <i>Biochim Biophys Acta 419</i> , 76, 1976 (1150)

Tissue Dissociation Guide

TISSUE TABLES

Species	Pancreas (con't)	Cell(s)	Enzyme(s)
	Rat, Wistar-Lewis, fetal 18-20 day gestation (also neonatal 3-12 day old)	Islets	Collagenase Type 4: 0.63%
	Rat, neonate	Islets	Trypsin: 0.05%
	Rat, male, albino, 200-300 g	Islets	Collagenase: 0.5%
	Rat, Lewis, inbred	Islets	Collagenase: 0.5%
	Rat, Wistar, albino, male, 400 - 500 g	Islets	Collagenase Type 4: 1.0% - 1.2%
	Rat	Ascites hepatoma	Trypsin: 0.1%

Parotid

Species		Cell(s)	Enzyme(s)
Rat	Rat, Wistar, male, 130 g	Acinar	Collagenase: 75 u/ml Hyaluronidase: 153 u/ml
	Rat, SD, 100-120 g	Parotid acinar cells	Collagenase Type 2: 90 u/ml
	Rat, SD, male, 100-150 g	Parotid acinar	Trypsin: 0.001%
	Rat, Wistar, male	Parotid acinar	Trypsin: 0.02%
	Rat	Parotid	Hyaluronidase: 0.025%
	Rat, Wistar, male, 250 - 350 g	Acinar, parotid	Hyaluronidase: 0.015%
	Rat, SD, male, 50 - 125 g	Acinar Exorbital lacrimal, parotid, pancreas	Trypsin: 0.01%

Pituitary

Species		Cell(s)	Enzyme(s)
Bovine	Calf, male, less than 6 months old	Pituitary	Collagenase: 0.3%
	Bovine	Pituitary	Collagenase: 0.1%
	Bovine	Follicular, anterior pituitary and pars tuberalis	Deoxyribonuclease I: 200 µg/ml
	Calf, male, 1-6 week old	Pituitary	Hyaluronidase: 0.1%
Mouse	Mouse, male	Pituitary	Collagenase: 0.4% Hyaluronidase: 0.1% Trypsin: 0.3%
Ovine	Ovine, adult	Somatotropes	Collagenase Type 1: 0.3% Hyaluronidase: Collagenase Type 1:
Rat	Rat, male, 12-15 week	Pituitary	Collagenase Type 2: 0.4% Deoxyribonuclease I: 0.04%
	Rat, Wistar, male, 2 month old	Pituitary	Trypsin: 0.1%
	Rat, SD, female, 200 - 250 g	Anterior pituitary gland	Trypsin: 0.1%
	Rat	Pituitary	Trypsin: 0.3 %
	Rat, SD, male, 250-450 g	Anterior pituitary	Trypsin: 0.25%

Tissue Dissociation Guide

Medium	Reference
EBSS (see reference)	Braaten, J., Jarlfors, U., Smith, D., and Mintz, D.: Purification of Monolayer Cell Cultures of the Endocrine Pancreas, <i>Tissue Cell</i> 7 (4), 747, 1975 (792)
Puck's saline buffered w/ EDTA 0.02%	Leonard, R., Lazarow, A., and Hegre, O.: Pancreatic Islet Transplantation in the Rat, <i>Diabetes</i> 22, 413, 1973 (684)
Hanks solution	Lacy, P., Walker, M., and Fink, J.: Perfusion of Isolated Rat Islets <i>in Vitro</i> , <i>Diabetes</i> 21 (10), 987, 1972 (1008)
HBSS	Ballinger, W., and Lacy, P.: Transplantation of Intact Pancreatic Islets in Rats, <i>Surgery</i> 72 (2), 175, 1972 (1119)
HBSS	Lacy, P., and Kostianovsky, M.: Method for the Isolation of Intact Islets of Langerhans from the Rat Pancreas, <i>Diabetes</i> 16, 35, 1967 (685)
Phosphate buffer (see reference)	Essner, E.: Experiments on an Ascites Hepatoma. I. Enzymatic Digestion and Alkaline Degradation of the Cementing Substance and Separation of Cells, in Tumor Islands, <i>Exp Cell Res</i> 7, 430, 1954 (403)

Parotid

Medium	Reference
RPMI 1640	Looms, D., Dissing, S., Tritsaris, K., Pedersen, A. and Nauntofte, B.: Adrenoceptor-Activated Nitric Oxide Synthesis in Salivary Acinar Cells., <i>Adv Dent Res Vol. 14</i> , 62, 2000 (10629)
Krebs-Henseleit Bicarbonate	D'Silva NJ, DiJulio DH, Belton CM, Jacobson KL, Watson EL: Immunolocalization of Rap1 in the rat parotid gland: detection on secretory granule membranes, <i>J Histochem Cytochem</i> 45, 965-73, 1997 (10231)
F12 medium	Yeh, C., Mertz, P., Oliver, C., Baum, B., and Kousvelari, E.: Cellular Characteristics of Long-Term Cultured Rat Parotid Acinar Cells, <i>In Vitro Cell Dev Biol</i> 27, 707, 1991 (473)
Solution B (see reference)	Foskett, J., Roifman, C. and Wong, D.: Activation of Calcium Oscillations by Thapsigargin in Parotid Acinar Cells, <i>J Biol Chem</i> 266, 2778, 1991 (573)
HBSS with 20mM HEPES	Takuma, T. and Ichida, T.: Amylase Secretion From Saponin-Permeabilized Parotid Cells Evoked by Cyclic AMP, <i>J Invest Dermatol</i> 103, 95, 1988 (676)
Earle's MEM	Melvin, J., Kawaguchi, M., Baum, B., and Turner, R.: A Muscarinic Agonist-Stimulated Chloride Efflux Pathway is Associated With Fluid Secretion in Rat Parotid Acinar Cells, <i>Biochem Biophys Res Commun</i> 145, 754, 1987 (308)
HBSS, CMF	Oliver, C., Waters, J., Tolbert, C., and Kleinman, H.: Growth of Exocrine Acinar Cells on a Reconstituted Basement Membrane Gel, <i>In Vitro Cell Dev Biol</i> 23, 465, 1987 (421)

Pituitary

Medium	Reference
DMEM	Hassan, H., and Merkel, R.: Perfusion Model System to Culture Bovine Hypothalamic Slices In Series with Dispersed Anterior Pituitary Cells, <i>In Vitro Cell Dev Biol</i> 30A, 435, 1994 (968)
EBSS, CMF	Mason, W. and Ingram, C.: Techniques for Studying the Role of Electrical Activity in Control of Secretion by Normal Anterior Pituitary Cells, <i>Vol. 124</i> , 207, 1986 (632)
HBSS, CMF	Ferrara, N., Goldsmith, P., Fujii, D., and Weiner, R.: Culture and Characterization of Follicular Cells of the Bovine Anterior Pituitary and Pars Tuberalis, <i>Vol. 124</i> , 245, 1986 (633)
DMEM	Ridgway, E., Klibanski, A., Marorana, M., Milbury, P., Kieffer, J., and Chin, W.: The Effect of Somatostatin on the Release of Thyrotropin and its Subunits from Bovine Anterior Pituitary Cells <i>in Vitro</i> , <i>Endocrinology</i> 112 (6), 1937, 1983 (1026)
DMEM/Han's F12	Stevenson Tami C, Ciccotosto Giuseppe D, Ma Xin-Ming, Mueller Gregory P, Mains Richard E, Eipper Betty A: Menkes protein contributes to the function of peptidylglycine alpha-amidating monooxygenase, <i>Endocrinology</i> 144, 188-200, 2003 (10241)
Medium 199	Xu Ruwei, Wang Qinling, Yan Ming, Hernandez Maria, Gong Changhong, Boon WahChin, Murata Yoko, Ueta Yoichi, Chen Chen: Orexin-A augments voltage-gated Ca2+ currents and synergistically increases growth hormone (GH) secretion with GH-releasing hormone in primary cultured ovine somatotropes, <i>Endocrinology</i> 143, 4609-19, 2002 (10246)
DMEM	Akieda-Asai, S., Zaima, N., Ikegami, K., Kahyo, T., Yao, I., Hatanaka, T., Iemura, S., Sugiyama, R., Yokozeki, T., Eishi, Y., Koike, M., Ikeda, K., Chiba, T., Yamaza, H., Shimokawa, I., Song, S., Matsuno, A., Mizutani, A., Sawabe, M. Chao, N., Tanaka, M., : SIRT1 Regulates Thyroid-Stimulating Hormone Release by Enhancing PIP5K gamma Activity through Deacetylation of Specific Lysine Residues in Mammals., <i>PLoS ONE</i> 5, e11755, 2010 (10644)
DMEM	Zhou, X., De Schepper, J., De Craemer, D., Delhase, M., Gys, G., Smitz, J., and Hooghe-Peters, E.: Pituitary Growth Hormone Release and Gene Expression in Cafeteria-Diet-Induced Obese Rats, <i>J Endocrinol</i> 159, 165, 1998 (1143)
EBSS, CMF	D'Emden, M. and Wark, J.: Culture Requirements for Optimal Expression of 1,25-Dihydroxyvitamin D3-Enhanced Thyrotropin Secretion, <i>In Vitro Cell Dev Biol</i> 27, 197, 1991 (459)
HEPES	Wilfinger, W., Larsen, W., Downs, T., and Wilbur, D.: An <i>In Vitro</i> Model for Studies of Intercellular Communication in Cultured Rat Anterior Pituitary Cells, <i>Tissue Cell</i> 16 (4), 483, 1984 (1224)
Krebs	Portanova, R., Smith, D., and Sayers, G.: A Trypsin for the Preparation of Isolated Rat Anterior Pituitary, <i>Proc Soc Exp Biol Med</i> 133, 573, 1970 (1207)

Tissue Dissociation Guide

Prostate			
Species		Cell(s)	Enzyme(s)
Human	Human, 52-56 yr	Prostatic stromal cells	Collagenase Type 1: 0.2%
	Human	Prostate stromal cells	Collagenase Type 1: 0.1%
	Human, fetal	Prostatic fibroblasts	Collagenase Type 1: 0.125%
Mouse	Mouse, 6-8 week	Prostate epithelial/stem	Collagenase Type 2: 0.5% Trypsin: 0.05%
	Mouse, 2 week	Prostatic epithelial	Collagenase Type 3: 170 u/ml
	Mouse, male	Prostatic stem	Collagenase Type 1: 170 u/ml
Reproductive			
Species		Cell(s)	Enzyme(s)
Bovine	Bovine	Corpus leuteal cells	Collagenase Type 4: 420 u/ml
	Bovine, female	Leuteal	Collagenase Type 1: 0.2%
	Calf, mid to late gestational	Fibroblasts	Collagenase: 0.1%
	Bovine	Epithelial Endometrial	Collagenase Type 2: 0.1%
	Bovine (also porcine, human)	Interna & corpus luteum Endometrium Ovarian Uterine	Pronase: 0.1%
Chicken	Chicken, Gallus Domesticus, 20-30 week	Primary follicles	Trypsin: 0.15% Collagenase Type 1: 0.125%
Frog	Xenopus laevis, female	Oocytes	Collagenase: 0.2%
	Xenopus laevis, female	Oocytes	Collagenase Type 1: 0.2%
	<i>Xenopus laevis</i> , female	Oocytes	Collagenase Type 1: 0.2%
	<i>Xenopus</i>	Oocytes	Collagenase Type 1: 1%
	<i>Xenopus laevis</i> , female	Oocytes	Collagenase: 0.1%
	<i>Xenopus laevis</i> , mature female	Oocytes	Collagenase: 0.2%
	Hamster	Hamster, Chinese	Ovary
Human	Human, female	Decidual	Collagenase: 0.25% Deoxyribonuclease I: 6.25 u/ml
	Human, female	Uterine epithelial	Pancreatin: 0.34% Hyaluronidase: 0.01% Collagenase: 0.16%

Tissue Dissociation Guide

Prostate

Medium	Reference
DMEM/F-12	Le Hanh, Arnold Julia T, McFann Kimberly K, Blackman Marc R: DHT and testosterone, but not DHEA or E2, differentially modulate IGF-I, IGFBP-2, and IGFBP-3 in human prostatic stromal cells, <i>Am J Physiol/Endo</i> 290, E952-60, 2006 (10126)
RPMI 1640	Nakashiro Koh-Ichi, Hara Shingo, Shinohara Yuji, Oyasu Miho, Kawamata Hitoshi, Shintani Satoru, Hamakawa Hiroyuki, Oyasu Ryoichi: Phenotypic switch from paracrine to autocrine role of hepatocyte growth factor in an androgen-independent human prostatic carcinoma cell line, CWR22R, <i>Am J Pathol</i> 165, 533-40, 2004 (10163)
DMEM/F12	Levine AC, Liu XH, Greenberg PD, Eliashvili M, Schiff JD, Aaronson SA, Holland JF, Kirschenbaum A: Androgens induce the expression of vascular endothelial growth factor in human fetal prostatic fibroblasts, <i>Endocrinology</i> 139, 4672-8, 1998 (10124)
HBSS	Burger, P., Gupta, R., Xiong, X., Ontiveros, C., Salm, S., Moscatelli, D. and Wilson, E.: High Aldehyde Dehydrogenase Activity: A Novel Functional Marker of Murine Prostate Stem/Progenitor Cells., <i>Stem Cells</i> 27, 2220-8, 2009 (10488)
DMEM	Imamov Otabek, Morani Andrea, Shim Gil-Jin, Omoto Yoko, Thulin-Andersson Christina, Warner Margaret, Gustafsson Jan-Ake: Estrogen receptor beta regulates epithelial cellular differentiation in the mouse ventral prostate, <i>Proc Natl Acad Sci U S A</i> 101, 9375-80, 2004 (10224)
DMEM	Dubey P, Wu H, Reiter RE, Witte ON: Alternative pathways to prostate carcinoma activate prostate stem cell antigen expression, <i>Cancer Res</i> 61, 3256-61, 2001 (10229)

Reproductive

Medium	Reference
M-199	Levy N, Gordin M, Mamluk R, Yanagisawa M, Smith M F, Hampton J H, Meidan R: Distinct cellular localization and regulation of endothelin-1 and endothelin-converting enzyme-1 expression in the bovine corpus luteum: implications for luteolysis, <i>Endocrinology</i> 142, 5254-60, 2001 (10169)
Ham's F-12	Tsang PC, Poff JP, Boulton EP, Condon WA: Four-day-old bovine corpus luteum: progesterone production and identification of matrix metalloproteinase activity in vitro, <i>Biol Reprod</i> 53, 1160-8, 1995 (10284)
Medium 199	Coplen, D., Howard, P., Duckett, J., Snyder, H., and Macarak, E.: Characterization of a Fibroblast Cell From the Urinary Bladder Wall, <i>In Vitro Cell Dev Biol</i> 30A, 604, 1994 (776)
DMEM/EBSS	Munson, L., Chandler, S., and Schlafer, D.: Long-Term Culture of Bovine Trophoblastic Cells, <i>J Tiss Cul Meth</i> 11 (3), 123, 1988 (868)
Moscona's BSS	Marcus, G., Connor, L., Domingo, M., Tsang, B., Downey, B., and Ainsworth, L.: Enzymatic Dissociation of Ovarian and Uterine Tissues, <i>Endocr Res</i> 10, 151, 1984 (372)
Dulbecco's phosphate buffered saline	Du Meihong, Han Haitang, Jiang Bin, Zhao Chen, Qian Changsong, Shen Haiyan, Xu Yan, Li Zandong: An efficient isolation method for domestic hen (<i>Gallus domesticus</i>) ovarian primary follicles, <i>J Reprod Dev</i> 52, 569-76, 2006 (10301)
(see reference)	Mruk, K. and Kobertz, W.: Discovery of a Novel Activator of KCNQ1-KCNE1 K Channel Complexes., <i>PLoS ONE</i> 4, e4236, 2009 (10511)
(see reference)	Pannaccione Anna, Castaldo Pasqualina, Ficker Eckhard, Annunziato Lucio, Tagliatela Maurizio: Histidines 578 and 587 in the S5-S6 linker of the human Ether-a-gogo Related Gene-1 K+ channels confer sensitivity to reactive oxygen species, <i>J Biol Chem</i> 277, 8912-9, 2002 (10166)
CF Medium	Alagem, N., Dvir, M., and Reuveny, E.: Mechanism of Ba ²⁺ Block of a Mouse Inwardly Rectifying K ⁺ Channel: Differential Contribution by Two Discrete Residues, <i>J Physiol</i> 534 (2), 381, 2001 (1148)
(see reference)	Tian, J., Kim, S., Heilig, E., and Ruderman, J.: Identification of XPR-1, A Progesterone Receptor Required for <i>Xenopus</i> Oocyte Activation, <i>Proc Natl Acad Sci U S A</i> 97, 14358, 2000 (1291)
Barth's solution, CF	Karknias, N, and Papke, R.: Subtype-Specific Effects of Lithium on Glutamate Receptor Function, <i>J Neurophysiol</i> 81, 1506, 1999 (1087)
CF Medium	Moriarty, T., Gillo, B., Carty, D., Premont, R., Landau, E., Iyengar, R.: Beta gamma Subunits of GTP-Binding Proteins Inhibit Muscarinic Receptor Stimulation of Phospholipase C, <i>Proc Natl Acad Sci U S A</i> 85, 8865, 1988 (661)
Dialyzed fetal calf serum, 10% and 0.5M Methotrexate	Wallis, R., and Drickamer, K.: Molecular Determinants of Oligomer Formation and Complement Fixation in Mannose-Binding Proteins, <i>J Biol Chem</i> 274 (6), 3580, 1999 (1125)
DMEM/F12	Lockwood, C., Arcuri, F., Toti, P., Felice, C., Krikun, G., Guller, S., Buchwalder, L. and Schatz, F.: Tumor Necrosis Factor-Alpha and Interleukin-1 Beta Regulate Interleukin-8 Expression in Third Trimester Decidual Cells: Implications for the Genesis of Chorioamnionitis., <i>Am J Pathol</i> 169, 1294-302, 2006 (10353)
HBSS	Meter, R., Wira, C. and Fahey, J.: Secretion of Monocyte Chemotactic Protein-1 by Human Uterine Epithelium Directs Monocyte Migration in Culture., <i>Fertil Steril Vol.</i> 84, 191, 2005 (10583)

Tissue Dissociation Guide

Human	Sertoli cells	Trypsin: 2.5% Collagenase Type 1: 2% Hyaluronidase: 1%
Human, female, 34-51 yr	Endometrial epithelial and stromal cells	Collagenase Type 3: 0.03% Deoxyribonuclease I: 0.004%
Human	Endothelial placental	Collagenase Type 1: 0.2% Trypsin: 0.2% Deoxyribonuclease I: 0.1%
Human	Amnion epithelial and fibroblast	Trypsin: 0.125% Collagenase: 0.1% Deoxyribonuclease I: 0.02%
Human	Uterine epithelial cells	Pancreatin: 0.34% Collagenase: 0.16% Hyaluronidase: 0.16%
Human, embryo	Endothelial Hematopoietic Stromal	Collagenase Type 1/2/4: 0.1%
Human, female	Mesothelial	Collagenase Type 1: 0.1% Deoxyribonuclease I: 0.05%
Human	Stem, embryonic	Neutral Protease: 0.01% - 0.02%
Human, 20-40 yr	Endometrium epithelial and stromal cells	Collagenase Type 1: 0.2%
Human	Chorionic villi	Trypsin: see reference Collagenase Type 3: 100 u/ml
Human	Endometrial endothelial cells	Collagenase Type 1: 0.2%
Human, female, 20-40 year	Microvascular endothelial cells	Collagenase Type 2: 0.2% Deoxyribonuclease I: 0.0015% Trypsin: 0.05%
Human, female	Endometrial epithelial cells	Collagenase Type 3: 45 u/ml Deoxyribonuclease I: .00035%
Human	Theca cells	Collagenase Type 1: 0.3% Deoxyribonuclease I: 0.0005% Hyaluronidase: 0.1%
Human	Corpus luteum cells	Collagenase Type 2: 0.25% Deoxyribonuclease I: .005%
Human, females, 25-45 yr	Luteal cells	Collagenase Type 2: 0.25% Deoxyribonuclease I: 0.005%
Human, female, 25-41 year	Follicles	Collagenase Type 2: 0.025-0.1%
Human	Placental	Deoxyribonuclease I: 0.04%
Human, female	Endometrial stromal cells	Collagenase: 4000 u/ml
Human	Stromal endometrial	Collagenase Type 3: 45 u/ml Deoxyribonuclease I: .00035%
Human	Epithelial, fallopian tube	Collagenase Type 1: 1%

Tissue Dissociation Guide

Medium	Reference
DMEM/F-12	Teng Yan, Xue Wu-jun, Ding Xiao-ming, Feng Xin-shun, Xiang He-li, Jiang Ya-zhuo, Tian Pu-xun: Isolation and culture of adult Sertoli cells and their effects on the function of co-cultured allogeneic islets in vitro, <i>Chin Med J (Engl)</i> 118, 1857-62, 2005 (10322)
DMEM/F-12	Chan Rachel W S, Schwab Kijana E, Gargett Caroline E: Clonogenicity of human endometrial epithelial and stromal cells, <i>Biol Reprod</i> 70, 1738-50, 2004 (10137)
DMEM	Wang Xin, Athayde Neil, Trudinger Brian: Microvascular endothelial cell activation is present in the umbilical placental microcirculation in fetal placental vascular disease, <i>Am J Obstet Gynecol</i> 190, 596-601, 2004 (10211)
PBS	Sun Kang, Myatt Leslie: Enhancement of glucocorticoid-induced 11beta-hydroxysteroid dehydrogenase type 1 expression by proinflammatory cytokines in cultured human amnion fibroblasts, <i>Endocrinology</i> 144, 5568-77, 2003 (10097)
HBSS	Fahey John V, Wira Charles R: Effect of menstrual status on antibacterial activity and secretory leukocyte protease inhibitor production by human uterine epithelial cells in culture, <i>J Infect Dis</i> 185, 1606-13, 2002 (10106)
DMEM	Oberlin, E., Tavian, M., Blazsek, I., and Peault, B.: Blood-forming potential of vascular endothelium in the human embryo, <i>Development</i> 129, 4147, 2002 (1059)
Eagle's MEM	Witz Craig A, Allsup Karen T, Montoya-Rodriguez Iris A, Vaughn Shelby L, Centonze Victoria E, Schenken Robert S: Culture of menstrual endometrium with peritoneal explants and mesothelial monolayers confirms attachment to intact mesothelial cells, <i>Hum Reprod</i> 17, 2832-8, 2002 (10287)
DMEM	Zhang, S., Wernig, M., Duncan, I., Brustle, O., and Thomson, J.: <i>In Vitro</i> Differentiation of Transplantable Neural Precursors from Human Embryonic Stem Cells, <i>Nat Biotechnol</i> 19, 1129, 2001 (1135)
HBSS	Arnold, J., Kaufman, D., Seppala, M., and Lessey, B.: Endometrial stromal cells regulate epithelial cell growth in vitro: a new co-culture model, <i>Hum Reprod</i> 16, 836, 2001 (9820)
HBSS	Yusuf RZ, Naeem R: Cytogenetic studies of spontaneous miscarriages: a seven year study to compare significance of primary vs. secondary culture methods for assessment of fetal karyotype yield and maternal cell contamination, <i>Early Pregnancy</i> 5, 121-31, 2001 (10290)
McCoys medium	Nikitenko LL, MacKenzie IZ, Rees MC, Bicknell R: Adrenomedullin is an autocrine regulator of endothelial growth in human endometrium, <i>Mol Hum Reprod</i> 6, 811, 2000 (10029)
PBS	Gargett CE, Bucak K, Rogers PA: Isolation, characterization and long-term culture of human myometrial microvascular endothelial cells, <i>Hum Reprod</i> 15, 293-301, 2000 (10148)
DMEM/F-12	Zhang J, Lathbury LJ, Salamonsen LA: Expression of the chemokine eotaxin and its receptor, CCR3, in human endometrium, <i>Biol Reprod</i> 62, 404-11, 2000 (10217)
PBS	Runesson E, Ivarsson K, Janson P, Brannstrom M: Gonadotropin- and cytokine-regulated expression of the chemokine interleukin 8 in the human preovulatory follicle of the menstrual cycle, <i>J Clin Endocrinol Metab</i> 85, 4387-95, 2000 (10272)
PBS	Friden BE, Runesson E, Hahlin M, Brannstrom M: Evidence for nitric oxide acting as a luteolytic factor in the human corpus luteum, <i>Mol Hum Reprod</i> 6, 397-403, 2000 (10332)
PBS	Friden BE, Hagstrom H, Lindblom B, Sjoblom P, Wallin A, Brannstrom M, Hahlin M: Cell characteristics and function of two enriched fraction of human luteal cells prolonged culture, <i>Mol Hum Reprod</i> 5, 714-9, 1999 (10147)
EBSS	Hovatta O, Wright C, Krausz T, Hardy K, Winston RM: Human primordial, primary and secondary ovarian follicles in long-term culture: effect of partial isolation, <i>Hum Reprod</i> 14, 2519-24, 1999 (10323)
PSS	Bradbury, R., Sunn, K., Crossley, M., Bai, M., Brown, E., Delbridge, D., and Conigrave, A.: Expression of the Parathyroid Ca ²⁺ - Sensing Receptor in Cytotrophoblasts From Human Term Placenta, <i>J Endocrinol</i> 156, 425-430, 1998 (741)
DMEM/F-12	Huang JC, Liu DY, Dawood MY: The expression of vascular endothelial growth factor isoforms in cultured human endometrial stromal cells and its regulation by 17beta-oestradiol, <i>Mol Hum Reprod</i> 4, 603-7, 1998 (10154)
DMEM/Ham's F12	Zhang J, Nie G, Jian W, Woolley DE, Salamonsen LA: Mast cell regulation of human endometrial matrix metalloproteinases: A mechanism underlying menstruation, <i>Biol Reprod</i> 59, 693-703, 1998 (10291)
Medium 199	Takeuchi, K., Maruyama, I., Yamamoto, S., Oki, T., Nagata, Y.: Isolation and Monolayer Culture of Human Fallopian Tube Epithelial Cells, <i>In Vitro Cell Dev Biol</i> 27, 720, 1991 (475)

Tissue Dissociation Guide

Mouse	Human	Trophoblasts, placental	Trypsin: 0.25%
	Human	Trophoblasts, placental	Trypsin: 0.25%
	Human	Chorionic, placental	Deoxyribonuclease I: 0.003%
	Human, female	Endometrial	Collagenase: 2%
	Human	Placental	Trypsin: 0.25%
	Human (also porcine, bovine)	Interna & corpus luteum Endometrium Ovarian Uterine	Pronase: 0.1%
	Human, female, 27-49 years	Epithelial Ovary	Trypsin: 0.125%
	Human	Epithelial Stromal	Collagenase: 0.25%
	Human, infant and neonate	Epithelial Prostate	Trypsin: 0.1%
	Human, female, 27 years	Smooth muscle, uterine	Trypsin: 0.05%
	Human	Epithelial Stromal	Collagenase Type 1: 180 u/ml
	Mouse, male	Testis, meiotic	Collagenase Type 1: 120 u/ml Deoxyribonuclease I: 0.001% Trypsin: 0.1%
	Mouse	Germ cells	Collagenase Type 1: 100 u/ml
	Mouse, 6 day	Sertoli	Collagenase Type 2: 500 u/ml Hyaluronidase: 0.1% Deoxyribonuclease I: .0005%
	Mouse, adult, neonatal and fetal	Testicular cells	Collagenase Type 1: 0.1%
	Mouse, male, 3.5 months old	Leydig	Collagenase: 0.06%
	Mouse, male, 12-14-week-old	Seminiferous tubules	Trypsin: 0.05%
	Mouse, 6-12 day	Oocyte-granulosa	Collagenase Type 1: 0.1% Deoxyribonuclease I: 0.02%
	Mouse, female, 6-8 weeks	Uterine	Trypsin: 0.2%
	Mouse, CF1, female	Cumulus, one-cell embryos	Hyaluronidase: 0.1%
	Mouse BALB/cCRGL, male, 2-3 months	Epithelial, prostate gland	Hyaluronidase: 0.1%
	Mouse C57B/T, fetus, 16-17 days old	Prostate	Trypsin: 1.0%
	Mouse, outbred, CD-1, 21-23 days old	Epithelial	Trypsin: 0.5%
	Mouse, BALB/c	Epithelial Mesencymal	Trypsin: 1%
	Mouse, BALB/cCrg, 40 days old	Epithelial	Trypsin: 1%
	Mouse, male, 10-13 wk	Leydig Testis	Deoxyribonuclease I: 0.001%

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Medium	Reference
EBSS, CMF	Branchaud, C.L., Goodyer, C.G., Guyda, H.J. and Lefebvre, Y.: A Serum-Free System for Culturing Human Placental Trophoblasts, <i>In Vitro Cell Dev Biol</i> 26, 865, 1990 (453)
PBS	Jie, Z., Fey, S., Hager, H., Hollsberg, P., Ebbesen, P., and Larsen, P.: Markers For Human Placental Trophoblasts in Two-Dimensional Gel Electrophoresis, <i>In Vitro Cell Dev Biol</i> 26, 937, 1990 (455)
HBSS	Egan, D., Grzegorzcyk, V., Tricarico, K., Rueter, A.H., Olleman, W., and Marcotte, P.: Human Placental Chorionic Renin: Production, Purification and Characterization, <i>Biochim Biophys Acta</i> 965, 68, 1988 (335)
RPMI 1640	Rinehart, C., Lyn-Cook, B., and Kaufman, D.: Gland formation from human endometrial epithelial cells in vitro, <i>In Vitro Cell Dev Biol</i> 24 (10), 1037, 1988 (1295)
DMEM	Morrish, D., and Siy, O.: Critical Factors in Establishing Monolayer Cultures of Normal Human Placental Cells in Serum-Free Medium, <i>Endocr Res</i> 12 (3), 229, 1986 (979)
Moscona's BSS	Marcus, G., Connor, L., Domingo, M., Tsang, B., Downey, B., and Ainsworth, L.: Enzymatic Dissociation of Ovarian and Uterine Tissues, <i>Endocr Res</i> 10, 151, 1984 (372)
HBSS, CMF	Auersperg, N., Siemens, C.H., and Myrdal, S.E.: Human Ovarian Surface Epithelium In Primary Culture, <i>In Vitro</i> 20, 743, 1984 (535)
(see reference)	Siegfried, J., Nelson, K., Martin, J., and Kaufman, D.: Histochemical Identification of Cultured Cells From Human Endometrium, <i>In Vitro</i> 20 (1), 25, 1984 (985)
HBSS	Lechner, J., Babcock, M., Marnell, M., Narayan, K., and Kaighn, M.: Normal Human Prostate Epithelial Cell Cultures, <i>Methods Cell Biol</i> 21, 195, 1980 (631)
EBSS	Rifas, L., Fant, J., Makman, M., and Seifter, S.: The Characterization of Human Uterine Smooth Muscle Cells in Culture, <i>Cell Tissue Res</i> 196, 385, 1979 (355)
DMEM	Kirk, D., King, R., Heyes, J., Peachey, L., Hirsch, P., and Taylor, W.: Normal Human Endometrium in Cell Culture, <i>In Vitro</i> 14 (8), 651, 1978 (984)
Gey's BSS	Getun, I., Torres, B. and Bois, P.: Flow Cytometry Purification of Mouse Meiotic Cells., <i>J Vis Exp</i> 50, 2602, 2011 (10658)
HBSS	Breault, D., Min, I., Carlone, D., Farilla, L., Ambruzs, D., Henderson, D., Algra, S., Montgomery, R., Wagers, A. and Hole, N.: Generation of mTert-GFP Mice as a Model to Identify and Study Tissue Progenitor Cells., <i>Proc Natl Acad Sci U S A</i> 105, 10420, 2008 (10522)
DMEM	Naibandian Angele, Dettin Luis, Dym Martin, Ravindranath Neelakanta: Expression of vascular endothelial growth factor receptors during male germ cell differentiation in the mouse, <i>Biol Reprod</i> 69, 985-94, 2003 (10162)
DMEM/F-12	O'Shaughnessy PJ, Fleming LM, Jackson G, Hochgeschwender U, Reed P, Baker PJ: Adrenocorticotrophic hormone directly stimulates testosterone production by the fetal and neonatal mouse testis, <i>Endocrinology</i> 144, 3279-84, 2003 (10165)
Medium E 199	Faldikova, L., Diblikova, I., Canderle, J., Zraly, Z., Veznik, Z., and Sulcova, A.: Effects of Nutrition, Social Factors and Chronic Stress on the Mouse Leydig Cell Testosterone Production, <i>Vet Med</i> 46 (6), 160, 2001 (1118)
DMEM	Lin, Q., Sirotkin, A., and Skoultchi, A.: Normal Spermatogenesis in Mice Lacking the Testis-Specific Linker Histone H1t, <i>Mol Cell Biol</i> 20 (6), 2122, 2000 (1147)
Waymouth	Eppig JJ, O'Brien MJ: Development in vitro of mouse oocytes from primordial follicles, <i>Biol Reprod</i> 54, 197-207, 1996 (10256)
HBSS	Ghosh, D., Danielson, K., Alston, J., Heyner, S.: Functional Differentiation of Mouse Uterine Epithelial Cells Grown On Collagen Gels Or Reconstituted Basement Membranes, <i>In Vitro Cell Dev Biol</i> 27, 713, 1991 (474)
PBS, CMF	Spindle, A.: In vitro Development of One-Cell Embryos from Outbred Mice: Influence of Culture Medium Composition, <i>In Vitro Cell Dev Biol</i> 26, 151, 1990 (424)
Medium 199	Turner, T., Bern, H., Young, P., and Cunha, G.: Serum-Free Culture of Enriched Mouse Anterior and Ventral Prostatic Epithelial Cells in Collagen Gel, <i>In Vitro Cell Dev Biol</i> 26, 722, 1990 (449)
HBSS/DMEM	Thompson, T.C., Southgate, J., Kitchener, G., and Land, H.: Multistage Carcinogenesis Induced by ras and myc Oncogenes in a Reconstituted Organ, <i>Cell</i> 56, 917, 1989 (360)
Medium 199	Tomooka, Y., DiAugustine, R., and McLachlan, J.: Proliferation of Mouse Uterine Epithelial Cells <i>in Vitro</i> , <i>Endocrinology</i> 118 (3), 1011, 1986 (914)
DMEM	Bigsby, R., Cooke, P., and Cunha, G.: A Simple Efficient Method For Separating Murine Uterine Epithelial and Mesenchymal Cells, <i>Am J Physiol</i> 251, E630, 1986 (915)
Medium 199	Cooke, P., Uchima, F., Fujii, D., Bern, H., and Cunha, G.: Restoration of Normal Morphology and Estrogen Responsiveness in Cultured Vaginal and Uterine Epithelia Transplanted with Stroma, <i>Proc Natl Acad Sci U S A</i> 83, 2109, 1986 (931)
Medium 199 w/ BSA	Stalvey, J. and Payne, A.: Luteinizing Hormone Receptors and Testosterone Production in Whole Testes and Purified Leydig Cells from the Mouse: Differences among Inbred Strains, <i>Endocrinology</i> 112, 1696, 1983 (376)

Tissue Dissociation Guide

Species

Reproductive (con't)

Cell(s)

Enzyme(s)

Ovine	Mouse BALB/cCrgl, female, 50 - 60 days	Epithelial, vagina	Collagenase Type 3: 0.1%	
	Sheep	Epithelial	Collagenase: 125 - 190 u/ml	
Porcine	Porcine, male, 8 day	Seminiferous epithelial cells	Collagenase: 0.15% Deoxyribonuclease I: .0001% Hyaluronidase: 0.15% Trypsin: 0.05%	
	Porcine, female	Corpus Leuteum	Collagenase Type 4: 600 u/ml	
	Porcine (also bovine, human)	Interna & corpus luteum Endometrium Ovarian Uterine	Pronase: 0.1%	
Rabbit	Porcine, 3-4 week	Leydig Testis	Trypsin: 0.0003%	
	Rabbit, New Zealand, 3 month	Testicular germ	Collagenase Type 1: 0.1% Trypsin: 0.25% Deoxyribonuclease I: 0.7%	
	Rabbit, New Zealand white, 4-6 month old,	Ovarian Mesothelial	Collagenase Type 1: 300 u/ml	
	Rabbit, New Zealand white, adult, female (nonpregnant), 3-4 kg	Myocytes, uterine	Deoxyribonuclease I: 200 µg/ml	
	Rabbit, New Zealand, mature, female, 4-5 Kg	Ovarian surface epithelial and peritoneal mesothelial	Collagenase Type 1: 300 IU/ml (280 IU/mg)	
	Rabbit, mixed breed	Endometrial epithelial	Collagenase Type 1: 0.005%	
	Rabbit, New Zealand white, mature, 3-4 Kg	Myometrial	Trypsin: 0.02%, 0.03%, 0.0375%	
	Rabbit, New Zealand white estrous, female, 4-5 months	Mesothelial and surface epithelial Ovaries	Trypsin: 0.125%-0.5%	
	Rat	Rat, SD, 350-450 g	Leydig cells	Collagenase: .05-0.1%
		Rat, SD, adult, male, 8 - 10 week	Seminiferous tubules	Trypsin: 0.05%
Rat, SD, immature		Ovary	Collagenase Type 1: 144 u/ml	
Rat, Han-Wistar, adult, male, 250 g		Testes	Trypsin: 0.1%	
Rat, SD, female, immature, 26 day (also rat, pregnant and pseudopregnant)		Corpus luteum	Neutral Protease: 2.4 u/ml Deoxyribonuclease: 200u/ml	
Rat, SD, 25 day		Ovarian theca-interstitial	Collagenase Type 1: 0.5% Deoxyribonuclease I: 0.02%	
Rat, SD, male, 90 days		Sertoli	Hyaluronidase: 0.1%	
Rat, SD, male		Leydig	Trypsin: 0.02%	
Rat, neonatal		Uterine	Trypsin: 1%	
Rat, SD, male, 120-160 g		Leydig Adrenal	Collagenase Type 2: 0.03% (adrenal)	
Rat, Wistar, adult	Uterine	Trypsin: 0.5%		

Tissue Dissociation Guide

Medium	Reference
HBSS	Iguchi, T., Uchima, F.A., Ostrander, P., and Bern, H.: Growth of Normal Mouse Vaginal Epithelial Cells in and on Collagen Gels, <i>Proc Natl Acad Sci U S A</i> 80, 3743, 1983 (655)
DMEM	Salamonsen, L., Sum O, W., Doughton, B., and Findlay, J.: The Effects of Estrogen and Progesterone In Vivo on Protein Synthesis and Secretion by Cultured Epithelial Cells from Sheep Endometrium, <i>Endocrinology</i> 117 (5), 2148, 1985 (1193)
DMEM/F12	Dirami G, Ravindranath N, Pursel V, Dym M: Effects of stem cell factor and granulocyte macrophage-colony stimulating factor on survival of porcine type A spermatogonia cultured in KSOM, <i>Biol Reprod</i> 61, 225-30, 1999 (10142)
Medium 199	Ciereszko, R., Petroff, B., Ottobre, A., Guan, Z., Stokes, B., and Ottobre, J.: Assessment of the Mechanism by Which Prolactin Stimulates Progesterone Production by Early Corpora Lutea of Pigs, <i>J Endocrinol</i> 159, 201, 1998 (1088)
Moscona's BSS	Marcus, G., Connor, L., Domingo, M., Tsang, B., Downey, B., and Ainsworth, L.: Enzymatic Dissociation of Ovarian and Uterine Tissues, <i>Endocr Res</i> 10, 151, 1984 (372)
Lebovitz L-15 Medium	Mather, J., Saez, J., and Haour, F.: Regulation of Gonadotropin Receptors and Steroidogenesis in Cultured Porcine Leydig Cells, <i>Endocrinology</i> 110, 933, 1982 (374)
HBSS	Kubota, H., Wu, X., Goodyear, S., Avarbock, M. and Brinster, R.: Glial Cell Line-Derived Neurotrophic Factor and Endothelial Cells Promote Self-Renewal of Rabbit Germ Cells with Spermatogonial Stem Cell Properties., <i>FASEB J</i> 25, 2604, 2011 (10640)
HBSS	Setrakian, S., Oliveros-Saunders, B., and Nicosia, S.: Growth Stimulation of Ovarian and Extraovarian Mesothelial Cells by Corpus Luteum Extract, <i>In Vitro Cell Dev Biol</i> 29A, 879, 1993 (785)
HBSS-HEPES buffer	Phillippe, M., Saunders, T., and Bangalore, S.: Alpha-1, Alpha-2, and Beta Adrenergic Signal Transduction in Cultured Uterine Myocytes, <i>In Vitro Cell Dev Biol</i> 26, 369, 1990 (439)
HBSS, CMF	Piquette, G., and Timms, B.: Isolation and Characterization of Rabbit Ovarian Surface Epithelium, Granulosa Cells, and Peritoneal Mesothelium in Primary Culture, <i>In Vitro Cell Dev Biol</i> 26, 471, 1990 (443)
DMEM	Mulholland, J., Winterhager, E., and Beier, H.: Changes in Proteins Synthesized by Rabbit Endometrial Epithelial Cells Following Primary Culture, <i>Cell Tissue Res</i> 252, 123, 1988 (905)
HBSS	Boulet, A., and Fortier, M.: Preparation and Characterization of Rabbit Myometrial Cells in Primary Culture: Influence of Estradiol and Progesterone Treatment, <i>In Vitro Cell Dev Biol</i> 23, 93, 1987 (1286)
Medium 199	Nicosia, S., Johnson, J., and Streibel, E.: Isolation and Ultrastructure of Rabbit Ovarian Mesothelium (Surface Epithelium), <i>Int J Gynecol Pathol</i> 3, 348, 1984 (542)
Medium 199	Sharma RS, Pal PC, Rajalakshmi M.: Isolation and Culture of Leydig Cells from Adult Rats, <i>Indian J Clinical Biochem</i> 21, 27, 2006 (10026)
Krebs-Ringer bicarbonate buffer (see reference)	Abou-Haila, A., and Tulsiani, D.: Acid Glycohydrolases in Rat Spermatozoa, Spermatozoa: Enzyme Activities, Biosynthesis and Immunolocalization, <i>Biol Proced Online</i> 3 (1), 35, 2001 (1074)
McCoy's 5a	Ando, M., Kol, S., Irahara, M., Sirois, J., and Adashi, E.: Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) Block the Late, Prostanoid-Dependent/Ceramide-Independent Component of Ovarian IL-1 Action: Implications for the Ovulatory Process, <i>Mol Cell Endocrinol</i> 157, 21, 1999 (1141)
Medium 199 w/ Hank's salts	Leckie, C., Welberg, L., and Seckl, J.: 11 β -Hydroxysteroid Dehydrogenase is a Predominant Reductase in Intact Rat Leydig Cells, <i>J Endocrinol</i> 159, 233, 1998 (1089)
Serum-free medium (see reference)	Telleria, C., Ou, J., Sugino, N., Ferguson, S., and Gibori, G.: The Expression of Interleukin-6 in the Pregnant Rat Corpus Luteum and Its Regulation by Progesterone and Glucocorticoid, <i>Endocrinology</i> 139 (8), 3597, 1998 (1090)
Medium 199	Duleba AJ, Spaczynski RZ, Olive DL, Behrman HR: Effects of insulin and insulin-like growth factors on proliferation of rat ovarian theca-interstitial cells, <i>Biol Reprod</i> 56, 891-7, 1997 (10331)
DMEM	Onoda, M. and Djakiew, D.: Pachytene Spermatozoa Protein(s) Stimulate Sertoli Cells Grown in Bicameral Chambers: Dose-Dependent Secretion of Ceruloplasmin, Sulfated Glycoprotein-1, Sulfated Glycoprotein-2, and Transferrin, <i>In Vitro Cell Dev Biol</i> 27, 215, 1991 (460)
DMEM	Abayasekara, D., Kurlak, L., Band, A., Sullivan, M., and Cooke, B.: Effect of Cell Purity, Cell Concentration, and Incubation Conditions on Rat Testis Leydig Cell Steroidogenesis, <i>In Vitro Cell Dev Biol</i> 27, 253, 1991 (461)
HBSS	Branham, W., Lyn-Cook, B., Andrews, A., McDaniel, M., Sheehan, D.: Growth of Neonatal Rat Uterine Luminal Epithelium on Extracellular Matrix, <i>In Vitro Cell Dev Biol</i> 27, 442, 1991 (465)
Krebs Ringer bicarbonate buffer	Ng, T. and Liu, W.: Toxic Effect of Heavy Metals on Cells Isolated from the Rat Adrenal and Testis, <i>In Vitro Cell Dev Biol</i> 26, 24, 1990 (435)
PBS	Pampfer, S., Vanderheyden, I., Michiels, B., and DeHertogh, R.: Co-Culture of Two-Cell Rat Embryos on Cell Monolayers, <i>In Vitro Cell Dev Biol</i> 26, 944, 1990 (456)

Tissue Dissociation Guide

Species

Reproductive (con't)

Cell(s)

Enzyme(s)

Rat, SD, 10 day	Sertoli cells	Collagenase Type 2: 500 u/ml Deoxyribonuclease I: 0.0005% Hyaluronidase: 0.1%
Rat, immature	Luteal, ovaries	Collagenase: 0.3%
Rat, male, 20 day	Sertoli	Trypsin: 0.15%
Rat, SD, female	Luminal epithelial	Trypsin: 0.5%
Rat, Wistar, virgin, female, 250 g	Vaginal epithelial	Trypsin: 0.5%
Rat, SD, female, 21 day	Luteal, ovaries	Deoxyribonuclease I: 0.0004%
Rat, SD, male, 10 day	Sertoli	Trypsin: 0.025%
Rat, Holtzman, female, immature	Uterine	Deoxyribonuclease I: 0.025%
Rat, SD, adult, male, 50-70 days	Testicular	Deoxyribonuclease I: 10 µg/ml
Rat, SD, female, pseudopregnant, 21 day	Luteal, ovaries	Hyaluronidase: 0.1%
Rat, SD, 4-6 day, male	Sertoli, seminiferous tubules	Collagenase: 0.03%
Rat, SD, female, 26 days	Luteal	Deoxyribonuclease I: 0.0004%
Rat, SD, male	Leydig	Collagenase: 0.1%

Scales

Species

Cell(s)

Enzyme(s)

Fish	Goldfish (<i>Carassius Auratus L.</i>)	Pigment, xanthopores	Deoxyribonuclease I: 0.005%
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Skin

Species

Cell(s)

Enzyme(s)

Frog	Frog	Epidermal	Trypsin: 0.18%
Human	Human, 6-12 year	Dermal fibroblasts	Neutral Protease: 0.1% Collagenase Type 1: 0.1%
	Human	fibroblasts	Collagenase: 0.5% Trypsin: 0.25%
	Human, adult	Dermal fibroblasts	Collagenase: 0.5-1.0%
	Human	Hair follicular epithelial	Neutral Protease: 1.25% Trypsin: 0.25%
	Human, neonatal	Keratinocytes	Neutral Protease: 0.4% Collagenase: 0.3%
	Human	Mast cells	Neutral Protease: 0.05% Collagenase Type 4: 1.0%
	Human	Fibroblasts	Trypsin: 0.25%
	Human	Keratinocytes, fibroblasts, endothelial	Neutral Protease: see reference Collagenase: see reference

Tissue Dissociation Guide

Medium	Reference
PBS	Hadley MA, Weeks BS, Kleinman HK, Dym M: Laminin promotes formation of cord-like structures by Sertoli cells in vitro, <i>Dev Biol</i> 140, 318-27, 1990 (10150)
McCoy's	Rajan, V. and Menon, K.: Differential Uptake and Metabolism of Free and Esterified Cholesterol from High-density Lipoproteins in the Ovary, <i>Biochim Biophys Acta</i> 959, 206, 1988 (330)
(see reference)	Skinner, M., Fetterolf, P., and Anthony, C.: Purification of a Paracrine Factor, P-Mod-S, Produced by Testicular Peritubular Cells That Modulates Sertoli Cell Function, <i>J Biol Chem</i> 263, 2884, 1988 (561)
HBSS	Glasser, S., Julian, J., Decker, G., Tang, J., and Carson, D.: Development of Morphological and Functional Polarity in Primary Cultures of Immature Rat Uterine Epithelial Cells, <i>J Cell Biol</i> 107 (6), 2409, 1988 (920)
PBS	Conti, C., and Tasat, D.: Regulation of Cultured Rat Vaginal Epithelial Cells By 17 β -Estradiol and Progesterone, <i>J Steroid Biochem</i> 24 (3), 747, 1986 (921)
McCoy's	Rajan, V. and Menon, K.: Involvement of Microtubules in Lipoprotein Degradation and Utilization for Steroidogenesis in Cultured Rat Luteal Cells, <i>Endocrinology</i> 117, 2408, 1985 (382)
DMEM	Hadley, M., Byers, S., Suarez-Quian, C., Kleinman, H., and Dym, M.: Extracellular Matrix Regulates Sertoli Cell Differentiation, Testicular Cord Formation and germ cell development in vitro, <i>J Cell Biol</i> 101, 1511, 1985 (575)
DMEM buffered with HEPES	Kassis, J., Walent, J., and Gorski, J.: Estrogen Receptors in Rat Uterine Cell Cultures: Effects of Medium on Receptor Concentration, <i>Endocrinology</i> 115, 762, 1984 (381)
HEPES	Hsueh, A., Bambino, T., Zhuang, L., Welsh, T., and Ling, N.: Mechanism of the Direct Action of Gonadotropin Releasing Hormone and Its Antagonist on Androgen Biosynthesis by Cultured Rat Testicular Cells, <i>Endocrinology</i> 112, 1653, 1983 (375)
EBSS	Rajendran, K., Hwang, J., and Menon, K.: Binding, Degradation and Utilization of Plasma High Density and Low Density Lipoproteins for Progesterone Production In Cultured Rat Luteal Cells, <i>Endocrinology</i> 112, 1746, 1983 (377)
Serum-free medium	Rich, K., Bardin, C., Gunsalus, G., and Mather, J.: Age-Dependent Pattern of Androgen-Binding Protein Secretion from Rat Sertoli Cells in Primary Culture, <i>Endocrinology</i> 113, 2284, 1983 (379)
Medium 199	Azhar, S. and Reaven, E.: Effect of Antimicrotubule Agents on Microtubules and Steroidogenesis in Luteal Cells, <i>Am J Physiol</i> 243, E380, 1982 (290)
Krebs Ringer bicarbonate buffer	Ramachandran, J., and Sairam, M.R.: The Effects Of Interstitial Cell-Stimulating Hormone, Its Subunits, and Recombinants on Isolated Rat Leydig Cells, <i>Arch Biochem Biophys</i> 167, 294, 1975 (303)

Scales

Medium	Reference
Medium 199 w/BSA	Lo, S., Grabowski, S., Lynch, T., Kern, D., Taylor, J.T., and Chen, T.: Isolation of xanthophores from the goldfish, <i>In Vitro</i> 18, 356, 1982 (518)

Skin

Medium	Reference
Barth's solution, CMF	Nishikawa, A., Shimizu-Nishikawa, K., and Miler, L.: Isolation, characterization, and in vitro culture of larval and adult epidermal cells of the frog <i>Xenopus laevis</i> , <i>In Vitro Cell Dev Biol</i> 26, 1128, 1990 (1287)
DMEM	Chen, F., Zhang, W., Bi, D., Liu, W., Wei, X., Chen, F., Zhu, L., Cui, L. and Cao, Y.: Clonal Analysis of Nestin(-) Vimentin(+) Multipotent Fibroblasts Isolated from Human Dermis., <i>J Cell Sci</i> 120, 2875, 2007 (10548)
DMEM/F12	Clark, R., Chong, B., Mirchandani, N., Yamanaka, K., Murphy, G., Dowgiert, R. and Kupper, T.: A Novel Method for the Isolation of Skin Resident T cells from Normal and Diseased Human Skin., <i>J Invest Dermatol</i> 126, 1059, 2006 (10678)
DMEM	Wang, H., Van Bliitterswijk, C., Bertrand-De Haas, M., Schuurman, A. and Lamme, E.: Improved Enzymatic Isolation of Fibroblasts for the Creation of Autologous Skin Substitutes., <i>In Vitro Cell Dev Biol Anim</i> 40, 268, (10556)
DMEM/F-12	Yu Hong, Fang Dong, Kumar Suresh M, Li Ling, Nguyen Thiengga K, Acs Geza, Herlyn Meenhard, Xu Xiaowei: Isolation of a novel population of multipotent adult stem cells from human hair follicles, <i>Am J Pathol</i> 168, 1879-88, 2006 (10334)
DMEM	Li, A., Pouliot, N., Redvers, R., and Kaur, P.: Extensive tissue-regenerative capacity of neonatal human keratinocytes stem cells and their progeny, <i>J Clin Invest</i> 113, 390-400, 2004 (10128)
RPMI	Babina Magda, Guhl Sven, Starke Andrae, Kirchhof Loreen, Zuberbier Torsten, Henz Beate M: Comparative cytokine profile of human skin mast cells from two compartments--strong resemblance with monocytes at baseline but induction of IL-5 by IL-4 priming, <i>J Leukoc Biol</i> 75, 244-52, 2004 (10218)
DMEM	Tuan Tai-Lan, Wu Huayang, Huang Eunice Y, Chong Sheree S N, Laug Walter, Messadi Diana, Kelly Paul, Le Anh: Increased plasminogen activator inhibitor-1 in keloid fibroblasts may account for their elevated collagen accumulation in fibrin gel cultures, <i>Am J Pathol</i> 162, 1579-89, 2003 (10279)
(see reference)	Supp Dorothy M, Wilson-Landy Kaila, Boyce Steven T: Human dermal microvascular endothelial cells form vascular analogs in cultured skin substitutes after grafting to athymic mice, <i>FASEB J</i> 16, 797-804, 2002 (10277)

Tissue Dissociation Guide

Species

Skin (con't)

Cell(s)

Enzyme(s)

Human, adult	Keratinocytes	Neutral Protease: 0.25%
Human	Keratinocytes	Neutral Protease: 0.25% Trypsin: 0.17% Thermolysin: 0.05%
Human	Human skin mast cells	Neutral Protease: 0.1% Collagenase Type 1: see reference
Human	Epidermal	Collagenase Type 2: 0.1% Neutral Protease: 0.5-1.0%
Human	Keratinocytes	Trypsin: 0.05%
Human	Epidermis plus dermis Abdomen or inner forearm	Trypsin: 0.125%
Human	Keratinocytes	Trypsin: 0.25%
Human, adult, female, breast skin	Keratinocytes	Trypsin: 0.25%
Human, neonatal foreskin	Fibroblasts	Trypsin: 0.25%
Human, 18-50 years	Sweat duct	Collagenase: 0.03%
Human	Fibroblasts	Trypsin: 0.25%
Human	Sweat gland	Collagenase Type 2: 0.015%
Human, ages 16-30	Human sweat duct	Collagenase Type 2: 0.2%
Human	Melanocytes, skin/ foreskin	Trypsin: 0.25%
Human	Human sweat glands	Collagenase: 0.2%
Human	Keratinocytes	Trypsin: 0.25%
Human, adult	Epidermal keratinocytes	Trypsin: 0.25% Collagenase: 0.2% Deoxyribonuclease I: 0.001%
Human, fetal	Smooth muscle, fibroblasts	Trypsin: 0.055%
Human, 18-30 years, male, female	Fibroblasts	Trypsin: 0.1%
Human, newborn	Keratinocytes	Trypsin: 0.3%
Human	Keratinocytes	Trypsin: 0.25%
Mouse	Mouse	Epidermal and dermal
		Collagenase Type 4: 0.18% Collagenase/Dispase: 0.18%
	Mouse	Dermal
		Collagenase Type 3: 0.3% Deoxyribonuclease I: 0.0005%
	Mouse, neonatal	Microvascular endothelial
		Neutral Protease: 0.005% Collagenase Type 1: 4%
	Mouse, neonatal	Dermal
		Collagenase: 0.35% Deoxyribonuclease I: see reference

Tissue Dissociation Guide

Medium	Reference
PBS	Baudoux, B., Castanares-Zapatero, D., Leclercq-Smekens, M., Berna, N., and Poumay, Y.: The Tetraspanin CD9 Associates with the Integrin $\alpha 6\beta 4$ in Cultured Human Epidermal Keratinocytes and is Involved in Cell Motility, <i>Eur J Cell Biol</i> 79, 41, 2000 (1103)
(see reference)	Hybbinette S, Bostrom M, Lindberg K: Enzymatic dissociation of keratinocytes from human skin biopsies for in vitro cell propagation, <i>Exp Dermatol</i> 8, 30-8, 1999 (10156)
PBS	Grutzkau A, Kruger-Krasagakes S, Baumeister H, Schwarz C, Kogel H, Welker P, Lippert U, Henz BM, Moller A: Synthesis, storage, and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by human mast cells: implications for the biological significance of VEGF206, <i>Mol Biol Cell</i> 9, 875-84, 1998 (10149)
PBS	Reece J C, Handley A J, Anstee E J, Morrison W A, Crowe S M, Cameron P U: HIV-1 selection by epidermal dendritic cells during transmission across human skin, <i>J Exp Med</i> 187, 1623-31, 1998 (10168)
Dulbecco's PBS	Judd, D., Battista, P., and Behm, D.: Culture of Human Keratinocytes in Defined Serum-Free Medium, <i>Focus</i> 19 (1), 2, 1997 (1208)
DMEM, M199	Harley, C., and Sherwood, S.: <i>Methods in Molecular Biology, Basic Cell Culture Protocols, 2nd ed. Vol. 75</i> , Pollard, J., and Walker, J., Humana Press, 23, 1997 (1294)
DMEM	Regnier, M: Culture of Human Karatinocytes, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons Ltd, 11B:4.1, 1995 (1270)
PBS	Hirel, B., Chesne, C., Pailgheret, J., and Guillouzo, A.: Expression of Differentiation Markers in Human Adult Keratinocytes Cultured in Submerged Conditions, <i>In Vitro Cell Dev Biol</i> 30A, 372, 1994 (1035)
DMEM	Hansbrough, J., Cooper, M., Cohen, R., Spielvogel, R., Greenleaf, G., Bartel, R., and Naughton, G.: Evaluation of a Biodegradable Matrix Containing Cultured Human Fibroblasts as a Dermal Replacement Beneath Meshed Skin Grafts on Athymic Mice, <i>Surgery</i> 111 (1), 438, 1992 (1106)
MEM	Bell, C. and Quinton, P.: Effects of Media Buffer Systems on Growth and Electrophysiologic Characteristics of Cultured Sweat Duct Cells, <i>In Vitro Cell Dev Biol</i> 27, 47, 1991 (466)
CMF solution	Limat, A., Hunziker, T., Boillat, C., Noser, F., and Wiesmann, U.: Postmitotic Human Dermal Fibroblasts Preserve Intact Feeder Properties for Epithelial Cell Growth After Long-Term Cryopreservation, <i>In Vitro Cell Dev Biol</i> 26, 709, 1990 (447)
(see reference)	Krouse, M., Hagiqara, G., Chen, J., Lewiston, N., and Wine, J.: Ion Channels in Normal Human and Cystic Fibrosis Sweat Gland Cells, <i>Am J Physiol</i> 257, C129, 1989 (299)
RPMI 1640 (see reference)	Pedersen, P.: Human Sweat Duct Cells in Primary Culture. Basic Bioelectric Properties of Cultures Derived From Normals and Patients with Cystic Fibrosis, <i>In Vitro Cell Dev Biol</i> 25 (4), 342, 1989 (987)
PBS	Peacocke, M., Yaar, M., Mansur, C., Chao, M., and Gilchrest, B.: Induction of Nerve Growth Factor Receptors on Cultured Human Melanocytes, <i>Proc Natl Acad Sci U S A</i> 85, 5282, 1988 (660)
HBSS	Lee, C., Carpenter, F., Coaker, T., and Kealey, T.: The Primary Culture of Epithelia From the Secretory Coil and Collecting Duct of Normal Human and Cystic Fibrotic Eccrine Sweat Glands, <i>J Cell Sci</i> 83, 103, 1986 (978)
DMEM	Dover, R. and Potten, C.: Cell Cycle Kinetics of Cultured Human Epidermal Keratinocytes, <i>J Invest Dermatol</i> 80, 423, 1983 (683)
Eagle's MEM	Alitalo K, Kuismanen E, Myllyla R, Kiistala U, Asko-Seljavaara S, Vaheri A: Extracellular matrix proteins of human epidermal keratinocytes and feeder 3T3 cells, <i>J Cell Biol</i> 94, 497-505, 1982 (10105)
DMEM	Davies, P. and Kerr, C.: Modification of LDL Metabolism by Growth Factors in Cultured Vascular Cells and Human Skin Fibroblasts, <i>Biochim Biophys Acta</i> 712, 26, 1982 (322)
HBSS	McCoy, B., Galdun, J., and Cohen, I.: Effects of Density and Cellular Aging On Collagen Synthesis and Growth Kinetics in Keloid and Normal Skin Fibroblasts, <i>In Vitro</i> 18 (1), 79, 1982 (1129)
DMEM	Liu, S., Eaton, M., and Karasek, M.: Growth Characteristics of Human Epidermal Keratinocytes from Newborn Foreskin in Primary and Serial Cultures, <i>In Vitro</i> 15 (10), 813, 1979 (1206)
(see reference)	Rheinwald, J., and Green, H.: Serial Cultivation of Strains of Human Epidermal Keratinocytes: The Formation of Keratinizing Colonies from Single Cells, <i>Cell</i> 6, 331, 1975 (361)
PBS	King, I., Kroenke, M. and Segal, B.: GM-CSF-Dependent, CD103+ Dermal Dendritic Cells Play a Critical Role in Th Effector Cell Differentiation After Subcutaneous Immunization., <i>J Exp Med</i> 207, 953, 2010 (10588)
HBSS	Eidsmo, L., Allan, R., Caminschi, I., Van Rooijen, N., Heath, W. and Carbone, F.: Differential Migration of Epidermal and Dermal Dendritic Cells During Skin Infection., <i>J Immunol</i> 182, 3165, 2009 (10587)
DMEM	Cha, S., Talavera, D., Demir, E., Nath, A. and Sierra-Honigmann, M.: A Method of Isolation and Culture of Microvascular Endothelial Cells from Mouse Skin., <i>Microvasc Res</i> 70, 198, 2005 (10635)
PBS	Crigler Lauren, Kazhanie Amita, Yoon Tae-Jin, Zakhari Julia, Anders Joanna, Taylor Barbara, Virador VictoriaM: Isolation of a mesenchymal cell population from murine dermis that contains progenitors of multiple cell lineages, <i>FASEB J</i> 21, 2050-63, 2007 (10310)

Tissue Dissociation Guide

TISSUE TABLES

Species	Skin (con't)	Cell(s)	Enzyme(s)	
Porcine	Mouse, male 6-24 week	Skin side population	Collagenase Type 4: 0.2% Neutral Protease: 1.2 u/ml	
	Mouse	Ear epidermal	Trypsin: 0.1% Collagenase: 0.2%	
	Mouse	Dermal fibroblasts	Trypsin: 0.25% Collagenase Type 1: 0.25%	
	Mouse, 3-7 month	Fibroblasts, mesangial, smooth muscle	Trypsin: 0.25% Collagenase: see reference Soybean Trypsin Inhibitor: .05%	
	Porcine, 2-6 month	Keratinocytes	Neutral Protease: 0.25%	
	Rat	Rat, Wistar, 4-8 day	Dermal fibroblasts and keratinocytes	Trypsin: 0.25%
		Rat, SD, male, 40-60 days	Sebaceous	Trypsin: 0.2%
		Rat, neonatal, 12-24 hr post partum	Fibroblasts	Trypsin: 0.2%
Rat, albino, one day old, CFN		Keratinocytes	Trypsin: 1%	

Spleen

Species		Cell(s)	Enzyme(s)
Mouse	Mouse	Spleen, bone marrow endothelial	Collagenase Type 4: 0.3-1.0% Deoxyribonuclease I: 20 u/ml
	Mouse, female, 6-8 week	Dendritic	Collagenase Type 4: 0.05%
	Mouse	Dendritic	Collagenase Type 1: 0.5%
	Mouse	Dendritic	Collagenase: 300 u/ml Deoxyribonuclease I: 0.002%
	Mouse	Leukocytes	Collagenase Type 4: 43 u/ml
	Mouse	Splenic stromal	Collagenase Type 3: 100-400 u/ml
	Mouse, 4-6 week	Dentritic	Collagenase Type 1: 0.1% Deoxyribonuclease I: 0.001%
	Mouse	Dentritic	Collagenase Type 3: 0.1% Deoxyribonuclease I: 325 u/ml
	Mouse	Dentritic	Collagenase: 100 u/ml

Stem

Species		Cell(s)	Enzyme(s)
Canine	Canine, 20-25 kg	Adipose stem cell	Collagenase: see reference
	Equine	Adipose derived stem cells	Collagenase Type 1: 0.1%
Equine	Equine, 1-5 year	Adipose derived stem cells	Collagenase Type 1: 0.1%

Medium	Reference
PBS	Montanaro F, Liadaki K, Volinski J, Flint A, and Kunkel LM.: Skeletal muscle engraftment potential of adult mouse skin side population cells, <i>Proc Natl Acad Sci U S A</i> 100, 9336, 2003 (10020)
PBS	Takanami-Ohnishi Yoko, Amano Shinya, Kimura Sadao, Asada Sachie, Utani Atsushi, Maruyama Masumi, Osada Hiroyuki, Tsunoda Hajime, Irukayama-Tomobe Yoko, Goto Katsutoshi, Karin Michael, Sudo Tatsuhiko, Kasuya Yoshitoshi: Essential role of p38 mitogen-activated protein kinase in contact hypersensitivity, <i>J Biol Chem</i> 277, 37896-903, 2002 (10278)
DMEM	Baxter Ruth M, Crowell Thomas P, McCrann Margaret E, Frew Erica M, Gardner Humphrey: Analysis of the tight skin (Tsk1/+) mouse as a model for testing antifibrogenic agents, <i>LAB Invest</i> 85, 1199-209, 2005 (10309)
DMEM	Bradshaw AD, Francki A, Motamed K, Howe C, Sage EH: Primary mesenchymal cells isolated from SPARC-null mice exhibit altered morphology and rates of proliferation, <i>Mol Biol Cell</i> 10, 1569-79, 1999 (10136)
Dulbecco-Vogt MEM	Regauer S, Compton C: Cultured porcine epithelial grafts: an improved method, <i>J Invest Dermatol</i> 94, 230-4, 1990 (10179)
Ham's F-12	Sugihara, H and Toda, S: Primary Tissue Intact and Dissociated Cell Culture, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 3A:2.1, 1995 (1284)
DMEM with FBS PBS	Laurent, S., Mednieks, M., and Rosenfield, R.: Growth of Sebaceous Cells in Monolayer Culture, <i>In Vitro Cell Dev Biol</i> 28, 83, 1992 (488)
HEPES buffered DMEM	Acheson, A., Barker, P., Alderson, R., Miller, F., and Murphy, R.: Detection of Brain-Derived Neurotrophic Factor-like Activity in Fibroblasts and Schwann Cells: Inhibition by Antibodies to NGF, <i>Neuron</i> 7, 265, 1991 (675)
EBSS	Vaughan, F., Gray, R., and Bernstein, I.: Growth and Differentiation of Primary Rat Keratinocytes on Synthetic Membranes, <i>In Vitro Cell Dev Biol</i> 22 (3), 141, 1986 (1057)

Spleen

Medium	Reference
PBS	Shi, C., Jia, T., Mendez-Ferrer, S., Hohl, T., Serbina, N., Lipuma, L., Leiner, I., Li, M., Frenette, P. and Pamer, E.: Bone Marrow Mesenchymal Stem and Progenitor Cells Induce Monocyte Emigration in Response to Circulating Toll-Like Receptor Ligands., <i>Immunity</i> 34, 590, 2011 (10641)
RPMI 1640	Abou Fakher, F., Rachinel, N., Klimczak, M., Louis, J. and Doyen, N.: TLR9-Dependent Activation of Dendritic Cells by DNA from Leishmania Major Favors Th1 Cell Development and the Resolution of Lesions., <i>J Immunol</i> 182, 1386, 2009 (10585)
HBSS	Flano, E., Jewell, N., Durbin, R. and Durbin, J.: Methods Used to Study Respiratory Virus Infection., <i>Curr Protoc Cell Biol Vol. Chapter 26</i> , , Unit 26.3, 2009 (10648)
RPMI 1640	Abe, K., Nguyen, K., Fine, S., Mo, J., Shen, C., Shenouda, S., Corr, M., Jung, S., Lee, J., Eckmann, L. and Raz, E.: Conventional Dendritic Cells Regulate the Outcome of Colonic Inflammation Independently of T Cells., <i>Proc Natl Acad Sci U S A</i> 104, 17022, 2007 (10356)
RPMI 1640	Siragam, V., Crow, A., Brinc, D., Song, S., Freedman, J. and Lazarus, A.: Intravenous Immunoglobulin Ameliorates ITP via Activating Fc Gamma Receptors on Dendritic Cells., <i>Nat Med</i> 12, 688, 2006 (10686)
HBSS	Benedict Chris A, De Trez Carl, Schneider Kirsten, Ha Sukwon, Patterson Ginelle, Ware Carl F: Specific remodeling of splenic architecture by cytomegalovirus, <i>PLoS Pathog</i> 2, e16, 2006 (10226)
RPMI-1640	McLellan AlexanderD, Kapp Michaela, Eggert Andreas, Linden Christian, Bommhardt Ursula, Brückner Eva-B, Köpfer Ulrike, Köpfer Eckhart: Anatomic location and T-cell stimulatory functions of mouse dendritic cell subsets defined by CD4 and CD8 expression, <i>Blood</i> 99, 2084-93, 2002 (10234)
RPMI 1640	Schiavoni, F, Mattei, F, Sestili, P, Borghi, P, Venditti, M, Morse, H, Belardelli, F, and Gabrieli, L: ICSBP is Essential for the Development of Mouse Type I Interferon-producing Cells and for the Generation and Activation of CD8a+ Dendritic Cells, <i>J Exp Med</i> 196, 1415, 2002 (10235)
HBSS	Brasel K, De Smedt T, Smith JL, Maliszewski CR: Generation of murine dendritic cells from flt3-ligand-supplemented bone marrow cultures, <i>Blood</i> 96, 3029-39, 2000 (10227)

Stem

Medium	Reference
Media-199	Fischer, L., McIlhenny, S., Tulenko, T., Golesorkhi, N., Zhang, P., Larson, R., Lombardi, J., Shapiro, I. and DiMuzio, P.: Endothelial Differentiation of Adipose-Derived Stem Cells: Effects of Endothelial Cell Growth Supplement and Shear Force., <i>J Surg Res</i> 152, 157, 2009 (10599)
PBS	Vidal, M., Robinson, S., Lopez, M., Paulsen, D., Borkhsenius, O., Johnson, J., Moore, R. and Gimble, J.: Comparison of Chondrogenic Potential in Equine Mesenchymal Stromal Cells Derived from Adipose Tissue and Bone Marrow., <i>Vet Surg Vol. 37</i> , 713, 2008 (10561)
PBS	Vidal, M., Kilroy, G., Lopez, M., Johnson, J., Moore, R. and Gimble, J.: Characterization of Equine Adipose Tissue-Derived Stromal Cells: Adipogenic and Osteogenic Capacity and Comparison with Bone Marrow-Derived Mesenchymal Stromal Cells., <i>Vet Surg Vol. 36</i> , 613, 2007 (10533)

Tissue Dissociation Guide

Species

Stem (con't)

Cell(s)

Enzyme(s)

Human	Human, male 40-60 years	Adipose derived stem cells	Collagenase: 0.25% Deoxyribonuclease I: 0.002%
	Human	Umbilical cord stromal stem	Collagenase Type 4: 0.08% Neutral Protease: 0.138% Hyaluronidase: 0.02%
	Human	Glioma stem cells	PDS kit: per instructions
	Human	Dental pulp and apical papilla stem cells	Collagenase Type 1: 0.3% Neutral Protease: 0.4%
	Human	Corneal stromal stem	Neutral Protease: 1.2 u/ml Collagenase: 0.1%
	Human, 40-65 year	Adult human adipose stem cells	Collagenase Type 2: 0.075%
	Human	Filum terminale neural progenitor	Trypsin: see reference
	Human, male	Spermatogonial stem cells	Collagenase: 1% Deoxyribonuclease I: 0.22% Trypsin: 0.4%
	Human	Muscle derived multiprogenitor cells	Collagenase Type 2: 0.05%
	Human	Adipose derived stem cells	Collagenase Type 1: 0.075%
	Human	Hepatic stem cells and hepatoblasts	Collagenase Type 4: 0.014-0.06%
	Human, male	Adipose derived adult stem cells	Collagenase Type 1: 0.1%
	Human	Pancreatic cancer stem cells	Collagenase Type 4: 200 u/ml
	Human, female	Adipose derived adult stem cells	Collagenase Type 1: 0.1%
	Human	Adult stem cells	Collagenase Type 1: 0.1%
	Human, female	Adipose derived adult stem cells	Collagenase Type 1: 0.1%
	Human, fetal	Epithelial progenitor	Collagenase: 0.03%
	Human	Central nervous system stem	Collagenase: 0.1% Hyaluronidase: 0.1%
	Human	Bone marrow derived MSC	Trypsin: 0.05% Papain: 0.0025%
	Human, 8-12 year	Tendon stem/progenitor	Collagenase Type 1: 0.3% Neutral Protease: 0.4%
	Human, adult	Adipose-derived adult stem	Collagenase Type 1: 0.1%
	Human	Placental mesenchymal stem	Collagenase Type 2: 270 u/ml Neutral Protease: 2.4 u/ml

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Medium	Reference
PBS	Blasi, A., Martino, C., Balducci, L., Saldarelli, M., Soleti, A., Navone, S., Canzi, L., Cristini, S., Invernici, G., Parati, E. and Alessandri, G.: Dermal Fibroblasts Display Similar Phenotypic and Differentiation Capacity to Fat-Derived Mesenchymal Stem Cells, but Differ in Anti-Inflammatory and Angiogenic Potential, <i>Vasc Cell Vol. 3, 5, 2011 (10486)</i>
DMEM	Farias, V., Linares-Fernandez, J., Penalver, J., Paya Colmenero, J., Ferron, G., Duran, E., Fernandez, R., Olivares, E., O'Valle, F., Puertas, A., Oliver, F. and Ruiz de Almodovar, J.: Human Umbilical Cord Stromal Stem Cell Express CD10 and Exert Contractile Properties., <i>Placenta 32, 86, 2011 (10683)</i>
Neurobasal medium	Hjelmeland, A., Wu, Q., Wickman, S., Eyler, C., Heddleston, J., Shi, Q., Lathia, J., Macswords, J., Lee, J., McLendon, R. and Rich, J.: Targeting A20 Decreases Glioma Stem Cell Survival and Tumor Growth., <i>PLoS Biol Vol. 8, e1000319, 2010 (10536)</i>
MEM	Huang, G., Yamaza, T., Shea, L., Djouad, F., Kuhn, N., Tuan, R. and Shi, S.: Stem/Progenitor Cell-Mediated De Novo Regeneration of Dental Pulp with Newly Deposited Continuous Layer of Dentin in an In Vivo Model., <i>Tissue Eng Part A Vol. 16, 605, 2010 (10633)</i>
DMEM	Du, Y., Roh, D., Funderburgh, M., Mann, M., Marra, K., Rubin, J., Li, X. and Funderburgh, J.: Adipose-Derived Stem Cells Differentiate to Keratocytes In Vitro., <i>Mol Vis 16, 2680, 2010 (10602)</i>
DMEM	Sun, N., Panetta, N., Gupta, D., Wilson, K., Lee, A., Jia, F., Hu, S., Cherry, A., Robbins, R., Longaker, M. and Wu, J.: Feeder-Free Derivation of Induced Pluripotent Stem Cells from Adult Human Adipose Stem Cells., <i>Proc Natl Acad Sci U S A 106, 15720, 2009 (10525)</i>
DMEM/F-12	Varghese, M., Olstorn, H., Berg-Johnsen, J., Moe, M., Murrell, W. and Langmoen, I.: Isolation of Human Multipotent Neural Progenitors from Adult Filum Terminale., <i>Stem Cells Dev Vol. 18, 603, 2009 (10529)</i>
DMEM	Kossack, N., Meneses, J., Shefi, S., Nguyen, H., Chavez, S., Nicholas, C., Gromoll, J., Turek, P. and Reijo-Pera, R.: Isolation and Characterization of Pluripotent Human Spermatogonial Stem Cell-Derived Cells., <i>Stem Cells, 2008 (10352)</i>
DMEM	Nesti, L., Jackson, W., Shanti, R., Koehler, S., Aragon, A., Bailey, J., Sracic, M., Freedman, B., Giuliani, J. and Tuan, R.: Differentiation Potential of Multipotent Progenitor Cells Derived from War-Traumatized Muscle Tissue., <i>J Bone Joint Surg Am Vol. 90, 2390, 2008 (10490)</i>
DMEM	Jeong, J.: Adipose Stem Cells as a Clinically Available and Effective Source of Adult Stem Cell Therapy, <i>Int J Stem Cells 1, 43, 2008 (10530)</i>
Various	Wauthier, E., Schmelzer, E., Turner, W., Zhang, L., LeCluyse, E., Ruiz, J., Turner, R., Furth, M., Kubota, H., Lozoya, O., Barbier, C., McClelland, R., Yao, H., Moss, N., Bruce, A., Ludlow, J. and Reid, L.: Hepatic Stem Cells and Hepatoblasts: Identification, Isolation, and Ex Vivo Maintenance., <i>Methods Cell Biol 86, 137, 2008 (10557)</i>
DMEM	Lei, L., Liao, W., Sheng, P., Fu, M., He, A. and Huang, G.: Biological Character of Human Adipose-Derived Adult Stem Cells and Influence of Donor Age on Cell Replication in Culture., <i>Sci China C Life Sci Vol. 50, 320, 2007 (10517)</i>
Medium 199	Li, C., Heidt, D., Dalerba, P., Burant, C., Zhang, L., Adsay, V., Wicha, M., Clarke, M. and Simeone, D.: Identification of Pancreatic Cancer Stem Cells., <i>Cancer Res 67, 1030, 2007 (10514)</i>
DMEM/F-12	Guilak, F., Lott, K., Awad, H., Cao, Q., Hicok, K., Fermor, B. and Gimble, J.: Clonal Analysis of the Differentiation Potential of Human Adipose-Derived Adult Stem Cells., <i>J Cell Physiol 206, 229, 2006 (10520)</i>
PBS	Devireddy, R., Thirumala, S. and Gimble, J.: Cellular Response of Adipose Derived Passage-4 Adult Stem Cells to Freezing Stress., <i>J Biomech Eng 127, 1081, 2005 (10600)</i>
DMEM-Ham's F-12	Aust, L., Devlin, B., Foster, S., Halvorsen, Y., Hicok, K., du Laney, T., Sen, A., Willingmyre, G. and Gimble, J.: Yield of Human Adipose-Derived Adult Stem Cells from Liposuction Aspirates., <i>Cytotherapy Vol. 6, 7-14, 2004 (10518)</i>
DMEM	Malhi, H., Irani, A., Gagandeep, S. and Gupta, S.: Isolation of Human Progenitor Liver Epithelial Cells with Extensive Replication Capacity and Differentiation into Mature Hepatocytes., <i>J Cell Sci 115, 2679, 2002 (10368)</i>
HBSS	Uchida, N., Buck, D., He, D., Reitsma, M., Masek, M., Phan, T., Tsukamoto, A., Gage, F. and Weissman, I.: Direct Isolation of Human Central Nervous System Stem Cells., <i>Proc Natl Acad Sci U S A 97, 14720, 2000 (10527)</i>
DMEM	Welter Jean F, Solchaga Luis A, Penick Kitsie J: Simplification of aggregate culture of human mesenchymal stem cells as a chondrogenic screening assay, <i>Biotechniques 42, 732, 734-7, 2007 (10317)</i>
DMEM	Bi Yanming, Ehrichiou Driss, Kilts Tina M, Inkson Colette A, Embree Mildred C, Sonoyama Wataru, Li Li, Leet Arabella I, Seo Byoung-Moo, Zhang Li, Shi Songtao, Young Marian F: Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche, <i>Nat Med 13, 1219-27, 2007 (10337)</i>
DMEM/F-12 Ham's	Mitchell James B, McIntosh Kevin, Zvonic Sanjin, Garrett Sara, Floyd Z Elizabeth, Kloster Amy, Di Halvorsen Yuan, Storms Robert W, Goh Brian, Kilroy Gail, Wu Xiyang, Gimble Jeffrey M: Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers, <i>Stem Cells 24, 376-85, 2006 (10204)</i>
MEM	Portmann-Lanz CBettina, Schoeberlein Andreina, Huber Alexander, Sager Ruth, Malek Antoine, Holzgreve Wolfgang, Surbek DanielV: Placental mesenchymal stem cells as potential autologous graft for pre- and perinatal neuroregeneration, <i>Am J Obstet Gynecol 194, 664-73, 2006 (10167)</i>

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Species

Stem (con't)

Cell(s)

Enzyme(s)

	Human	Mesenchymal stem	Collagenase Type 1: 0.075%
	Human	Stromal stem cells	Collagenase Type 1: 0.2%
	Human, adult, male	Human epidermal keratinocyte stem cells	Neutral Protease: 0.5%
	Human	Umbilical vein mesenchymal stem cells	Collagenase: 1%
	Human, 7-8 yr	Stem cells Human exfoliated deciduous teeth	Collagenase Type 1: 0.3% Neutral Protease: 0.4%
	Human, adult	Human skin mast cells	Collagenase Type 2: 0.15% Hyaluronidase: 0.07% Deoxyribonuclease I: 0.03%
	Human	Embryonic stem	Neutral Protease: 1% Collagenase Type 4: 0.1%
	Human	Muscle-derived stem cells	Trypsin: 0.25%
Monkey	Monkey	Embryonic stem	Collagenase Type 4: 0.08%
	Baboons, 1yr, 7yr, 14 yr	Primate spermatogonial	Collagenase Type 2: 0.1% Trypsin: 0.05% Deoxyribonuclease I: 0.1%
Mouse	Mouse, 8-10 week	Adipose derived stem	Collagenase Type 1: 0.025%
	Mouse	Spleen, bone marrow endothelial	Collagenase Type 4: 0.3-1.0% Deoxyribonuclease I: 20 u/ml
	Mouse	Stem and progenitor	Collagenase Type 2: 0.2%
	Mouse, 6-8 week	Bone marrow mesenchymal stem	Collagenase Type 1: 0.25%
	Mouse, adult	Adult neural stem	Trypsin: 0.05%
	Mouse, 6-8 week	Prostate epithelial/stem	Collagenase Type 2: 0.5% Trypsin: 0.05%
	Mouse	Germ cells	Collagenase Type 1: 100 u/ml
	Mouse	Neural progenitor cell	Papain: 10 ul/ml
	Mouse	Neural stem cells	Papain: see reference
	Mouse, embryonic	HES-BC cells	Trypsin: 0.05%
	Mouse, 6-8 week	Tendon stem/progenitor	Collagenase Type 1: 0.3% Neutral Protease: 0.4%
	Mouse	Liver epithelial progenitor cells	Collagenase Type 4: 0.1% Deoxyribonuclease I: 0.05%

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Medium	Reference
DMEM	Kern Susanne, Eichler Hermann, Stoeve Johannes, Kluter Harald, Bieback Karen: Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue, <i>Stem Cells</i> 24, 1294-301, 2006 (10329)
HBSS	Boquest Andrew C, Shahdadfar Aboulghassem, Brinchmann Jan E, Collas Philippe: Isolation of stromal stem cells from human adipose tissue, <i>Methods Mol Biol</i> 325, 35-46, 2006 (10345)
DMEM	Papini S, Cecchetti D, Campani D, Fitzgerald W, Grivel J, Chen S, Margolis L, Revoltella R.: Isolation and Clonal Analysis of Human Epidermal Keratinocyte Stem Cells in Long-term Culture, <i>Stem Cells</i> 21, 481, 2003 (9804)
PBS	Covas DT, Siufi JL C, Silva AR L, Orellana MD: Isolation and culture of umbilical vein mesenchymal stem cells, <i>Braz J Med Biol Res</i> 36, 1179-83, 2003 (10139)
(see reference)	Miura, M., Gronthos, S, Zhao, M, Lu, B, Fisher, L, Robey, P, and Shi, S.: SHED: Stem Cells from Human Exfoliated Deciduous Teeth, <i>Proc Natl Acad Sci U S A</i> 100, 5807, 2003 (9800)
HBSS	Kambe, N, Kambe, M, Kochan, J, and Schwartz ,L.: Human Skin-derived Mast Cells Can Proliferate While Retaining Their Characteristic Functional and Protease Phenotypes, <i>Blood</i> 97, 2045, 2001 (9803)
DMEM	Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM: Embryonic stem cell lines derived from human blastocysts, <i>Science</i> 282, 1145-7, 1998 (10318)
DMEM/F12	Alessandri Giulio, Pagano Stefano, Bez Alessandra, Benetti Anna, Pozzi Stefano, Iannolo Gioacchin, Baronio Manuela, Invernici Gloria, Caruso Arnaldo, Muneretto Claudio, Bisleri Gianluigi, Parati Eugenio: Isolation and culture of human muscle-derived stem cells able to differentiate into myogenic and neurogenic cell lineages, <i>Lancet</i> 364, 1872-83, (10342)
DMEM	Chen, S., Revoltella, R., Papini, S., Michelini, M., Fitzgerald, W., Zimmerberg, J., and Margolis, L.: Multilineage Differentiation of Rhesus Monkey Embryonic Stem cells in Three-dimensional Culture Systems, <i>Stem Cells</i> 21(3), 281, 2003 (9805)
DMEM	Nagano, M., McCarrey, J., and Brinster, R.: Primate Spermatogonial Stem Cells Colonize Mouse Testes, <i>Biol Reprod</i> 64, 1409, 2001 (9799)
HBSS	Sugii, S., Kida, Y., Berggren, W. and Evans, R.: Feeder-Dependent and Feeder-Independent iPS Cell Derivation from Human and Mouse Adipose Stem Cells., <i>Nat Protoc</i> 6, 346, 2011 (10493)
PBS	Shi, C., Jia, T., Mendez-Ferrer, S., Hohl, T., Serbina, N., Lipuma, L., Leiner, I., Li, M., Frenette, P. and Pamer, E.: Bone Marrow Mesenchymal Stem and Progenitor Cells Induce Monocyte Emigration in Response to Circulating Toll-Like Receptor Ligands., <i>Immunity</i> 34, 590, 2011 (10641)
HBSS	Han, J., Koh, Y., Moon, H., Ryoo, H., Cho, C., Kim, I. and Koh, G.: Adipose Tissue is an Extramedullary Reservoir for Functional Hematopoietic Stem and Progenitor Cells., <i>Blood</i> 115, 957, 2010 (10494)
RPMI 1640	Xu, S., De Becker, A., Van Camp, B., Vanderkerken, K. and Van Riet, I.: An Improved Harvest and In Vitro Expansion Protocol for Murine Bone Marrow-Derived Mesenchymal Stem Cells., <i>J Biomed Biotechnol Vol.</i> 2010, 105940, 2010 (10617)
DMEM	Deleyrolle, L. and Reynolds, B.: Isolation, Expansion, and Differentiation of Adult Mammalian Neural Stem and Progenitor Cells using the Neurosphere Assay., <i>Methods Mol Biol</i> 549, 91, 2009 (10521)
HBSS	Burger, P., Gupta, R., Xiong, X., Ontiveros, C., Salm, S., Moscatelli, D. and Wilson, E.: High Aldehyde Dehydrogenase Activity: A Novel Functional Marker of Murine Prostate Stem/Progenitor Cells., <i>Stem Cells</i> 27, 2220-8, 2009 (10488)
HBSS	Breault, D., Min, I., Carlone, D., Farilla, L., Ambruzs, D., Henderson, D., Algra, S., Montgomery, R., Wagers, A. and Hole, N.: Generation of mTert-GFP Mice as a Model to Identify and Study Tissue Progenitor Cells., <i>Proc Natl Acad Sci U S A</i> 105, 10420, 2008 (10522)
PBS	Hutton, S. and Pevny, L.: Isolation, Culture, and Differentiation of Progenitor Cells from the Central Nervous System., <i>Cold Spring Harb. Protoc.</i> 11, 5077, 2008 (10532)
DMEM/F-12	Meletis, K., Wirta, V., Hede, S., Nister, M., Lundberg, J. and Frisen, J.: p53 Suppresses the Self-Renewal of Adult Neural Stem Cells., <i>Development</i> 133, 363, 2006
DMEM	Lu Shi-Jiang, Feng Qiang, Caballero Sergio, Chen Yu, Moore Malcolm A, Grant Maria B, Lanza Robert: Generation of functional hemangioblasts from human embryonic stem cells, <i>Nat Methods</i> 4, 501-9, 2007 (10082)
DMEM	Bi Yanming, Ehrichiou Driss, Kilts Tina M, Inkson Colette A, Embree Mildred C, Sonoyama Wataru, Li Li, Leet Arabella I, Seo Byoung-Moo, Zhang Li, Shi Songtao, Young Marian F: Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche, <i>Nat Med</i> 13, 1219-27, 2007 (10337)
DMEM	Li Wen-Lin, Su Juan, Yao Yu-Cheng, Tao Xin-Rong, Yan Yong-Bi, Yu Hong-Yu, Wang Xin-Min, Li Jian-Xiu, Yang Yong-Ji, Lau Joseph T Y, Hu Yi-Ping: Isolation and characterization of bipotent liver progenitor cells from adult mouse, <i>Stem Cells</i> 24, 322-32, 2006 (10248)

Tissue Dissociation Guide

Species	Stem (con't)	Cell(s)	Enzyme(s)
Rat	Mouse, 6 week	Adipose mesenchymal stem	Collagenase: 0.2%
	Mouse, day 7	Cerebellar stem cells	Papain: 10u/ml Deoxyribonuclease I: 250 u/ml
	Mouse, embryonic	Embryonic fibroblast feeder cells	Collagenase Type 4: 0.1%
	Mouse, 15 day or 3 month	Neural stem cells	Papain: 0.1%
	Mouse, 4-8 month	Neural subventricular zone	Trypsin: 0.13% Hyaluronidase: 0.067%
	Mouse, 6 week	Muscle hematopoietic stem cells	Collagenase: 0.2% Trypsin: 0.1%
	Rat, SD, newborn	Neural stem cells, Schwann cells	Trypsin: 0.25% Collagenase: 0.16%
	Rat, male, 200-250 g	Neural stem cells	Papain: 0.09% Deoxyribonuclease I: 0.1%
	Rat, adult	Neural stem cells	Papain: 2.5 u/ml Deoxyribonuclease I: 250 u/ml Neutral Protease: 1 u/ml

Thymus

Species		Cell(s)	Enzyme(s)
Human	Human	Dentritic	Collagenase Type 2: 0.1% Deoxyribonuclease I: 0.002%
Mouse	Mouse	Stromal	Collagenase Type 3: 0.2% Hyaluronidase: 0.1%
	Mouse	Dentritic	Collagenase Type 3: 0.1% Deoxyribonuclease I: 325 u/ml
	Mouse, 3-6 week	Thymic	Collagenase Type 3: 100-400 u/ml
	Mouse BALB/c, C3H, C57BL/6, 1-28 day	Epithelial, thymus	Neutral Protease: 1.5 µg/ml
	Mouse, C3H, 16 wk old, female	Epithelial	Collagenase Type 3: 0.1%
	Mouse, Swiss, 6 wk	Thymus	Collagenase Type 3: 150 u/ml
Rat	Rat, postnatal	Thymic	Trypsin: 0.05%
	Rat	Thymic epithelial	Collagenase Type 3: 0.1%
	Rat ACI/NMs X BUF/Mna F1, male, 28 months Rat, ACI/MNs male, 8 weeks (also bovine, adult)	Epithelial	Collagenase Type 3: 0.1%

Thyroid/Parathyroid

Species		Cell(s)	Enzyme(s)
Bovine	Bovine, adult	Parathyroid	Deoxyribonuclease I: 0.005%,
	Bovine	Parathyroid glands	Deoxyribonuclease I: 0.0075%
	Bovine	Parathyroid	Deoxyribonuclease I: 0.004%

Medium	Reference
PBS	Di Rocco Giuliana, Iachininoto Maria Grazia, Tritarelli Alessandra, Straino Stefania, Zacheo Antonella, Germani Antonia, Crea Filippo, Capogrossi Maurizio C: Myogenic potential of adipose-tissue-derived cells, <i>J Cell Sci</i> 119, 2945-52, 2006 (10327)
Dulbecco's PBS	Lee, A, Kessler, J, Read, T, Kaiser, C, Corbeil, D, Huttner ,W, Johnson, J, Wechsler-Reya, R.: Isolation of Neural Stem Cells from the Postnatal Cerebellum, <i>Nat Neurosci</i> 8, 723, 2005 (9801)
DMEM	Schatten Gerald, Smith Joseph, Navara Christopher, Park Jong-Hyuk, Pedersen Roger: Culture of human embryonic stem cells, <i>Nat Methods</i> 2, 455-63, 2005 (10120)
DMEM/ F-12	Gritti, A, Bonfanti, L, Doetsch, F, Caille, I, Alvarez-Buylla, A, Lim, D, Galli, R, Verdugo J, Herrera, D, and Vescovi A.: Multipotent Neural Stem Cells Reside into the Rostral Extension and Olfactory Bulb of Adult Rodents, <i>J Neurosci</i> 22(2), 437, 2002 (9808)
DMEM/F12	Gritti A, Frolichsthal-Schoeller P, Galli R, Parati E, Cova L, Pagano S, Bjornson C, and Vescovi A.: Epidermal and Fibroblast Growth Factors Behave as Mitogenic Regulators for a Single Multipotent Stem Cell-like Population from the Subventricular Region of the Adult Mouse forebrain, <i>J Neurosci</i> 19(9), 3287, 1999 (9806)
DMEM	Jackson, K., and Goodell, M.: Hematopoietic Potential of Stem Cells Isolated from Murine Skeletal Muscle, <i>Proc Natl Acad Sci U S A</i> 96, 14482, 1999 (9802)
DMEM/F12	Zeng Yuan-Shan, Ding Ying, Wu Li-Zhi, Guo Jia-Song, Li Hai-Biao, Wong Wai-Man, Wu Wu-Tian: Co-transplantation of schwann cells promotes the survival and differentiation of neural stem cells transplanted into the injured spinal cord, <i>Dev Neurosci</i> 27, 20-6, 2005 (10109)
EBSS	Gobbel GT, Choi SJ, Beier S, Niranjan A: Long-term cultivation of multipotential neural stem cells from adult rat subependyma, <i>Brain Res</i> 980, 221, 2003 (10051)
DMEM/F-12	Palmer, T., Markakis, E., Willhoite, A., Safar, F., and Gage, F.: Fibroblast Growth Factor-2 Activates a Latent Neurogenic Program in Neural Stem Cells from Diverse Regions of the Adult CNS, <i>J Neurosci</i> 19, 8487, 1999 (9798)

Thymus

Medium	Reference
RPMI 1640	Vandenabeele S, Hochrein H, Mavaddat N, Winkel K, Shortman K: Human thymus contains 2 distinct dendritic cell populations, <i>Blood</i> 97, 1733-41, 2001 (10245)
RPMI 1640	Phillips, Joy, Brondstetter, T., English, C., Lee, H., Virts, E, and Thoman, M.: IL-7 Gene Therapy in Aging Restores Early thymopoiesis without Reversing Involution, <i>J Immunol</i> , 4869, 2004 (10237)
RPMI 1640	Schiavoni, F, Mattei, F, Sestili, P, Borghi, P, Venditti, M, Morse, H, Belardelli, F, and Gabrieli, L: ICSPB is Essential for the Development of Mouse Type I Interferon-producing Cells and for the Generation and Activation of CD8a+ Dendritic Cells, <i>J Exp Med</i> 196, 1415, 2002 (10235)
HBSS	Smith KM, Olson DC, Hirose R, Hanahan D: Pancreatic gene expression in rare cells of thymic medulla: evidence for functional contribution to T cell tolerance, <i>Int Immunol</i> 9, 1355-65, 1997 (10239)
DMEM	Ropke, C., van Deurs, B., and Petersen, O.: Short-term Cultivation of Murine Thymic Epithelial Cells in a Serum-Free Medium, <i>In Vitro Cell Dev Biol</i> 26, 671, 1990 (1288)
DMEM	Ehmann, U., Shiurba, R., and Peterson, W.: Long-Term Proliferation of Mouse Thymic Epithelial Cells in Culture, <i>In Vitro Cell Dev Biol</i> 22 (12), 738, 1986 (916)
DMEM	Jones, K. and Pierre, R.: Analysis of Cellular Heterogeneity in Mouse Thymus Cultures, <i>In Vitro</i> 17, 431, 1981 (511)
HBSS	Bonfanti, P., Claudinot, S., Amici, A., Farley, A., Blackburn, C. and Barrandon, Y.: Microenvironmental Reprogramming of Thymic Epithelial Cells to Skin Multipotent Stem Cells., <i>Nature</i> 466, 978, 2010 (10595)
DMEM	Masuda, A and Matsuyama, M: Epithelial Cell Lines From Rat Thymoma and Rat Thymus, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 2</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 21C:4.1, 1995 (1280)
Eagle's MEM Serum-free	Masuda, A., Ohtsuka, K., and Matsuyama, M.: Establishment of Functional Epithelial Cell Lines from a Rat Thyoma and a Rat Thymus, <i>In Vitro Cell Dev Biol</i> 26, 713, 1990 (448)

Thyroid/Parathyroid

Medium	Reference
HEPES Ham's F10	Nygren, P., Gylfe, E., Larsson, R., Johansson, H., Juhlin, C., Klareskoq, L., Akerstrom, G., and Rastad, J.: Modulation of the Ca ²⁺ -Sensing Function of Parathyroid Cells <i>In Vitro</i> and in Hyperparathyroidism, <i>Biochim Biophys Acta</i> 968, 253, 1988 (337)
HEPES buffer	Wallace, J., and Scarpa, A.: Regulation of Parathyroid Hormone Secretion <i>in Vitro</i> by Divalent Cations, <i>J Biol Chem</i> 257, 10613, 1982 (555)
Eagle's #2 medium without bicarbonate	Brown, E., Hurwitz, S., and Aurbach, G.: Preparation of Viable Isolated Bovine Parathyroid Cells, <i>Endocrinology</i> 99, 1582, 1976 (385)

Tissue Dissociation Guide

TISSUE TABLES

Species	Thyroid/Parathyroid (con't)	Cell(s)	Enzyme(s)
Chicken	Bovine (also porcine)	Thyroid	Trypsin: 0.004%
	Chick embryo	Thyroid Muscle Heart	Collagenase: 0.25%
Human	Chicken, Rhode Island Red, embryo	Thyroid follicular	Collagenase: 0.2%
	Human	Endothelial	Collagenase Type 2: 0.1%
	Human	Thyocytes	Collagenase Type 1: 130 u/ml Neutral Protease: 0.5 u/ml
	Human	Thyocytes	Neutral Protease: 0.5% Trypsin: 0.25% Collagenase: 0.1%
Mouse	Human	Thyroid	Collagenase: 300 u/ml
	Mouse, 6 week	Thyroid	Neutral Protease: 0.0012 u/ml Collagenase Type 2: 0.25 u/ml
Ovine	Sheep	Thyroid	Collagenase: 0.2%
Porcine	Porcine (also bovine)	Thyroid	Trypsin: 0.004%
Rat	Rat, Lewis, male, 4 week	Thyroid	Collagenase Type 2: 0.15% Collagenase Type 4: 0.15%

Tonsil

Species		Cell(s)	Enzyme(s)
Human	Human, female, 25-45 year	Tonsillar mononuclear cells	Collagenase Type 1: 210 u/ml Deoxyribonuclease I: 90 u/ml
	Human	Tonsillar mononuclear cells	Collagenase Type 1: 210 u/ml Deoxyribonuclease I: 90 u/ml

Tumor

Species		Cell(s)	Enzyme(s)
Hamster	Hamster, 90-100 g	Tumor	Hyaluronidase: 0.1%
	Hamster, 6 week old	Buccal pouch	Neutral Protease: 0.24%
Human	Human	Tumorigenic melanoma	Collagenase Type 4: 200 u/ml Trypsin: 0.05% Deoxyribonuclease I: 50-100 u/ml
	Human	Pancreatic tumor	Collagenase Type 4: 200 u/ml
	Human	Tumor	Collagenase Type 4: 0.1% Hyaluronidase: 0.07% Deoxyribonuclease I: 0.04%

Thyroid/Parathyroid (con't)

Medium	Reference
EBSS	Tong, W.: The Isolation and Culture of Thyroid Cells, <i>Meth Enzymol</i> 32, 745, 1974 (636)
Tyrode's saline, potassium free	Hilfer, S., and Brown, J.: Collagenase. Its Effectiveness as a Dispersing Agent for Embryonic Chick Thyroid and Heart, <i>Exp Cell Res</i> 65, 246, 1971 (401)
Tyrode's solution, CMF	Spooner, B.: The Expression of Differentiation by Chick Embryo Throid in Cell Culture. I. Functional and Fine Structural Stability in Mass and Clonal Culture, <i>J Cell Physiol</i> 75, 33, 1970 (682)
DMEM	Patel, V., Logan, A., Watkinson, J., Uz-Zaman, S., Sheppard, M., Ramsden, J. and Eggo, M.: Isolation and Characterization of Human Thyroid Endothelial Cells., <i>Am J Physiol Endocrinol Metab</i> Vol. 284, E168, 2003 (10586)
HBSS	Gianoukakis, A., Cao, H., Jennings, T. and Smith, T.: Prostaglandin Endoperoxide H Synthase Expression in Human Thyroid Epithelial Cells., <i>Am J Physiol Cell Physiol</i> 280, C701, 2001 (10594)
EBSS	Howie, A., Walker, S., Akesson, B., Arthur, J. and Beckett, G.: Thyroidal Extracellular Glutathione Peroxidase: A Potential Regulator of Thyroid-Hormone Synthesis., <i>Biochem J</i> 308 (Pt 3), 713, 1995 (10593)
Ham's F-12/MEM	Miller, R., Hiraoka, T., Nakamura, N., Tenou, H., Kopecky, K., Jones, M. and Gould, M.: In Vitro Culture of Human Thyroid Cells; Methods and Application to Radiation Biology., <i>J Radiat Res (Tokyo)</i> Vol. 26, 269, 1985 (10365)
RPMI 1640	Martin, A., Coronel, E., Sano, G., Chen, S., Vassileva, G., Canasto-Chibuque, C., Sedgwick, J., Frenette, P., Lipp, M., Furtado, G. and Lira, S.: A Novel Model for Lymphocytic Infiltration of the Thyroid Gland Generated by Transgenic Expression of the CC Chemokine CCL21., <i>J Immunol</i> 173, 4791, 2004 (10653)
Puck's Saline F	Kerkof, P.: Preparation of Primary Cultures of Ovine Thyroid Gland Cells, <i>J Tiss Cul Meth</i> 7, 23, 1982 (1289)
EBSS	Tong, W.: The Isolation and Culture of Thyroid Cells, <i>Meth Enzymol</i> 32, 745, 1974 (636)
DMEM	Arauchi, A., Shimizu, T., Yamato, M., Obara, T. and Okano, T.: Tissue-Engineered Thyroid Cell Sheet Rescued Hypothyroidism in Rat Models After Receiving Total Thyroidectomy Comparing with Nontransplantation Models., <i>Tissue Eng Part A</i> Vol. 15, 3943, 2009 (10655)

Tonsil

Medium	Reference
RPMI	Grammer Amrie C, Slota Rebecca, Fischer Randy, Gur Hanan, Girschick Hermann, Yarboro Cheryl, Illei Gabor G, Lipsky PeterE: Abnormal germinal center reactions in systemic lupus erythematosus demonstrated by blockade of CD154-CD40 interactions, <i>J Clin Invest</i> 112, 1506-20, 2003 (10223)
RPMI	Grammer AC, McFarland RD, Heaney J, Darnell BF, Lipsky PE: Expression, regulation, and function of B cell-expressed CD154 in germinal centers, <i>J Immunol</i> 163, 4150-9, 1999 (10230)

Tumor

Medium	Reference
Waymouth's MB	Gonzalez, A., Oberley, T., Schultz, J., Ostrom, J., and Li, J.: <i>In Vitro</i> Characterization of Estrogen Induced Syrian Hamster Renal Tumors: Comparison with an Immortalized Cell Line Derived from Diethylstilbestrol-Treated Adult Hamster Kidney, <i>In Vitro Cell Dev Biol</i> 29A, 562, 1993 (1180)
CMF HBSS	Min, B., Kim, K., Cherrick, H., and Park, N.: Three Cell Lines from Hamster Buccal Pouch Tumors Induced by Topical 7,12-Dimethylbenz(a)Anthracene, Alone or in Conjunction with Herpes Simplex Virus Inoculation, <i>In Vitro Cell Dev Biol</i> 27A, 128, 1991 (457)
PBS	Quintana, E., Shackleton, M., Foster, H., Fullen, D., Sabel, M., Johnson, T. and Morrison, S.: Phenotypic Heterogeneity Among Tumorigenic Melanoma Cells from Patients that is Reversible and Not Hierarchically Organized., <i>Cancer Cell</i> Vol. 18, 510, 2010 (10601)
RPMI-1640	Kim, M., Evans, D., Wang, H., Abbruzzese, J., Fleming, J. and Gallick, G.: Generation of Orthotopic and Heterotopic Human Pancreatic Cancer Xenografts in Immunodeficient Mice., <i>Nat Protoc</i> 4, 1670, 2009 (10524)
(see reference)	Sauvageot, C., Weatherbee, J., Kesari, S., Winters, S., Barnes, J., Dellagatta, J., Ramakrishna, N., Stiles, C., Kung, A., Kieran, M. and Wen, P.: Efficacy of the HSP90 Inhibitor 17-AAG in Human Glioma Cell Lines and Tumorigenic Glioma Stem Cells., <i>Neuro Oncol</i> Vol. 11, 109, 2009 (10592)

Tissue Dissociation Guide

Species

Tumor (con't)

Cell(s)

Enzyme(s)

	Human	Breast epithelial	Collagenase Type 3: 200 u/ml
	Human	Prostate stromal cells	Collagenase Type 1: 0.1%
	Human, adult	Human synovial sarcoma	Collagenase Type 2: 200 u/ml
	Human	Colonocytes	Collagenase:
	Human	Epithelial, fibroblasts	Trypsin: 0.25%
	Human	Colon adenocarcinoma	Hyaluronidase: 100 u/ml
	Human	Tumor, breast	Hyaluronidase: 100 u/ml
	Human	Glioma	Hyaluronidase: 0.01%
	Human	Epithelial	Collagenase: 2.0%
	Human	Tumor	Neutral Protease: 0.24%
	Human, 9-74 year	Neurofibroma	Neutral Protease: 1.25 u/ml Collagenase Type 1: 0.05% Hyaluronidase: 0.1%
	Human	Tumor	Trypsin: 0.05%
	Human	Tumor, colon	Trypsin: 0.25%
	Human	Epithelial and tumor Colon	Collagenase: 300 u/ml
	Human	Tumor, breast	Neuraminidase: 0.8 u/ml
	Human	Melanoma Metastatic tumors	Collagenase Type 3: 0.10%
	Human	Mammary tumors, hard	Collagenase: 0.10%
Mouse	Mouse, 4-6 week	Pancreatic tumor	Collagenase Type 4: 200 u/ml

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Medium	Reference
HBSS	Liu, R., Wang, X., Chen, G., Dalerba, P., Gurney, A., Hoey, T., Sherlock, G., Lewicki, J., Shedden, K. and Clarke, M.: The Prognostic Role of a Gene Signature from Tumorigenic Breast-Cancer Cells., <i>N Engl J Med</i> 356, 217, 2007 (10551)
RPMI 1640	Nakashiro Koh-Ichi, Hara Shingo, Shinohara Yuji, Oyasu Miho, Kawamata Hitoshi, Shintani Satoru, Hamakawa Hiroyuki, Oyasu Ryoichi: Phenotypic switch from paracrine to autocrine role of hepatocyte growth factor in an androgen-independent human prostatic carcinoma cell line, CWR22R, <i>Am J Pathol</i> 165, 533-40, 2004 (10163)
DMEM/F-12	Nishio Jun, Iwasaki Hiroshi, Ishiguro Masko, Ohjimi Yuko, Fujita Chikako, Isayama Teruto, Naito Masatoshi, Oda Yoshinao, Kaneko Yasuhiko, Kikuchi Masahiro: Establishment of a new human synovial sarcoma cell line, FU-SY-1, that expresses c-Met receptor and its ligand hepatocyte growth factor, <i>Int J Oncol</i> 21, 17-23, 2002 (10164)
DMEM/F12	Emenaker N, Calaf G, Cox D, Basson M and Qureshi N: Short chain fatty acids differentially modulate cellular phenotype and c-myc protein levels in primary human nonmalignant and malignant colonocytes, <i>J Nutr</i> 46, 96-105, 2001 (10143)
Ham's F-12	MacLeod, R: Rapid Monolayer Primary Cell Culture from Tissue Biopsy, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons Ltd, 3E:2.1, 1995 (1269)
DMEM	Hague, A and Paraskeva, C: Colon Adenocarcinoma Cells, <i>Cell & Tissue Culture: Laboratory Procedures Vol. 1</i> , Doyle, A., Griffiths, J., and Newell, D., John Wiley and Sons, Ltd., 12C:1.1, 1995 (1277)
DMEM	Beaupain, R., Mainquene, C., Brouty-Boye, D., Planchon, P., and Magniew, V.: "Normal" Breast Cells Adjacent to a Tumor Grown in Long-term Three Dimensional Culture, <i>In Vitro Cell Dev Biol</i> 29, 100, 1993 (490)
HBSS	Kruse, C., Mitchell, D., Kleinschmidt-DeMasteis, B., Franklin, W., Morse, H., Spector, E., and Lillehei, K.: Characterization of a Continuous Human Glioma Cell Line DBTRG-OSMG: Growth Kinetics, Karyotype, Receptor Expression and Tumor Suppressor Gene Analyses, <i>In Vitro Cell Dev Biol</i> 28, 609, 1992 (485)
DMEM/Ham's F-12	Emerman, J. and Wilkinson, D.: Routine Culturing of Normal, Dysplastic and Malignant Human Mammary Epithelial Cells from Small Tissue Samples, <i>In Vitro Cell Dev Biol</i> 26, 1186, 1990 (429)
DMEM/Ham's F-12	Boyd, J., Rinehart Jr., C., Walton, L., Siegal, G. and Kaufman, D.: Ultrastructural Characterization of Two New Human Endometrial Carcinoma Cell Lines and Normal Human Endometrial Epithelial Cells Cultured on Extracellular Matrix, <i>In Vitro Cell Dev Biol</i> 26, 701, 1990 (446)
L-15	Sheela S, Riccardi VM, Ratner N: Angiogenic and invasive properties of neurofibroma Schwann cells, <i>J Cell Biol</i> 111, 645-53, 1990 (10295)
DMEM	Sacks, P., Parnes, S., Gallick, G., Mansouri, Z., Lichtner, R., Satya-Prakash, K., Pathak, S, and Parsons, D.: Establishment and Characterization of Two New Squamous Cell Carcinoma Cell Lines Derived from Tumors of the Head and Neck, <i>Cancer Res</i> 48, 2858, 1988 (1181)
McCoy's	Brattain, M., Marks, M., McCombs, J., Finely, W., and Brattain, D.: Characterization of Human Colon Carcinoma Cell Lines Isolated From a Single Primary Tumour, <i>Br J Cancer</i> 47, 373, 1983 (1183)
PBS medium 199 or medium F 12	Friedman, E., Higgins, P., Lipkin, M., Shinya, H., and Gelb, A.: Tissue Culture of Human Epithelial Cells from Benign Colonic Tumors, <i>In Vitro</i> 17, 632, 1981 (514)
HBSS	Leung, C., and Shiu, R.: Morphological and Proliferative Characteristics of Human Breast Tumor Cells Cultured on Plastic and in Collagen Matrix, <i>In Vitro</i> 18, 476, 1981 (521)
DMEM	Creasey, A., Smith, H., Hackett, A., Fukuyama, K., Epstein, W., and Madin, S.: Biological Properties of Human Melanoma Cells in Culture, <i>In Vitro</i> 15, 342, 1979 (503)
RPMI-1640 w/ 5% Fetal Calf Serum	Lasfargues, E.: , <i>Tissue Culture Methods/Applications</i> , Kruse, P., and Patterson, M., Academic Press, 45, 1973 (1293)
RPMI-1640	Kim, M., Evans, D., Wang, H., Abbruzzese, J., Fleming, J. and Gallick, G.: Generation of Orthotopic and Heterotopic Human Pancreatic Cancer Xenografts in Immunodeficient Mice., <i>Nat Protoc</i> 4, 1670, 2009 (10524)

Tissue Dissociation Guide

Species

Tumor (con't)

Cell(s)

Enzyme(s)

	Mouse	Tumor	Collagenase Type 3: 200 u/ml
	Mouse	Granule cell precursors, pre-neoplastic and tumor cells	Papain: 10 u/ml Deoxyribonuclease I: 250 u/ml
	Mouse, 30 week	Tumor endothelial	Collagenase Type 4: 500 u/ml Collagenase Type 2: 550 u/ml Deoxyribonuclease I: 3 u/ml
	Mouse, 8-10 week	Tumor associated endothelial cells	Collagenase Type 2:
	Mouse	Pancreatic tumor	Collagenase Type 2: 0.5% Collagenase Type 4: 0.5% Deoxyribonuclease I: 0.2%
	Mouse	Tumor-infiltrating lymphocyte	Collagenase: 200 u/ml
	Mouse, male	Melanoma tumor cells	Collagenase Type 2: 0.5%
	Mouse BALB/cfC3H	Mammary tumors Epithelial	Collagenase: 1.0%
	Mouse, BALB/cfC3H/Crgl	Neoplastic Epithelial tumor	Trypsin:
	Mouse, lactating, 14 day	Mammary	Trypsin NF 1:250: 0.25%
Rat	Rat, male 12 week	Sponge infiltrating cells	Collagenase Type 4: 0.15% Deoxyribonuclease I: 0.02%
	Rat ACI/NMs X BUF/Mna F1, male, 28 months, rat, ACI/MNs male, 8 weeks	Epithelial	Collagenase Type 3: 0.1%
	Rat	Yolk sac tumor	Trypsin: 0.01%
	Rat	Tumor, islet	Trypsin: 0.05%
	Rat	Ascites hepatoma	Trypsin: 0.1%

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Medium	Reference
RPMI-1640	Prince, M., Sivanandan, R., Kaczorowski, A., Wolf, G., Kaplan, M., Dalerba, P., Weissman, I., Clarke, M. and Ailles, L.: Identification of a Subpopulation of Cells with Cancer Stem Cell Properties in Head and Neck Squamous Cell Carcinoma., <i>Proc Natl Acad Sci U S A</i> 104, 973, 2007 (10526)
Neurobasal/B27	Oliver, T., Read, T., Kessler, J., Mehmeti, A., Wells, J., Huynh, T., Lin, S. and Wechsler-Reya, R.: Loss of Patched and Disruption of Granule Cell Development in a Pre-Neoplastic Stage of Medulloblastoma., <i>Development</i> 132, 2425, 2005 (10555)
PBS	Berger M., Bergers G., Arnold B., Hammerling G. and Ganss R.: Regulator of G-protein Signaling-5 Induction in Pericytes Coincides With Active Vessel Remodeling During Neovascularization, <i>Blood</i> 105, 1094, 2005 (10249)
(see reference)	Hida Kyoko, Hida Yasuhiro, Amin Dhara N, Flint Alan F, Panigrahy Dipak, Morton Cynthia C, Klagsbrun Michael: Tumor-associated endothelial cells with cytogenetic abnormalities, <i>Cancer Res</i> 64, 8249-55, 2004 (10170)
PBS	Bergers Gabriele, Song Steven, Meyer-Morse Nicole, Bergsland Emily, Hanahan Douglas: Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors, <i>J Clin Invest</i> 111, 1287-95, 2003 (10132)
RPMI 1640	Uekusa Yasuhiro, Yu Wen-Gong, Mukai Takao, Gao Ping, Yamaguchi Nobuya, Murai Masako, Matsushima Kouji, Obika Satoshi, Imanishi Takeshi, Higashibata Yuji, Nomura Shintaro, Kitamura Yukihiko, Fujiwara Hiromi, Hamaoka Toshiyuki: A pivotal role for CC chemokine receptor 5 in T-cell migration to tumor sites induced by interleukin 12 treatment in tumor-bearing mice, <i>Cancer Res</i> 62, 3751-8, 2002 (10285)
DMEM	Arbiser JL, Raab G, Rohan RM, Paul S, Hirschi K, Flynn E, Price ER, Fisher DE, Cohen C, Klagsbrun M: Isolation of mouse stromal cells associated with a human tumor using differential diphtheria toxin sensitivity, <i>Am J Pathol</i> 155, 723-9, 1999 (10114)
HBSS	Yang, J., Guzman, R., Richards, J., and Nandi, S.: Primary Cultures of Mouse Mammary Tumor Epithelial Cells Embedded in Collagen Gels, <i>In Vitro</i> 16, 502, 1980 (507)
DMEM	Hosick, H.: A Note on Growth Patterns of Epithelial Tumor Cells in Primary Culture, <i>Cancer Res</i> 34, 259, 1974 (932)
HBSS	Kopelovich, L., Abraham, S., McGrath, H., DeOme, K., Chaikoff, I.: Metabolic Characteristics of a Naturally Occurring Preneoplastic Tissue. I. Glycolytic Enzyme Activators of Hyperplastic Alveolar Nodule Outgrowths and Adenocarcinomas of Mouse Mammary Gland, <i>Cancer Res</i> 26, 1534, 1966 (352)
RPMI 1640	Sharma N, Luo J, Kirschmann DA, O'Malley Y, Robbins ME, Akporiaye ET, Lubaroff DM, Heidger PM, Hendrix MJ: A novel immunological model for the study of prostate cancer, <i>Cancer Res</i> 59, 2271-6, 1999 (10273)
Eagle's MEM Serum-free	Masuda, A., Ohtsuka, K., and Matsuyama, M.: Establishment of Functional Epithelial Cell Lines from a Rat Thyoma and a Rat Thymus, <i>In Vitro Cell Dev Biol</i> 26, 713, 1990 (448)
DMEM	Brennan, M., Oldberg, A., Hayman, E., and Ruoslahti, E.: Effect of a Proteoglycan Produced by Rat Tumor Cells on Their Adhesion to Fibronectin-Collagen Substrata, <i>Cancer Res</i> 43, 4302, 1983 (353)
Medium 199	Gazdar, A., Chick, W., Oie, H., Sims, H., King, D., Weir, G., and Lauris, V.: Continuous, Clonal, Insulin-and Somatostatin-Secreting Cell Lines Established from a Transplantable Rat Islet Cell Tumor, <i>Proc Natl Acad Sci U S A</i> 77 (6), 3519, 1980 (1182)
Phosphate buffer (see reference)	Essner, E.: Experiments on an Ascites Hepatoma. I. Enzymatic Digestion and Alkaline Degradation of the Cementing Substance and Separation of Cells, in Tumor Islands, <i>Exp Cell Res</i> 7, 430, 1954 (403)

FREE Collagenase Sampling Program and Online Lot Selection Tool

Simple as 1, 2, 3... and completely Free!

1. Go to: www.worthington-biochem.com/cls/clssamp.html

Worthington Collagenase Sampling Program

The lot-to-lot variation which is typical of crude enzyme preparations such as Worthington crude collagenase makes it important to pre-test a particular lot of enzyme you are planning to use in your experiment. Many years ago we found that the most practical approach for the researcher is to presample several different lots of collagenase at a time and select the best of the group. As the world's leading manufacturer of collagenase, Worthington is able to offer the greatest number of different lots at any given time and recommend specific lots for an application.

There is no charge for participating in the collagenase sampling program. Under the program, individual researchers are provided with 100 mg samples of up to three different lots of collagenase for evaluation in their own assay systems. A period of 60 days is allowed for your evaluation of these samples. A minimum of 3 grams of each lot will be placed on HOLD, reserved in your name. When you determine which lot performs best for you, simply specify the lot desired when ordering.

To become part of this program, or to discuss any of the Worthington products, just call our **Technical Service group toll-free at 800.445.9603** from anywhere in the United States or Canada or e-mail techservice@worthington-biochem.com

International customers should check our International Distributor listing for a distributor. If you do not have a Worthington Distributor for your country, please contact International Sales or Technical Service.

2. Consider using the interactive lot selection tool...

Collagenase Lot Selection Tool Now Available Online!

Worthington's 'Collagenase Lot Selection Tool' is now available online at our website. This new feature was designed to help researchers select and evaluate current collagenase lots that match previous lots or desired activity profiles. Users may enter target values for collagenase, caseinase, clostripain, and tryptic activities or specify previous lot numbers. Each value can be weighted based on the relative level of importance to the application. After the search for matches is completed, a ranked list of collagenase lots currently available is generated. The selected lots can then be sampled simply by using the built in link to the **Free Collagenase Sampling Program**. As always, Worthington Customer and Technical Service personnel are available via phone at **800.445.9603/732.942.1660** and e-mail to assist with collagenase or any other products.

2. Complete the online Sampling request form...

...samples will be shipped and reserved amount of each lot placed on hold for 60 days pending your evaluation. Completely free and without obligation!

Collagenase Lot Selection Tool

(optional) Look-up assay values for historical lot number:
If you are looking for a lot similar to one you have used in the past, enter it here:
Lot Number:
 (Assay values will be entered below. You may then a matches.)

Find lot matches for specified assay values:

1. Enter amount of collagenase required: mg

2. Select desired collagenase type:

3. Enter target assay values and relative levels of importance:

	u/mg dw	u/mg dw	u/mg dw	u/mg
	caseinase	clostripain	tryptic	
Values:	<input type="text" value="235"/>	<input type="text" value="327"/>	<input type="text" value="2.4"/>	<input type="text" value="0.3"/>
Weights:	<input type="text" value="3"/>	<input type="text" value="3"/>	<input type="text" value="3"/>	<input type="text" value="3"/>

(Report will open in a new window.)

Collagenase Sampling Program Request

Mailing Address:

Email:

Contact Name:

Phone:

Requested Collagenase Type:

Specify up to 3 lot numbers if known:

How much would you like us to reserve for you? grams
(Minimum 3 grams)

Describe tissue to be dissociated:

Related Worthington Products

Product	Activity	Catalog No.	Package	Code
Cell Isolation Optimizing System				CIT
A complete method development kit containing an assortment of enzymes most frequently used in tissue dissociation and cell isolation procedures. Includes instructions, references and strategies for the handling, use and optimization of enzymatic cell isolation methods to achieve maximum yield of viable cells. Kit includes 500 mg of each of four types of collagenase, 500 mg trypsin, 50 ku hyaluronidase, 100 mg elastase, 100 mg papain, 25 mg DNase I, 10 mg neutral protease (Dispase) and 100 mg trypsin inhibitor. Store at 2-8°C.		LK003200	1 bx	
Chymotrypsin, Alpha, 1X				CDAG
1X crystallized as zymogen and activated. Dialyzed against 1mM HCl and lyophilized. Store at 2-8°C.		≥35 units per mg protein	LS001333 LS001334 LS001332	1 gm 10 gm Bulk
Chymotrypsin, Alpha, 3X				CDI
3X crystallized alpha chymotrypsin which is an activation product of a 3X crystallized zymogen. Dialyzed against 1mM HCl and lyophilized. Store at 2-8°C.		≥45 units per mg protein	LS001448 LS001450 LS001451 LS001453	250 mg 1 gm 10 gm Bulk
Chymotrypsin, Alpha, Purified				CDS
Chromatographically prepared by the procedure of Yapel, <i>et al.</i> , <i>J. Amer. Chem. Soc.</i> , 88, 2573 (1966). A lyophilized powder. Store at 2-8°C.		≥45 units per mg protein	LS001475 LS001479 LS001477	100 mg 1 gm Bulk
Chymotrypsin, Alpha, TLCK Treated				CDTLCK
3X crystallized and treated with 1-chloro-3-tosylamido-7-amino-2-heptanone (TLCK) to inhibit trypsin activity [Shaw, <i>et al.</i> , <i>Biochemistry</i> , 4, 2219 (1965)]. Dialyzed against 1 mM HCl to remove autolysis products and low molecular weight contaminants. Supplied as a dialyzed, lyophilized powder. Store at 2-8°C.		≥45 units per mg protein	LS001430 LS001432 LS001434 LS001438	25 mg 100 mg 1 gm Bulk
Collagen				CL
Type I collagen prepared by the method of Einbinder and Schubert, <i>J. Biol. Chem.</i> , 188, 335 (1951). Supplied as a shredded, lyophilized, insoluble preparation. Store at 2-8°C.			LS001654 LS001652 LS001656 LS001658	1 gm 5 gm 10 gm Bulk
Collagen, Soluble				CLCS
Type I collagen supplied as a 6mg/ml liquid preparation in 75mM sodium citrate, pH 3.6 - 4.0, containing 0.01% merthiolate as a preservative. 2-8°C REQUIRES SPECIAL SHIPPING: ICE PACK. Note: Contains thimerisol as a preservative; proper disposal required.		Less than 20 minutes gel time	LS001663	Bulk

Tissue Dissociation Guide

Related Worthington Products

Product	Activity	Catalog No.	Package	Code
Collagenase, Animal Origin-Free				CLSAFA
Collagenase derived from cultures grown in animal-free medium. Suitable for applications needing to avoid introduction of animal derived pathogens into bioprocessing procedures. Store at 2-8°C.	≥150 units per mg dry weight	LS004152	100 mg	
		LS004154	1 gm	
		LS004156	5 gm	
		LS004158	Bulk	
Collagenase, Type 1				CLS-1
The original balance of enzymatic activities. Each lot assayed for collagenase, caseinase, clostripain and tryptic activities. Suggested for epithelial, liver, lung and adrenal primary cell isolations. A dialyzed, lyophilized powder. Store at 2-8°C.	≥125 units per mg dry weight	LS004194	100 mg	
		LS004196	1 gm	
		LS004197	5 gm	
		LS004200	Bulk	
Collagenase, Type 2				CLS-2
Prepared to contain higher clostripain activity. Suggested for bone, heart, liver, thyroid and salivary primary cell isolation. Supplied as a dialyzed, lyophilized powder. Store at 2-8°C.	≥125 units per mg dry weight	LS004174	100 mg	
		LS004176	1 gm	
		LS004177	5 gm	
		LS004179	Bulk	
Collagenase, Type 3				CLS-3
Lower in secondary proteolytic contaminant activities but with typical collagenase activity. Suggested for mammary primary cell isolation. A dialyzed, lyophilized powder. Store at 2-8°C.	≥100 units per mg dry weight	LS004180	100 mg	
		LS004182	1 gm	
		LS004183	5 gm	
		LS004185	Bulk	
Collagenase, Type 4				CLS-4
Prepared to contain lower tryptic activity levels to limit damage to membrane proteins and receptors but with normal to above normal collagenase activity. Suggested for pancreatic islet primary isolation. A dialyzed, lyophilized powder. Store at 2-8°C.	≥160 units per mg dry weight	LS004186	100 mg	
		LS004188	1 gm	
		LS004189	5 gm	
		LS004191	Bulk	
Collagenase, Purified				CLSPA
Chromatographically purified. ≤50 caseinase units per milligram. Supplied as a lyophilized powder. Store at 2-8°C.	≥500 units per mg dry weight	LS005275	4 ku	
		LS005273	10 ku	
		LS005277	Bulk	
Collagenase, Type 1, Filtered				CLSS-1
Collagenase, Type 1 (Code: CLS-1), which is filtered through a 0.22 micron membrane and lyophilized in vials to contain > 50 milligrams or 1 gram per vial. Store at 2-8°C.	≥125 units per mg dry weight	LS004214	50 mg	
		LS004216	5x50 mg	
		LS004217	1 gm	
Collagenase, Type 2, Filtered				CLSS-2
Collagenase, Type 2 (Code: CLS-2), which is filtered through a 0.22 micron membrane and lyophilized in vials to contain > 50 milligrams or 1 gram per vial. Store at 2-8°C.	≥125 units per mg dry weight	LS004202	50 mg	
		LS004204	5x50 mg	
		LS004205	1 gm	

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Related Worthington Products

Product	Activity	Catalog No.	Package	Code
Collagenase, Type 3, Filtered				CLSS-3
Collagenase, Type 3 (Code: CLS-3), which is filtered through a 0.22 micron membrane and lyophilized in vials to contain ≥ 50 milligrams per vial. Store at 2-8°C.	≥ 100 units per mg dry weight	LS004206 LS004208	50 mg 5x50 mg	
Collagenase, Type 4, Filtered				CLSS-4
Collagenase, Type 4 (Code: CLS-4), which is filtered through a 0.22 micron membrane and lyophilized in vials to contain > 50 milligrams or 1 gram per vial. Store at 2-8°C.	≥ 160 units per mg dry weight	LS004210 LS004212 LS004209	50 mg 5x50 mg 1 gm	
Deoxyribonuclease I, Ribonuclease & Protease Free, Solution				DPRFS
Molecular Biology Grade. Chromatographically purified to remove RNase and protease. Supplied as a solution at approximately 2 Kunitz units per microliter (approximately 1 mg/ml) containing 50% glycerol and 1mM calcium chloride. Store at 2-8°C or -20°C.	$\geq 2,000$ Kunitz units per ml	LS006342 LS006344 LS006348	100 un 500 un Bulk	
Deoxyribonuclease I, Ribonuclease & Protease Free				DPRF
Molecular Biology Grade. Chromatographically purified to remove RNase and protease. Lyophilized in vials. Each 10,000 unit vial contains 2 mg glycine, 2 μ moles calcium, and $\geq 10,000$ units of DNase I. Each 2,500 unit vial contains 0.5mg glycine, 0.5 μ moles calcium, and $\geq 2,500$ units of DNase I. Dissolving the entire 10,000 unit vial in 5 ml, or the entire 2,500 unit vial in 1.25 ml, provides the equivalent of a 1 mg/ml solution. (ku = 1000un) Store at 2-8°C. PROTECT FROM MOISTURE	$\geq 2,000$ units per mg dry weight	LS006331 LS006333 LS006334	2500 un 10 ku Bulk	
Deoxyribonuclease I				DPFF
Chromatographically purified. A lyophilized powder containing glycine as a stabilizer. Contains $\leq 0.0005\%$ RNase. Store at 2-8°C. PROTECT FROM MOISTURE. RNase & Protease Free	$\geq 2,000$ Kunitz units per mg dry weight	LS006330 LS006328 LS006332	25 ku 125 ku Bulk	
Deoxyribonuclease I				D
Chromatographically purified. A lyophilized powder with glycine as a stabilizer. Store at 2-8°C. PROTECT FROM MOISTURE	$\geq 2,000$ Kunitz units per mg dry weight	LS002004 LS002006 LS002007 LS002009	5 mg 20 mg 100 mg Bulk	
Deoxyribonuclease I, Filtered				DCLS
Filtered through a 0.22 micron membrane and lyophilized in vials. Material is not tested for pyrogenicity. Store at 2-8°C. PROTECT FROM MOISTURE	$\geq 2,000$ units per mg dry weight	LS002058 LS002060	11 mg 25 mg	
Deoxyribonuclease I, Standard Vial				DSV
Lyophilized in vials for assay standardization. Labeled to show established activity. Not suitable for assays at neutral pH. Store at 2-8°C. PROTECT FROM MOISTURE	$\sim 2,000$ Kunitz units per vial	LS002173 LS002172	2 ku 5x2 ku	

Tissue Dissociation Guide

Related Worthington Products

Product	Activity	Catalog No.	Package	Code
Deoxyribonuclease I				DP
Partially purified. A lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE	≥2,000 Kunitz units per mg dry weight	LS002138 LS002139 LS002140 LS002141	25 mg 100 mg 1 gm Bulk	
Deoxyribonuclease I				DPB
Partially purified. Supplied as lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE	≥1,250 Kunitz units per mg dry weight	LS002145 LS002147 LS002149	100 mg 1 gm Bulk	
Deoxyribonuclease I, Recombinant				DR1
Recombinant protein produced in <i>Pichia pastoris</i> . Free of animal derived components, RNases and proteases. Chromatographically purified. A lyophilized powder containing glycine as a stabilizer. Store at 2-8°C. PROTECT FROM MOISTURE	≥5,000 units per mg protein	LS006361 LS006362 LS006360	10 ku 50 ku Bulk	
Deoxyribonuclease I, Recombinant Solution				DR1S
Recombinant protein produced in <i>Pichia pastoris</i> . Free of animal derived components, RNases and proteases. Chromatographically purified. A liquid preparation in 5mM Calcium Acetate, 4mg/ml glycine, pH 5.0 and 50% glycerol. Supplied with 10x reaction buffer. Store at -20°C. REQUIRES SPECIAL SHIPPING: ICE PACK	≥2 units per microliter	LS006353 LS006355 LS006357	2 ku 5 x 2 ku Bulk	
Elastase, Suspension				ES
2X crystallized. Supplied as an aqueous suspension. This preparation must be diluted to dissolve the enzyme. The diluted enzyme should be 0.22 micron filtered before use. Suitable for the isolation of Type II lung cells. Store at 2-8°C. DO NOT FREEZE REQUIRES SPECIAL SHIPPING: ICE PACK.	≥3 units per mg protein	LS002274 LS002279 LS002280 LS002276	25 mg 100 mg 1 gm Bulk	
Elastase, Purified				ESFF
Chromatographically purified. A lyophilized powder. Store at 2-8°C. REQUIRES SPECIAL SHIPPING: ICE PACK	≥8 units per mg protein	LS006363 LS006365 LS006367	5 mg 20 mg Bulk	
Elastase, Lyophilized				ESL
2X Crystallized, (Code: ESL), supplied as a dialyzed, lyophilized powder. The enzyme should be 0.22 micron filtered after reconstitution and prior to use. Suitable for the isolation of Type II lung cells. Store at 2-8°C.	≥3 units per mg protein	LS002290 LS002292 LS002294 LS002298	25 mg 100 mg 1 gm Bulk	
Hepatocyte Isolation System				HIS
The package contains sufficient materials for five separate adult rat liver perfusions including five single use CLSH enzyme vials, Five single use DNase vials, 10X CMF-Hank's Balanced Salt Solution, L-15 Media Powder, 0.15M MOPS buffer. 7.5% Sodium Bicarbonate Solution and optimized protocol. Store at 2-8°C.		LK002060	bx	

Tissue Dissociation Guide

Related Worthington Products

Product	Activity	Catalog No.	Package	Code
HIS-Collagenase/Elastase				CLSH
Worthington collagenase (Code: CLS-1) and elastase (Code: ESL), filtered through 0.22µm pore size membrane, and lyophilized. Before use, reconstitute with the L-15/MOPS solution and swirl gently to dissolve contents as directed in the following procedure. Store unreconstituted vials at 2–8°C.		LK002066	1 VI	
		LK002067	5 VI	
HIS Kit, DNase Vial				D2
A component of the Hepatocyte Isolation kit containing 1,000 Units DNase I each, 5 Vials Worthington DNase I (Code: D), filtered through 0.22µm pore size membrane, and lyophilized. Before use, reconstitute with L-15/MOPS solution and swirl gently to dissolve contents as directed in the procedure. Store unreconstituted vials at 2–8°C.		≥1,000 units per vial	LK003170	1 vi
		LK003172	5 vi	
Hank's Balanced Salt Solution (HBSS-CMF) 10X Solution				HBSS10
10X CMF-HBSS Concentrate, 1 bottle, 500ml Sterile calcium- and magnesium-free Hank's Balanced Salt Solution (CMF-HBSS). The solution is used for washing and perfusing the liver prior to the addition of the dissociating enzyme solution.		LK002064	1 ea	
L-15 Media Powder				L15NK
Leibovitz L-15 media powder, a component of the HIS kit. Reconstitute entire contents of pouch, QS to 1 liter with cell culture grade water, and 0.22 micron filter. Suitable for cell isolation and culture applications. Store at 2-8°C.		LK003250	1 ea	
0.15m, MOPS Buffer, HIS				MOPS
0.15M MOPS, pH 7.5, 0.22u filtered. Buffer concentrate used to buffer the constituted Leibovitz L-15 media in Hepatocyte Isolation System. Store at 2-8°C.		LK002070	1 ea	
Sodium Bicarbonate 7.5%				NAH
7.5% Sodium Bicarbonate (NaHCO ₃), 1 bottle, 100ml 7.5% Sodium bicarbonate concentrate, used to buffer the diluted CMF-HBSS. Store at 2-8°C.		LK002069	1 ea	
Hyaluronidase				HSE
A partially purified, dialyzed, lyophilized powder. Store at -20°C.		≥300 USP/NF units per mg dry weight	LS002594	50 ku
			LS002592	300 ku
			LS002591	Bulk
Hyaluronidase, Purified				HSEP
Chromatographically purified. A dialyzed, lyophilized powder. Store at -20°C.		≥3,000 USP/NF units per mg dry weight	LS005477	5 ku
			LS005475	15 ku
			LS005474	30 ku
			LS005479	Bulk

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Related Worthington Products

Product	Activity	Catalog No.	Package	Code
Lysozyme				LY
A lyophilized powder containing sodium chloride and acetate. Store at 2-8°C.	≥5,000 units per mg dry weight	LS002880 LS002881 LS002883	1 gm 10 gm Bulk	
Lysozyme, Purified, Salt Free				LYSF
A dialyzed, lyophilized powder. Store at 2-8°C.	≥8,000 units per mg dry weight	LS002931 LS002933 LS002934	1 gm 5 gm Bulk	
Neonatal Cardiomyocyte Isolation System				NCIS
Kit for performing five separate tissue dissociations each containing up to twelve hearts. Contains single use vials of purified collagenase and trypsin, CMF-HBSS, Leibovitz L-15 media and Falcon cell strainers along with a detailed protocol. The kit is use-tested by Worthington to assure performance. Store at 2-8°C.		LK003300 LK003303	1 ki 3 ki	
Collagenase Vial, NCIS				CLSPANK
A component of the NCIS kit. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 5 ml of HBSS or equivalent yields a solution of 300 units/ml of collagenase, Code: CLSPA. Suitable for cell isolation and culture applications. Store at 2-8°C.	≥500 units per mg dry weight	LK003240 LK003245	1 vi 5 vi	
Trypsin Vial, NCIS				TRLSNK
A component of the NCIS kit. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 2 ml of HBSS yields a solution of 500µg/ml of trypsin, Code: TRLS. Suitable for cell isolation and culture applications. Store at 2-8°C.	≥180 units per vial	LK003220 LK003225	1 vi 5 vi	
Inhibitor Vial, NCIS				SICNK
A component of the NCIS kit. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 1 ml of HBSS or equivalent yields a solution of 2 mg/ml of trypsin inhibitor, Code: SIC. Suitable for cell isolation and culture applications. Store at 2-8°C.	1 mg inhibits at least 0.75 mg trypsin, Code: TRL	LK003230 LK003235	1 vi 5 vi	
HBSS Solution				HBSS
Sterile calcium and magnesium free Hank's balanced salt solution (CMFHBSS), pH 7.4, as supplied in the NCIS kit; 1 x 500 ml. Store at 2-8°C.		LK003210	1 ea	

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Related Worthington Products

Product	Activity	Catalog No.	Package	Code
L-15 Media Powder				L15NK
Leibovitz L-15 media powder, a component of the NCIS kit. Reconstitute entire contents of pouch, QS to 1 liter with cell culture grade water, and 0.22 micron filter. Suitable for cell isolation and culture applications. Store at 2-8°C.		LK003250	1 ea	
Cell Strainers (Falcon)				CELSTRNK
Cell strainers (Falcon), components of the NCIS kit. Suitable for removal of tissue debris in cell isolation applications. Store at room temperature.		LK003265	5 ea	
Neutral Protease (Dispase®), Purified				NPRO
Chromatographically purified. A lyophilized powder. Store at 2-8°C.	≥4 units per mg	LS02100	10 mg	
	dry weight	LS02104	50 mg	
		LS02108	Bulk	
Neutral Protease, Partially Purified				NPRO2
Partially purified. A lyophilized powder. Store at 2-8°C.	≥0.1 units per mg	LS02109	1 gm	
	dry weight	LS02111	5 gm	
		LS02112	Bulk	
Ovalbumin				OA
Major protein of egg white, with a molecular weight of 43,000. A lyophilized powder. Store at 2-8°C.		LS003049	1 gm	
		LS003048	5 gm	
		LS003050	Bulk	
Ovalbumin, Purified				OAC
Chromatographically purified. Major protein of egg white, with a molecular weight of 43,000. A dialyzed, lyophilized powder. Store at 2-8°C.		LS003056	100 mg	
		LS003054	1 gm	
		LS003052	Bulk	
Papain, Suspension				PAP
Supplied as a 2X crystalline suspension in 50mM sodium acetate, pH 4.5. To insure full activity, the enzyme should be incubated in a solution containing 1.1mM EDTA, 0.067mM mercaptoethanol and 5.5mM cysteine-HCl for 30 minutes. It is recommended that the enzyme be 0.22 micron filtered after dissolution and prior to use. Store at 2-8°C. REQUIRES SPECIAL SHIPPING: ICE PACK	Activates to at least 20 units per mg protein	LS003124	25 mg	
		LS003126	100 mg	
		LS003127	1 gm	
		LS003128	Bulk	
Papain, Lyophilized				PAPL
Supplied as a lyophilized powder prepared from a 2X crystalline suspension, Code: PAP. To insure full activity, the enzyme should be incubated in a solution containing 1.1mM EDTA, 0.067mM mercaptoethanol and 5.5mM cysteine-HCl for 30 minutes. It is recommended that the enzyme be 0.22 micron filtered after dissolution and prior to use. Store at 2-8°C.	Activates to at least 15 units per mg protein	LS003118	25 mg	
		LS003119	100 mg	
		LS003120	1 gm	
		LS003122	Bulk	

Tissue Dissociation Guide

Related Worthington Products

Product	Activity	Catalog No.	Package	Code
PDS Kit, Papain Vial				PAP2
A component of the Papain Dissociation System, for use in the tissue dissociation method of Huettner, J.E., and Baughman, R.W.: <i>J. Neuroscience</i> , 6, 3044 (1986). Contains papain, L-cysteine, and EDTA. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 5 ml of EBSS or equivalent yields a solution at 20 units of papain per ml in 1mM L-cysteine with 0.5mM EDTA. Store at 2-8°C.	≥100 units	LK003176	1 vi	
	per vial	LK003178	5 vi	
Papain Dissociation System				PDS
Set of five single use vials of papain and five single use vials of DNase, 100 ml of Earle's balanced salt solution (EBSS), and an inhibitor vial for use in the tissue dissociation method of Huettner, J.E., and Baughman, R.W.: <i>J. Neuroscience</i> , 6, 3044 (1986). Use tested by Worthington using new-born rat pup spinal cord. The package contains sufficient materials for dissociation of five separate tissue aliquots of up to 0.3-0.4 cm ³ each. Store at 2-8°C.		LK003150	1 bx	
		LK003153	3 bx	
Papain Dissociation System, Without EBSS				PDS2
Complete kit as described for product Code: PDS, but without the Earle's Balanced Salt Solution (EBSS). Store at 2-8°C.		LK003160	1 bx	
		LK003163	3 bx	
PDS Kit, DNase Vial				D2
A component of the Papain Dissociation System. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 0.5 ml of EBSS or equivalent yields a solution of 2000 units/ml of deoxyribonuclease (1 mg/ml). Store at 2-8°C.	≥1,000 units	LK003170	1 vi	
	per vial	LK003172	5 vi	
PDS Kit, Inhibitor Vial				OI-BSA
Ovomucoid protease inhibitor and bovine serum albumin which is 0.22 micron filtered and lyophilized in autoclaved vials to contain 10 mg/ml each upon reconstitution with 32 ml of EBSS. Store at 2-8°C.	≥300 mg TRL inhibited per vial	LK003182	1 vi	
PDS Kit, EBSS Vial				EBSS
Earle's balanced salt solution (EBSS) as supplied in the Papain Dissociation System. Store at 2-8°C.		LK003188	1 vi	
Proteinase K				PROK
A lyophilized powder. Purified to remove DNase and RNase. Store at 2-8°C.	≥20 units per	LS004220	25 mg	
	mg dry weight	LS004222	100 mg	
		LS004224	1 gm	
		LS004226	Bulk	

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Related Worthington Products

Product	Activity	Catalog No.	Package	Code
Trypsin 2X Lyo				TRL
Supplied as a chromatographically purified, diafiltered and lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE	≥180 units per mg protein	LS003702	100 mg	
		LS003703	1 gm	
		LS003704	Bulk	
		LS003706	Bulk	
Trypsin				TRL3
Supplied as a chromatographically purified, diafiltered and lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE	≥180 units per mg protein	LS003708	100 mg	
		LS003707	1 gm	
		LS003709	Bulk	
Trypsin, 0.22µ Filtered				TRLS
Trypsin chromatographically purified, diafiltered, (Code TRL3) filtered through a 0.22 micron pore size membrane and lyophilized in sterile vials. This product is not tested for pyrogenicity. Store at 2-8°C.	≥180 units per mg protein (at least 10,350 BAEE/3,450 USP/NF u/mgP)	LS003736	50 mg	
		LS003734	5x50 mg	
		LS003738	Bulk	
Trypsin, 2X, Sterile, Irradiated				TRLVMF
2X crystallized (Code: TRL), lyophilized, irradiated and tested for the absence of mycoplasma and extraneous virus according to 9 CFR113.53c. Each vial is filled to contain ≥100 mg. Store at 2-8°C.	≥180 units per mg protein (at least 10,350 BAEE/3450 USP/NF units per mg protein)	LS004454	100 mg	
		LS004452	5x100 mg	
		LS004458	Bulk	
Trypsin, Purified, Sequencing Grade II				TRSEQII
Bovine trypsin that has been treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inhibit contaminating chymotryptic activity and extensively purified to remove autolysis products. Supplied as a lyophilized powder. Store at -20°C. PROTECT FROM MOISTURE. REQUIRES SPECIAL SHIPPING: ICE PACK	≥150 units per mg protein (at least 8,625 BAEE/2875 USP/NF units per mg protein)	LS02115	4x25 µg	
		LS02117	4x100 µg	
		LS02118	Bulk	
Trypsin, Modified, Sequencing Grade				TRSEQZ
Trypsin, treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone to inhibit contaminating chymotryptic activity, chemically modified to promote stability and further purified to remove autolysis fragments, resulting in a highly stable trypsin product resistant to autolysis while retaining specificity. Store at -20°C PROTECT FROM MOISTURE. REQUIRES SPECIAL SHIPPING: ICE PACK.	≥4 units per mg protein	LS02120	4x25 µg	
		LS02122	4x100 µg	
		LS02124	Bulk	
Trypsin, TPCK Treated				TRTPCK
A chromatographically purified, diafiltered, lyophilized powder that has been treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inhibit contaminating chymotryptic activity [Kostka, V., and Carpenter, F.: <i>JBC</i> , 239, 1799 (1964)]. Store at 2-8°C. PROTECT FROM MOISTURE	≥180 units per mg protein (at least 10,350 BAEE/3,450 USP/NF u/mgP)	LS003740	100 mg	
		LS003741	500 mg	
		LS003744	1 gm	
		LS003742	Bulk	

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Related Worthington Products

Product	Activity	Catalog No.	Package	Code
TRTVMF				
Trypsin, TPCK-Treated, Irradiated				
Chromatographically purified trypsin treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inhibit contaminating chymotryptic activity according to Kostka, V., and Carpenter, F.H.: <i>JBC</i> , 239, 1799 (1964), Code: TRTPCK, lyophilized, irradiated and tested for the absence of mycoplasma and extraneous virus according to 9 CFR 113.53c. Each vial is filled to contain ≥100 mg. Store at 2-8°C.	≥180 units per mg protein	LS003750 LS003752	100 mg 5x100 mg	
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Trypsin Inhibitor, Lima Bean				
LBI				
Fraction III of the preparation described by Fraenkel-Conrat, <i>et al.</i> , <i>Arch. Biochem. Biophys.</i> , 37, 393 (1952). Supplied as a dialyzed, lyophilized powder. Store at 2-8°C.	1mg inhibits at least 2.2 mg trypsin, Code: TRL	LS002829 LS002830 LS002831	100 mg 1 gm Bulk	
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Trypsin Inhibitor, Ovomuroid				
OI				
Mucoprotein and antitryptic factor of egg white described by Lineweaver and Murray, <i>J. Biol. Chem.</i> , 171, 565 (1947). A dialyzed, dried powder. Store at 2-8°C.	1 mg inhibits at least 1.2 mg trypsin, Code: TRL	LS003085 LS003087 LS003086 LS003089	500 mg 1 gm 2 gm Bulk	
<hr/>				
Trypsin Inhibitor, Soybean, Purified				
SI				
Chromatographically purified as described by Frattali, V., and Steiner, R.: <i>Biochem.</i> , 7, 521 (1968). A dialyzed, lyophilized powder. Purity checked using SDS PAGE. Store at 2-8°C.	1 mg inhibits at least 1.2 mg trypsin, Code: TRL	LS003570 LS003571 LS003573	100 mg 1 gm Bulk	
<hr/>				
Trypsin Inhibitor, Soybean				
SIC				
A partially purified acetone powder. Store at 2-8°C.	1 mg inhibits at least 0.75 mg trypsin, Code: TRL	LS003587 LS003590	1 gm Bulk	

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Authorization for any product returns must be obtained from Worthington Biochemical Corporation (Customer Service Department), or its authorized representative, prior to the return of the product. This authorization is required to insure the proper return of material and, if applicable, the correct issuance of credit. There is no provision for credit of outdated material. Product must be returned in the same condition as received and within 30 days of the original shipment by Worthington Biochemical Corporation. A restocking fee may be charged for all returns.

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Worthington products can be supplied in a wide range of purity and activity specifications. Custom analysis and special package sizes can also be provided. Contact your representative or the Bulk Sales Office to discuss your specific requirements.

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For orders of greater than 25 packages or orders of material packed in bulk, contact your representative or the Bulk Sales Office for special pricing consideration. Standing orders may also qualify for discounts. We welcome long-term use projections upon which we can consider special rates. Large institutional buyers should contact us regarding special purchasing agreements.

Technical Assistance

Available 8:00 AM to 5:00 PM Eastern Time Monday through Friday to process orders and provide customer service. We can be contacted 24 hours a day by FAX or e-mail.

Worthington produces most of the products it sells and welcomes your questions and suggestions. Because we are a primary manufacturer, we have ready access to all production and quality control records of our products by lot number.

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Several Animal Origin Free (AOF) collagenases, nucleases, proteases and other products are also available to eliminate BSE/TSE and other mammalian viral risks. Please inquire.