

Chondrex

Enabling Arthritis and
Inflammation Research
Worldwide



OTC AND SELF TESTING
ALLERGOLOGY
LAB PRODUCTS
PROFESSIONAL DEVICES
VETERINARY
MEDICAL SUPPLIES
SCIENTIFIC PRODUCTS

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Ordering Information

To Order

- By Phone:** (425) 702-6365
(888) 246-6373
- By Fax:** (425) 882-3094
- By Email:** info@chondrex.com
- By Internet:** www.chondrex.com
- By Mail:** Chondrex, Inc.
2607 151st Place NE
Redmond, WA 98052
USA

Please provide the following information to expedite your order:

1. Customer name and telephone number
2. Shipping address
3. Billing address
4. Purchase order number
5. Catalog number and product description
6. Quantity of product
7. Custom broker's name and telephone number (if applicable)
8. VAT number (if applicable)

Shipping

Continental USA Orders are generally shipped within 1 - 2 working days by overnight delivery.

International Orders are normally shipped within 2 - 5 working days.

Shipping and Handling Charges are prepaid and added to the invoice. Products are shipped FCA Redmond, WA 98052 USA.

Pricing and Terms

We reserve the right to discontinue or change product specifications, including price, at any time without notice. For the most up-to-date pricing information on products, please contact Customer Service or visit our web site, www.chondrex.com.

Our payment terms are net 30 days from the date of shipment on pre-approved purchase order accounts, payable in US dollars, and subject to a 1.5% monthly late fee if payment is not received within 30 days after shipping.

Remit Payments to: Chondrex, Inc.
2607 151st Place NE
Redmond, WA 98052
USA

Credit Cards are welcome.

Visa, Mastercard, and American Express are accepted.



International Customers, please make payments in US dollars by check drawn from a US bank, by money order, or by wire transfer. The customer is responsible for all bank service charges.

Support

For customer and technical support, please call (425) 702-6365 or (888) 246-6373 during normal business hours. You may also email info@chondrex.com for support. We will be happy to discuss any questions you may have regarding our products and protocols.

Returns

Please call Customer Service for a Return Material Authorization number and shipping instructions. A 30% restocking fee may apply depending on the circumstances of the return.

Allergenic IgE Monoclonal Antibodies

Chondrex provides two IgE monoclonal antibodies specific to ovalbumin (OVA), clone E-C1 and clone E-G6, in addition to one IgG1 monoclonal antibody against OVA, clone L71. In general, the cross-linkage of IgE molecules bound to the receptor on mast cells by a polyvalent allergen is required to trigger degranulation of mast cells. Alternatively, IgG antibody to the allergens can trigger the activation of mast cells by cross-linking two antigens bound by IgE antibodies on mast cell surfaces. However, E-C1 alone is capable of inducing degranulation of mast cells in vitro (Figure 1), and severe hypersensitivity reaction in vivo (Figure 2). Therefore, it is assumed that E-C1 might recognize repetitive epitopes of OVA. Furthermore, it has been reported that aggregated OVA carrying multiple epitopes, increases the formation of cross-linkage of IgE on mast cell surfaces. On the other hand, E-G5 is not capable of inducing these hypersensitivity reactions by itself in vitro and in vivo (Figures 1 and 2), and can be used as a control.

Catalog #	Description	Antigen	In Vitro *1	In Vivo *2	Quantity
3006	Mouse Monoclonal IgE Antibody E-C1	OVA & Aggregated OVA	Yes	Yes	1 mg/vial Lyophilized with Mouse Serum Albumin
3007	Mouse Monoclonal IgE Antibody E-G5	OVA Only *3	No	No	1 mg/vial Lyophilized with Mouse Serum Albumin
3008	Mouse Monoclonal IgG1 Antibody L71	OVA & Aggregated OVA	No	No	1 mg/vial Lyophilized with Mouse Serum Albumin

*1: Mast cell activation, *2: Hypersensitivity reaction, *3: Does not react to aggregated OVA

Degranulation of mast cells by monoclonal IgE antibody

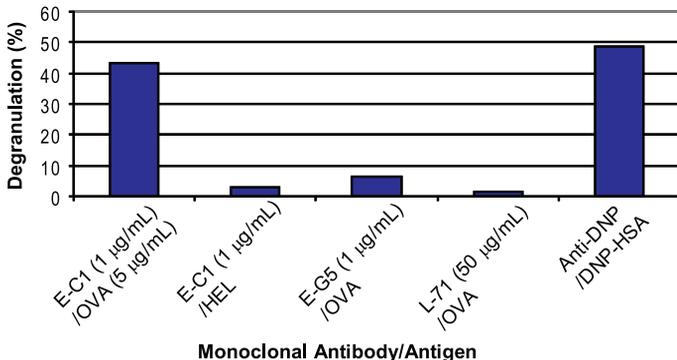


Figure 1A - Comparison of three monoclonal antibodies

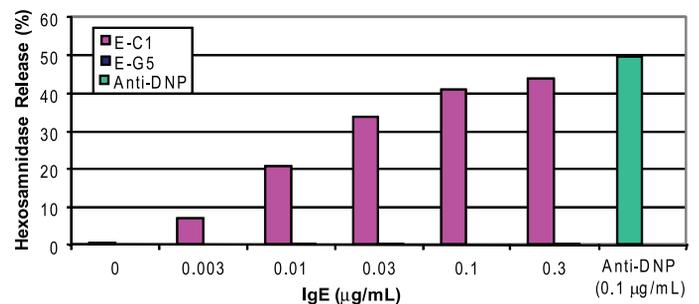


Figure 1B - Dose response of E-C1 and E-G5

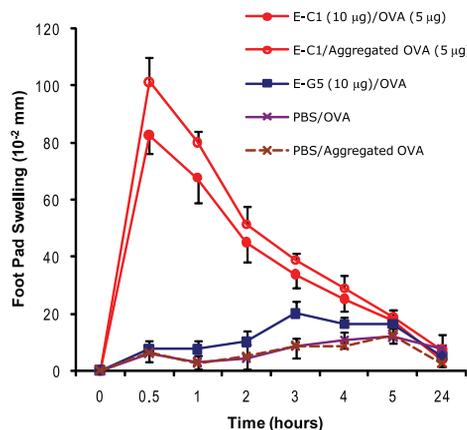


Figure 2 - Foot pad hypersensitivity reaction induced by E-C1 in Balb/c mice

Chondrex provides three types of IgE detection kits. These IgE detection kits employ an identical IgE standard (Clone E-C1). Therefore, the ratio of total IgE and ovalbumin (OVA) specific IgE in individual samples is easily and accurately compared. In addition, rat anti-mouse IgE monoclonal antibodies (Clone 345-2 and 77-1) used in these kits react to both IgE^a and IgE^b allotypes. Therefore, separate assays for IgE^a and IgE^b allotypes are not required.

Mouse Anti-OVA IgE Antibody Assay Kit (Catalog # 3004)

The Mouse Anti-OVA IgE Antibody Assay Kit is designed to simply determine anti-OVA IgE levels using an OVA-coated ELISA plate for samples used for in vitro experiments, such as an IgE solution used for stimulating mast cells. This ELISA kit includes reagents to measure 40 samples. The assay range is approximately 0.8 - 50 ng/mL.

Mouse Total IgE (IgE^a and IgE^b) Detection Kit (Catalog # 3005)

Mice are the most useful experimental animals for studying allergic diseases, since a variety of inbred strains and transgenic and gene knockout mice are available. Serum IgE level is often raised in allergic diseases and parasitic infections, although it alone does not reflect the allergic state and the clinical symptoms. The Mouse Total IgE (IgE^a and IgE^b) Detection Kit includes reagents to determine total IgE levels of 40 samples and the assay range is approximately 1.6 - 100 ng/mL.

Mouse Serum Anti-OVA IgE Antibody Assay Kit (Catalog # 3010)

OVA is widely used as an antigen for studying allergic diseases. However, mouse serum contains various types of antibodies, such as IgA, IgM, and IgG, against the same antigen at higher levels than IgE. Therefore, it is difficult to detect IgE antibody levels due to competitive binding of these different types of antibodies on the same antigenic determinants on the antigen. The Mouse Serum Anti-OVA IgE Antibody Assay Kit is designed to specifically detect IgE antibody in mouse serum, which contains a mixture of various types of anti-OVA antibodies. This kit measures anti-OVA IgE levels accurately in samples of which the total IgE levels are less than 500 ng/mL. This kit includes reagents for assaying 40 serum samples. The linear range of this assay is 0.4 - 25 ng/mL.

Mouse Anti-OVA IgG Assay Kit (Catalog # 3011)

The cross-linkage of IgE antibodies bound to the receptor on mast cells by polyvalent allergen triggers the degranulation of mast cells. On the other hand, the pathogenic roles of IgG antibodies against the allergen are not well characterized. The Mouse Anti-OVA IgG Assay Kit includes reagents to determine anti-OVA IgG antibody levels of 40 mouse serum samples and is used for studying the contributions of IgG in allergic diseases. The linearity range of this assay is 0.2 - 12.5 ng/mL.

Catalog #	Description
3004	Mouse Anti-OVA IgE Antibody Assay Kit
3005	Mouse Total IgE (IgE ^a and IgE ^b) Detection Kit
3010	Mouse Serum Anti-OVA IgE Antibody Assay Kit
3011	Mouse Anti-OVA IgG Antibody Assay Kit

ArthroGen-CIA[®] Arthritogenic Monoclonal Antibodies

Collagen-Induced Arthritis (CIA) and Collagen Antibody-Induced Arthritis (CAIA)

Collagen-induced arthritis (CIA) is mediated by autoantibodies against type II collagen, and a similar arthritis (Figures 1 and 2) can be induced by a cocktail of anti-type II collagen monoclonal antibodies alone or in combination with LPS. This collagen antibody-induced arthritis (CAIA) model has been widely used since 1998 when Chondrex started to distribute ArthroGen-CIA[®] arthritogenic monoclonal antibody cocktail, consisting of four monoclonal antibodies recognizing independent epitopes within the CB11 fragment of type II collagen.

Importantly, bacteria toxins, such as lipopolysaccharide (LPS), Staphylococcal enterotoxin B (SEB), and M. arthritis mitogen (MAM), play a synergistic effect with autoantibodies to type II collagen, and induces severe arthritis. Therefore, the combination of a sub-arthritogenic dose of monoclonal antibody cocktail and LPS has been widely used for inducing severe arthritis at high incidence in mice for a variety of purposes including evaluation of therapeutics (Figure 3A).



Figure 1 - Arthritis Induced by mAb Cocktail Without LPS in a DBA/1J Mouse

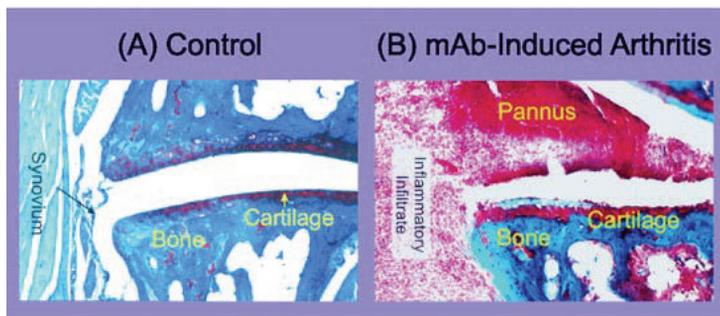


Figure 2 - Histopathology of mAb-Induced Arthritis

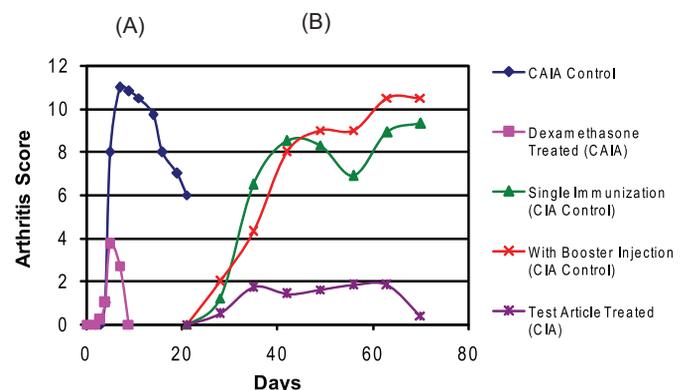
- (A) The control shows an uninvolved joint from a mouse injected with an ineffective pair of monoclonal antibodies.
- (B) The mAb-induced arthritis shows an arthritic joint with severe synovitis with inflammatory cells, pannus formation, and cartilage erosion.

Advantages of CAIA

- 1) Severe arthritis is induced within 24 - 48 hours (Figure 3A) instead of the four weeks required to induce CIA after immunization with type II collagen (Figure 3B).
- 2) Unlike the CIA model, which requires a restricted MHC haplotype, the CAIA model can be induced in almost all strains of mice, including CIA resistant, T-cell deficient, gene-knockout, and transgenic mice.
- 3) The severity of arthritis apparently correlates to the dose of monoclonal antibody cocktail, and more severe and consistent arthritis can be induced by increasing the dose of monoclonal antibody cocktail.
- 4) This model is ideal for studying the pathological role of individual gene products, cytokines, inflammatory cells, and environmental factors, as well as for screening and evaluating anti-inflammatory agents (Figure 3) without influences of complete or incomplete Freund's adjuvant, which strongly affects host immune systems.

Figure 3 - Comparison of Authentic CIA and mAb-LPS-Induced Arthritis

- (A) A cocktail of 4 monoclonal antibodies to type II collagen (2 mg) is injected intravenously on day 0 followed by an intraperitoneal injection of LPS (50 µg) on day 3. Arthritis develops on day 4 and reaches its peak on day 7 - 8. The therapeutic effects of test compounds are determined on day 7. Osteoblast formation and bone degradation will be more significant on day 14 and day 21 (not shown).
- (B) Authentic collagen-induced arthritis.

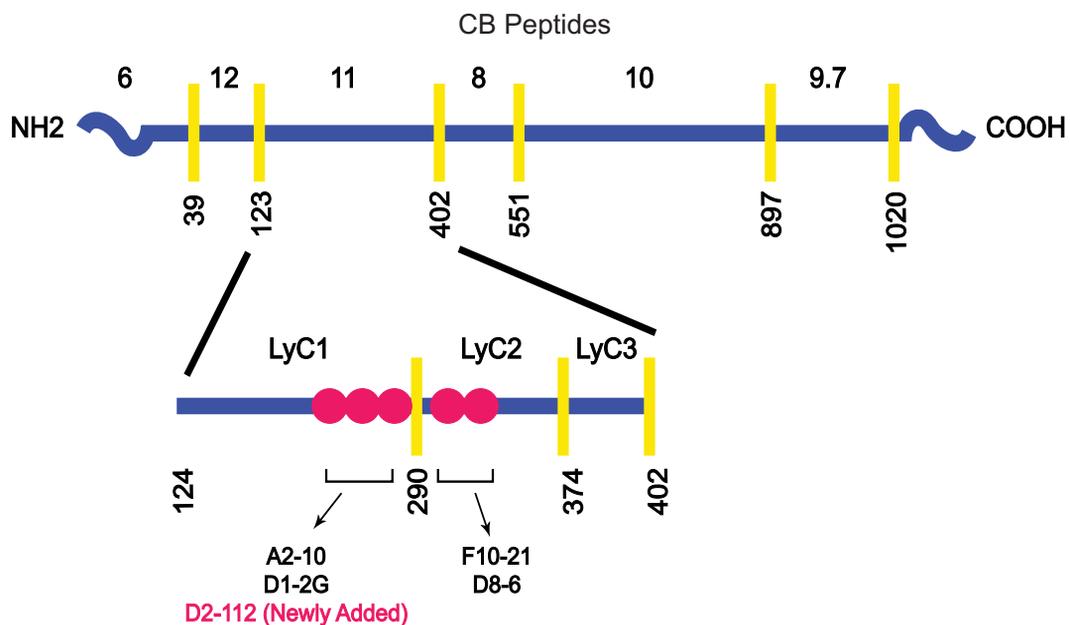


ArthroGen-CIA[®] Arthritogenic Monoclonal Antibodies

A Newly Improved 5-Clone Cocktail

In order to induce arthritis more effectively with or without LPS in a variety of mouse strains, Chondrex has developed a new 5-clone cocktail of arthritogenic monoclonal antibodies by adding a new clone to the current 4-clone cocktail. Three clones of this new cocktail recognize epitopes located within the LyC1 (CII 124-290) fragment, whereas the other two clones react with the LyC2 (CII 291-374) fragment of CB11 (CII 124-402).

Please note that the arthritogenic epitopes are not distributed along type II collagen molecule and are apparently clustered within a certain region of type II collagen depending upon the MHC types in mice, such as CB11 in DBA/1 (H-2^d) mice and CB8 in B10.RIII (H-2^r) mice. Importantly, a similar restriction of autoantibody-CB peptide reactivity is observed in patients with rheumatoid arthritis. For example, autoantibody from one group of patients reacts with CB11 dominantly, whereas autoantibodies from another group reacts with CB8 and CB10. In any case, autoantibodies from one patient do not recognize all of these epitopes distributed along the collagen molecule.



Arthritogenicity of New 5-Clone Cocktail

DBA/1 mice:

Chondrex's 4-clone cocktail is capable of inducing severe and consistent arthritis in CIA-susceptible DBA/1 mice at a dose of 2 mg per mouse by IV injection in combination with LPS (50 µg) (Figure 4 - red), whereas a competitor's cocktail induces only mild arthritis at the same dose, and the severity of arthritis varies significantly among individual animals (Figure 4 - blue). The new 5-clone cocktail is capable of inducing more severe and consistent arthritis at a dose of 2 mg per mouse in comparison with the other two products. The average arthritis score reaches 15 (maximum score: 20) and remains at that level until day 15 (Figure 4 - yellow).

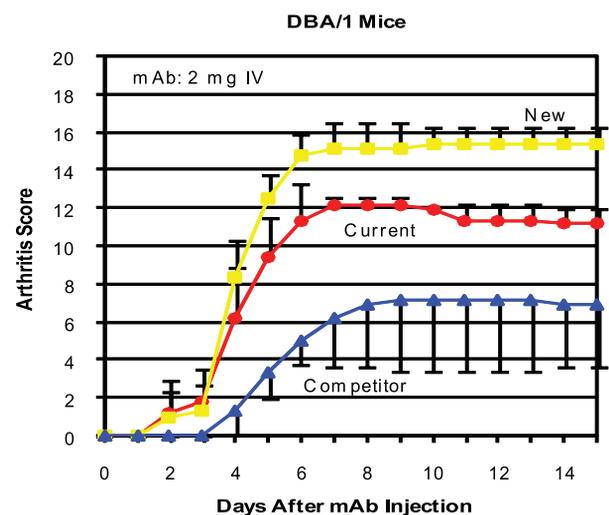


Figure 4

ArthroGen-CIA® Arthritogenic Monoclonal Antibodies

C.B-17/lcr mice:

T-cell deficient C.B-17/lcr mice are also highly susceptible to CAIA and develop severe arthritis by IP injection of 2 mg of Chondrex's 4-clone cocktail followed by an IP injection of LPS (50 µg) (Figure 5 - red), whereas a competitor's cocktail induced only mild arthritis at the same dose (Figure 5 - blue). Again, the new 5-clone cocktail induces more severe and consistent arthritis (Figure 5 - yellow) compared to the other two products, as observed in DBA/1 mice.

C57BL/6 mice:

A large amount of monoclonal antibody cocktail (8 - 10 mg per mouse) is required for inducing arthritis in C57BL/6 mice, indicating that C57BL/6 mice might be low responders to LPS. Arthritis induced by 4-clone cocktail is generally mild and the severity of arthritis varies significantly among individual mice, even at a high dose of 8 - 10 mg per mouse. For example, mice receiving 10 mg of competitor's 4-clone cocktail developed mild arthritis scored between 3 - 8 (Figure 6 - blue). Compared to Chondrex's 4-clone cocktail and a competitor's 4-clone cocktail, the new 5-clone cocktail is superior and capable of inducing severe arthritis at a dose of 10 mg per C57BL/6 mouse. All mice developed severe arthritis (score range: 11 - 18 out of 20) by IP injection of 10 mg of 5-clone cocktail followed by IP injection of 50 µg of LPS (Figure 6 - yellow).

Note: In vivo testing was performed by an independent lab. Monoclonal antibody cocktail was administered by IP injection in C.B-17/lcr and C57BL/6 mice, since it can be difficult to give IV injection accurately due to the black skin color of these mice.

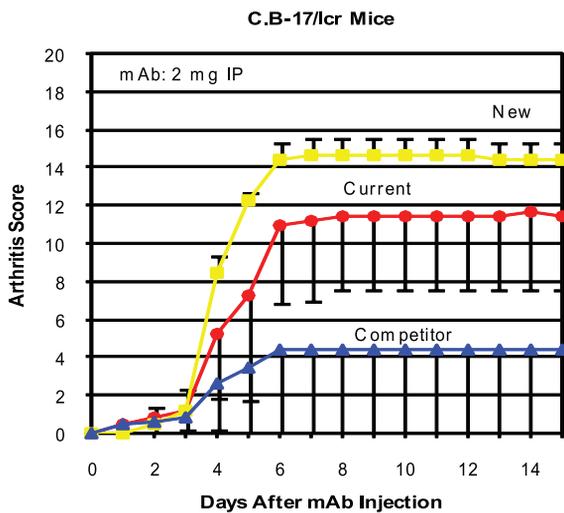


Figure 5

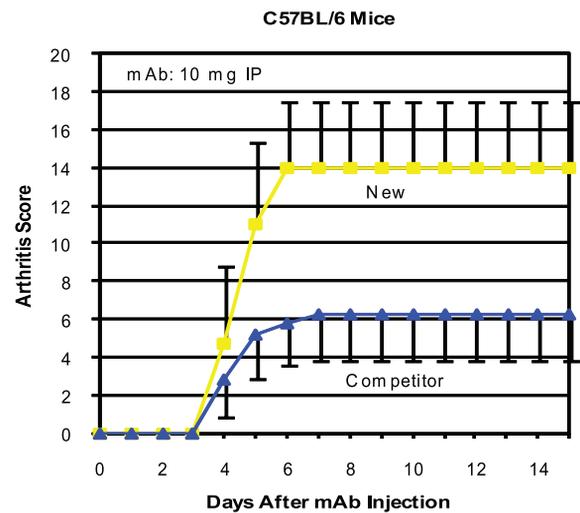


Figure 6

Catalog #	mAb Cocktail	LPS (E. Coli 0111:B4)	Price
10010	1 mL x 10 mg/mL - 4 clones	0.5 mL x 0.5 mg/mL	\$520
10040	4 mL x 10 mg/mL - 4 clones	2 mL x 0.5 mg/mL	\$1960
10100	10 mL x 10 mg/mL - 4 clones	5 mL x 0.5 mg/mL	\$4675
53010	1 mL x 10 mg/mL - 5 clones	1 mL x 0.5 mg/mL	\$730
53040	4 mL x 10 mg/mL - 5 clones	3 mL x 0.5 mg/mL	\$2755
53100	10 mL x 10 mg/mL - 5 clones	7 mL x 0.5 mg/mL	\$6540
9028	-----	5 mL x 0.5 mg/mL	\$25

Immunization Grade

For successfully inducing collagen-induced arthritis (CIA) in experimental animals, the quality of type II collagen is important as well as the quality of the animals. Chondrex provides various species of highly purified type II collagen which are suitable for the induction of CIA and for various applications such as bioengineering of artificial cartilage and cell cultures. Susceptibility to CIA in rodents is linked to the MHC class II molecules of animals, but also depends upon the species of type II collagen used for immunization. Choose the right species of type II collagen for inducing CIA according to the table on the right. Type II collagen is supplied lyophilized or in solution depending upon the experimental purposes.

In contrast to type II collagen, located in avascular tissues such as cartilage, type I collagen is most abundant and is widely used as a substrate for many purposes such as cell culture and bioengineering. Chondrex provides highly purified type I collagen for general research purposes. These type I collagen may be used as a control for a variety of applications in arthritis research.

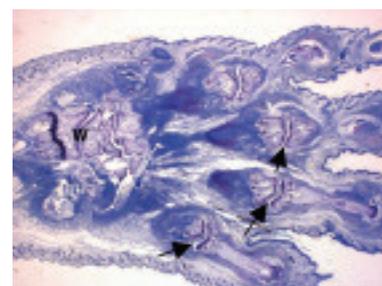
Species of Type II Collagen	Mouse		Rat		Primate Monkey (Outbred)
	DBA/1 (H-2 ^q)	B10.RIII (H-2 ^f)	LOU (RT1 ^u)	Lewis (RT1 ^l)	
Chick	+++	-	+++	++	+++
Bovine	+++	+++	+++	+++	+++
Porcine	+++	+++	+++	+++	Not Determined
Human	++	-	+	+	++

Immunization Grade Type II Collagen

Catalog #	Species	Quantity	Form
20011	Chick	10 mg	Lyophilized
20012	Chick	5 mL x 2 mg/mL	Solution, 0.05M acetic acid
20021	Bovine	10 mg	Lyophilized
20022	Bovine	5 mL x 2 mg/mL	Solution, 0.05M acetic acid
20031	Porcine	10 mg	Lyophilized
20032	Porcine	5 mL x 2 mg/mL	Solution, 0.05M acetic acid
20041	Rat	5 mg	Lyophilized
20042	Rat	2.5 mL x 2 mg/mL	Solution, 0.05M acetic acid
20051	Human	1 mg	Lyophilized
20052	Human	0.5 mL x 2 mg/mL	Solution, 0.05M acetic acid
20061	Mouse	1 mg	Lyophilized
20062	Mouse	0.5 mL x 2 mg/mL	Solution, 0.05M acetic acid

Immunization Grade Type I Collagen

Catalog #	Species	Quantity	Form
1061	Chick	10 mg	Lyophilized
1062	Bovine	10 mg	Lyophilized
1063	Porcine	10 mg	Lyophilized
1064	Rat	5 mg	Lyophilized
1065	Human	1 mg	Lyophilized
1066	Mouse	5 mg	Lyophilized



CIA Mouse Forepaw
Courtesy of HistoTox Labs, Inc.

Immunization Grade Type IX and XI Collagen

Chondrex provides chick, bovine, and porcine type IX and XI collagen. Please contact Customer Service for more information.

Collagen

Adjuvant

Complete Freund's adjuvant (CFA) containing various amounts of *M. tuberculosis* is provided. The selection of *M. tuberculosis* concentration depends on the experimental purposes and immunization schedules, and requires recommendation by each institution's animal care and use committee.

Catalog #	Description	M. Tuberculosis H-37 RA	Size
7002	Incomplete Freund's Adjuvant	-----	5 mL
7008	Complete Freund's Adjuvant	1 mg/mL	5 mL
7009	Complete Freund's Adjuvant	2 mg/mL	5 mL
7015	Complete Freund's Adjuvant	3 mg/mL	5 mL
7001	Complete Freund's Adjuvant	4 mg/mL	5 mL
7023	Complete Freund's Adjuvant	5 mg/mL	5 mL
7027	Complete Freund's Adjuvant	10 mg/mL	5 mL
7024	Complete Freund's Adjuvant	20 mg/mL	5 mL

ELISA Grade

CIA-susceptible and even CIA-resistant animals generate very high levels of antibodies to the immunizing collagen. Importantly, antibodies in CIA-susceptible animals recognize the conserved epitopes shared by various species of type II collagen and cross-react to autologous type II collagen by 50 - 60%. Therefore, we recommend monitoring the antibody levels against a heterologous type II collagen used for immunization and an autologous type II collagen to correlate the development of arthritis and antibody levels.



Similarly, antibodies to type II collagen in patients with rheumatoid arthritis (RA) react dominantly with a heterologous type II collagen such as bovine and chick type II collagen, and cross-react to human type II collagen to some degree, depending upon individual patients.

Type I collagen from various species of animals are also available. Type I and type II collagen share 80% homology in the amino acid sequences, but their immunological features differ significantly as well as their tissue distribution. These various species and types of collagen can be used to determine the antibody specificity in experimental animals and patients with a variety of autoimmune diseases.

Each vial of ELISA grade collagen contains enough collagen to coat ten 96-well plates. A 10X dilution buffer is also supplied to prevent fibril formation. Each vial of ELISA grade collagen contains 0.5 mg collagen in 1 mL of 0.05M acetic acid solution.

ELISA Grade Type II Collagen

Catalog #	Species	Price
2011	Chick	
2012	Bovine	
2013	Porcine	
2014	Rat	
2015	Human	
2016	Mouse	
2017	Monkey	

ELISA Grade Type I Collagen

Catalog #	Species
1001	Chick
1002	Bovine
1003	Porcine
1004	Rat
1005	Human
1006	Mouse

T-Cell Proliferation Grade

Since collagen is a T-cell dependent antigen, T-cell responses to type I and type II collagen can be assayed using ultra-pure, pepsin-free, denatured T-cell grade collagen by T-cell proliferation assay and by assaying cytokines released by activated T-cells.

T-Cell Proliferation Grade Type II Collagen

Catalog #	Species	Quantity	Form
2021	Chick	0.5 mg	Lyophilized
2022	Bovine	0.5 mg	Lyophilized
2023	Porcine	0.5 mg	Lyophilized
2024	Rat	0.5 mg	Lyophilized
2025	Human	0.5 mg	Lyophilized
2026	Mouse	0.5 mg	Lyophilized
2027	Monkey	0.5 mg	Lyophilized

T-Cell Proliferation Grade Type I Collagen

Catalog #	Species	Quantity	Form
1051	Chick	0.5 mg	Lyophilized
1052	Bovine	0.5 mg	Lyophilized
1053	Porcine	0.5 mg	Lyophilized
1054	Rat	0.5 mg	Lyophilized
1055	Human	0.5 mg	Lyophilized
1056	Mouse	0.5 mg	Lyophilized

Cyanogen Bromide-Cleaved Peptide Fragments of Type II Collagen (CB-Peptides)

CB-peptide fragments of type II collagen (chick, bovine, and human) are also available for studying B-cell and T-cell epitopes on type II collagen.

CB-11 Fragment of Type II Collagen

Catalog #	Species	Quantity	Form
9004	Chick	0.5 mg	Lyophilized
9006	Human	0.5 mg	Lyophilized
9007	Bovine	0.5 mg	Lyophilized

Other CB-Peptide Fragments of Type II Collagen

Chondrex also provides chick CB6, CB7, CB9, CB10, and CB12 fragments, bovine CB8, CB10, and CB12 fragments, and human CB8, CB9.7, CB10, and CB12 fragments. Please contact Customer Service for more information.

Anti-Collagen Antibody Assay Kits

Complete ELISA kits to measure serum antibody levels to various species and types of collagen are available. Generally, it is difficult or impossible to determine antibodies in human sera by ELISA and RIA using BSA and BSA-Tween 20 as a blocking agent, because of extremely high non-specific reactions caused by the hydrophobic binding of immunoglobulins in sample specimens onto plastic surfaces. These ELISA kits have been developed to minimize non-specific reactions caused by serum components. In human antibody assay kits, antigen-uncoated wells are included for determining the background values of individual samples. A standard mouse and rat kit with one species of collagen will measure 39 samples, and the human kit measures 38 samples. Each kit contains a reference standard and reagents to perform two partial assays. Customized kits to measure antibody levels to various species of type I and type II collagen can be ordered.

Mouse Anti-Type II Collagen IgG Assay Kit

Catalog #	Type II Collagen Coated Wells
2031	Chick
2032	Bovine
2033	Porcine
2035	Human
2036	Mouse

Mouse Anti-Type I Collagen IgG Assay Kit

Catalog #	Type I Collagen Coated Wells
1011	Chick
1012	Bovine
1013	Porcine
1015	Human
1016	Mouse

Rat Anti-Type II Collagen IgG Assay Kit

Catalog #	Type II Collagen Coated Wells
2041	Chick
2042	Bovine
2043	Porcine
2044	Rat
2045	Human

Rat Anti-Type I Collagen IgG Assay Kit

Catalog #	Type I Collagen Coated Wells
1021	Chick
1022	Bovine
1023	Porcine
1024	Rat
1025	Human

Individual kit components may be available upon request. Please contact Customer Service for more information.

Anti-Collagen Antibody Assay Kits

Human/Monkey Anti-Type II Collagen IgG Assay Kit

Catalog #	Type II Collagen Coated Wells
2051	Chick
2052	Bovine
2053	Porcine
2055	Human

Human/Monkey Anti-Type I Collagen IgG Assay Kit

Catalog #	Type I Collagen Coated Wells
1031	Chick
1032	Bovine
1033	Porcine
1035	Human

Human/Monkey Anti-Type II Collagen IgA Assay Kit

Catalog #	Type II Collagen Coated Wells
2061	Chick
2062	Bovine
2063	Porcine
2065	Human

Human/Monkey Anti-Type I Collagen IgA Assay Kit

Catalog #	Type I Collagen Coated Wells	Price
1041	Chick	
1042	Bovine	
1043	Porcine	
1045	Human	

Individual kit components may be available upon request. Please contact Customer Service for more information.



Mouse Anti-Collagen Antibody Subtype Assay Kits

Autoantibodies to type II collagen play a primary role in inducing autoimmune-mediated inflammatory arthritis in the collagen-induced arthritis (CIA) model. However, autoantibodies are not always capable of inducing arthritis due to their inability to activate complement, the first critical step in the activation of inflammatory cascades. In mice, complement fixable IgG2a and IgG2b subtype autoantibody levels are pertinent in the induction of arthritis, as well as their epitope specificity.

For example, mice immunized with type II collagen emulsified with Incomplete Freund's Adjuvant do not develop arthritis due to low IgG2a antibody levels. Therefore, to effectively induce CIA in mice, it is necessary to use complete Freund's Adjuvant to increase IgG2a subtype antibody production. In order to adequately study the pathological roles of antibodies to type II collagen, it is important to determine individual IgG subtype autoantibody levels to mouse type II collagen, as well as the antibody levels to the heterologous type II collagen used for immunization.

Chondrex provides IgG subtype (IgG1, IgG2a, IgG2b, and IgG3) anti-collagen antibody assay kits for further detailed analysis of antibodies in the mouse CIA model. These kits are designed to assay individual IgG subtype antibodies to type II collagen in mouse sera using monoclonal IgG1, IgG2a, IgG2b, and IgG3 antibodies to type II collagen as individual standards. All necessary reagents, buffers, and ELISA plate to measure 39 samples are included in the kit.

Mouse Anti-Type II Collagen IgG Subtype Assay Kit

Catalog #	Subtype	Type II Collagen Coated Wells
20311	IgG1	Chick
20312	IgG2a	Chick
20313	IgG2b	Chick
20321	IgG1	Bovine
20322	IgG2a	Bovine
20323	IgG2b	Bovine
20331	IgG1	Porcine
20332	IgG2a	Porcine
20333	IgG2b	Porcine
20351	IgG1	Human
20352	IgG2a	Human
20353	IgG2b	Human
20361	IgG1	Mouse
20362	IgG2a	Mouse
20363	IgG2b	Mouse

Mouse anti-type I collagen IgG subtype assay kits as well as IgG3 subtype assay kits are available. Individual kit components may be available upon request. Please contact Customer Service for more information.

Collagen Detection Kits

Human Type I Collagen Detection Kit

Type I collagen is a fibrillar collagen consisting of two identical $\alpha 1(I)$ chains and one $\alpha 2(I)$ chain. It is the most abundant collagen type and is found in most connective tissues such as skin, bone, tendon, ligament, and heart. Many tissues, except bone which consists of type I collagen, contain heterotypic fibrils with two or more distinct collagen types in coexistence. In general, type I collagen is dominant and type III collagen is a minor component.

The Human Type I Collagen Detection Kit is designed to quantify the amount of native type I collagen from human cell and/or tissue culture or from tissues by ELISA. All necessary reagents, buffers, and ELISA plate to measure 39 samples are included in the kit. The sensitivity of this assay is approximately 80 - 5000 ng/mL.

Type II Collagen Detection Kit

Type II collagen is unique among the collagen family, and its tissue distribution is limited to avascular tissues such as cartilage and the vitreous body of the eyes. Since type II collagen can induce arthritis in experimental animals, autoimmunity to type II collagen is suspected in the pathogenesis of certain autoimmune diseases in humans such as rheumatoid arthritis, eye diseases associated with rheumatoid arthritis, and relapsing polychondritis, which affects specific tissues containing type II collagen.

The Type II Collagen Detection Kit is designed to quantify the amount of native type II collagen from various species (such as human, monkey, porcine, bovine, rat, mouse, rabbit, equine, and chick) in cell and/or tissue culture or from tissues by ELISA. This research kit is recommended for studying type II collagen metabolism in both in vivo and in vitro systems. Each kit contains a standard type II collagen and all necessary reagents, buffers, and ELISA plate to measure 39 samples. The sensitivity of this assay is approximately 3.125 - 200 ng/mL.

Catalog #	Description
6008	Human Type I Collagen Detection Kit
6009	Type II Collagen Detection Kit

Note - Additional supplies such as guanidine hydrochloride, pepsin, and elastase are required for processing sample specimens such as cultured cells and tissues from animals.

Semi-Quantitative Collagen Assay Kit (Sirius Red/Fast Green Staining Kit)

This Semi-Quantitative Collagen Assay Kit provides a simple quantitative micro-assay tool for determining the amounts of collagen and non-collagenous proteins in cultured cell layers and tissue sections by differential staining with two dyes, Sirius Red and Fast Green. Sirius Red binds specifically to collagen, whereas Fast Green stains non-collagen proteins. This method has been applied to the measurement of collagen contents in various tissues. These dyes can be easily extracted from stained tissues, and the amounts (μg) of collagen and non-collagenous proteins in each section can be calculated based on OD 540 (Sirius Red) and OD 605 (Fast Green). The assay sensitivity is high enough to determine collagen and non-collagenous proteins in 10 - 20 μm tissue sections prepared for general histological studies. The assay sensitivity for collagen is more than 3 $\mu\text{g}/\text{section}$, and non-collagenous protein is more than 50 $\mu\text{g}/\text{section}$.

Catalog #	Description
9046	Semi-Quantitative Collagen Assay Kit

Individual kit components may be available upon request for all collagen detection kits. Please contact Customer Service for more information.

Collagen Immunostaining Kits and Reagents

Catalog #	Description
9001	Type II Collagen Staining Kit
9012	Type IX Collagen Staining Kit
9044	Human Type I Collagen Staining Kit

The four clone monoclonal antibody cocktail to type II collagen recognizes the conserved epitopes of various species of type II collagen such as human, monkey, porcine, bovine, rat, mouse, rabbit, equine, and chick, and cross-reacts to all of these species of type II collagen by more than 95%. Thus, this cocktail can be used for immunoblotting and immunostaining of tissue sections from these species.

Note: Since $\alpha 3$ (XI) chain of type XI collagen is identical to $\alpha 1$ (II) chain of type II collagen, these monoclonal antibodies cross-react to type XI collagen, but none of them cross-react to type I, type III, and type IX collagen.

The monoclonal antibody cocktail to type IX collagen recognizes the conserved epitopes on the low molecular weight fragment of human, bovine, rat, and chick type IX collagen. Thus, this cocktail can be used for immunoblotting and immunostaining of type IX collagen in tissue sections from human, bovine, rat, and chick.

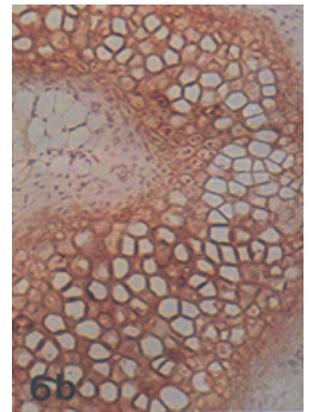
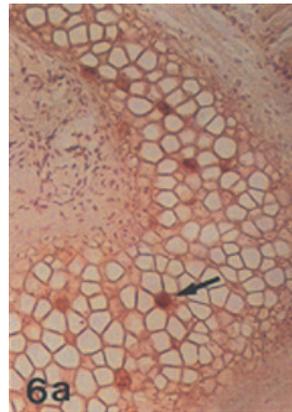
Note: None of these anti-type IX monoclonal antibodies cross-react to type I, type II, and type XI collagen. The cross-reactivity of type IX in other species (except human, bovine, rat, and chick) has not been studied.

Reagents Provided

1. Biotinylated monoclonal antibody to type II collagen or type IX collagen, 100 μ L x 250 μ g/mL
2. Streptavidin peroxidase, 50 μ L x 2
3. Blocking buffer, 15 mL
4. Antibody dilution buffer, 20 mL
5. Streptavidin peroxidase dilution buffer, 20 mL

Reagents Not Provided

1. Tissue fixative
2. Phosphate buffered saline (PBS)
3. 2% bovine testicular hyaluronidase
4. 1% hydrogen peroxide
5. Insoluble chromagen (such as DAB)
6. Mounting media



X.J. Ye, et. al. J. Histochem. Cytochem. 39: 265-271, 1991.

- (6a) Rat external ear (auricle) cartilage stained with a cocktail of monoclonal antibodies to type IX collagen.
- (6b) Rat external ear (auricle) cartilage stained with a cocktail of monoclonal antibodies to type II collagen.

Anti-Collagen Antibodies

Catalog #	Description
7025	Rat Monoclonal Anti-Human Type I Collagen Antibody, 100 μ g
7026	Rat Monoclonal Anti-Human Type I Collagen Antibody, Biotinylated, 100 μ g
7005	Mouse Monoclonal Anti-Type II Collagen Antibody, 100 μ g
7006	Mouse Monoclonal Anti-Type II Collagen Antibody, Biotinylated, 100 μ g
7018	Mouse Monoclonal Anti-Type IX Collagen Antibody, 100 μ g
7019	Mouse Monoclonal Anti-Type IX Collagen Antibody, Biotinylated, 100 μ g

Collagenase Assay Kits and Reagents

Rapid Collagenase Assay Kit

This kit contains all the necessary reagents to assay for collagenase activity including recombinant human neutrophil procollagenase (MMP-8) as a reference and two types of collagenase activators. Unlike antibody-based ELISA kits that only detect the presence of enzyme proteins, this kit is designed to assay for mammalian collagenase activity. The assay uses FITC-labeled soluble bovine type I or type II collagen as a substrate and only requires two hours. The enzyme reaction is ten times faster than substrate gel analysis and much more sensitive than assays using radiolabeled collagen. Each kit includes labeled collagen to perform 100 assays.

The rapid assay method consists of four steps:

1. Activation of latent collagenase in test samples.
2. Reaction with substrate for 10 - 60 minutes.
3. Denaturation and extraction of degraded products.
4. Measurement of fluorescence intensity in the extract.

Individual kit components may be available upon request. Please contact Customer Service for more information.

FITC-Labeled Collagen

FITC-labeled bovine type I and type II collagen are excellent substrates for examining collagenase activity. These labeled substrates are highly purified and telo-peptide free. To minimize background values in collagenase assays, FITC-labeled collagen has been further purified by ion-exchange chromatography to remove free FITC.

FITC-labeled collagen can also be used as a substrate for cell culture. Collagen degradation products in the culture supernatants can be directly determined by measuring the fluorescence at 520 nm (emission)/490 nm (excitation).

Catalog #	Description
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3001	Collagenase Assay Kit (Substrate: FITC-Bovine Type I Collagen, Reference: rMMP-8)
3002	Collagenase Assay Kit (Substrate: FITC-Bovine Type II Collagen, Reference: rMMP-8)
3003	MMP-13 Inhibitor Assay Kit (Substrate: Fluorogenic-Synthetic Peptide, Reference: Truncated rMMP-13)
4001	FITC-Bovine Type I Collagen, 10 mL x 1 mg/mL
4002	FITC-Bovine Type II Collagen, 10 mL x 1 mg/mL
5001	Recombinant Human MMP-8 (collagenase 2), 10 units
5002	Recombinant Truncated Human MMP-13 (collagenase 3), 10 µg

MMP-8 (Collagenase 2)

Recombinant latent human neutrophil procollagenase (MMP-8) can be used for various purposes such as a reference standard for assaying collagenase activity. The most critical process in assaying for collagenase is the activation of inactive collagenase. Since the activation method of collagenase depends on the sources and types of enzyme and selection of activators, it is important to have an inactive latent reference collagenase. Furthermore, activated MMP-8 can be used for testing inhibitors.

MMP-13 (Collagenase 3)

Recombinant human MMP-13 (truncated form) is available for studying inhibitors using a fluorogenic synthetic peptide as a substrate. However, this truncated enzyme is not capable of digesting native collagen. MMP-13 (collagenase 3) is a newly discovered matrix metalloproteinase in various tissues such as malignant tumors, osteoarthritic cartilage, rheumatoid synovium, and wounds. MMP-13 production in chondrocytes and synoviocytes is upregulated by stimulation with inflammatory mediators such as IL-1, TNF, and retinoic acid. MMP-13 has been shown to degrade type I and type II collagen although the degradation of type II occurs approximately 10 times faster than that of type I collagen. Although the typical 3/4 and 1/4 fragments are produced as with MMP-1 (collagenase 1), MMP-13 generates a second cleavage of type II collagen. MMP-13 also digests aggrecan, the major proteoglycan of cartilage.

Dendritic Cells Producing Collagenase



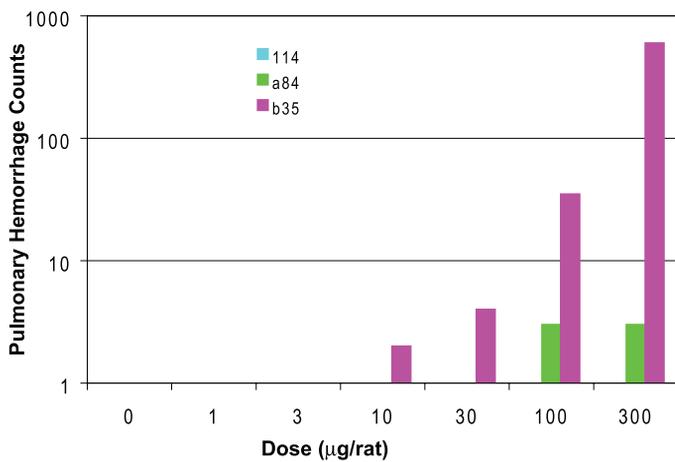
Rat Nephritogenic Monoclonal Antibodies

Chondrex provides three rat monoclonal antibodies against rat glomerular basement membrane, which induce nephritis in certain strains of rats. This nephritis is used as a model for Goodpasture Syndrome, severe renal disease often associated with pulmonary hemorrhage, which is mediated by autoantibodies against glomerular basement membrane. These monoclonal antibodies recognize non-collagenous domain 1 (NC1) of $\alpha 4$ chain of type IV collagen and deposit along the glomerular basement membrane. Individual monoclonal antibodies induce mild to severe nephritis in rats within 1 - 2 days by a single IP or IV injection.

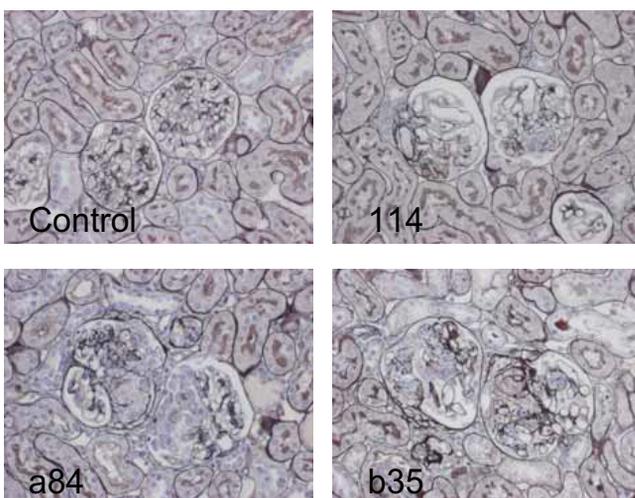
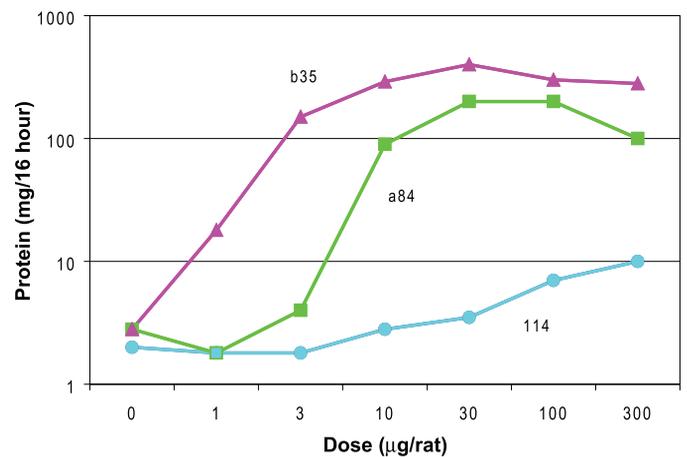
- 1) Monoclonal antibody, b35 (IgG2b), induces severe nephritis associated with hematuria and pulmonary hemorrhage.
- 2) Monoclonal antibody, a84 (IgG2a), induces severe nephritis associated with hematuria.
- 3) Monoclonal antibody, 114 (IgG1), induces mild nephritis at the same dose of b35 and a84.

Catalog #	Description	Size
70201	Nephritogenic Monoclonal Antibody 114	1 mL x 1 mg/mL
70205	Nephritogenic Monoclonal Antibody 114	5 mL x 1 mg/mL
70211	Nephritogenic Monoclonal Antibody a84	1 mL x 1 mg/mL
70215	Nephritogenic Monoclonal Antibody a84	5 mL x 1 mg/mL
70221	Nephritogenic Monoclonal Antibody b35	1 mL x 1 mg/mL
70225	Nephritogenic Monoclonal Antibody b35	5 mL x 1 mg/mL

Dose Effect of mAb on Pulmonary Hemorrhage



Dose Effect of mAb on Proteinuria



Severe histological changes can be observed in the kidneys after injection of nephritogenic monoclonal antibodies such as enlarged glomeruli with severe endocapillary hypercellularity, and extracapillary changes such as capsular adhesion and crescent formation.

Rat Urinary Protein Assay Kit

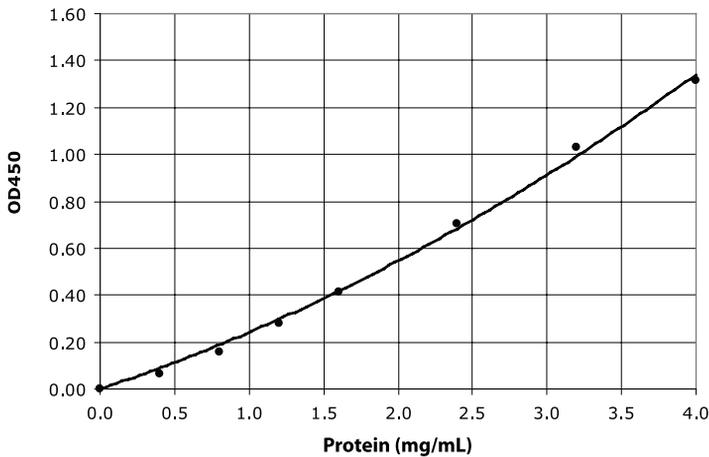
Turbidity assay method has been widely used to determine urinary protein levels in human specimens, because it is accurate, easy, and economical. However, urine volume collected from rats varies from 0.1 mL to 20 mL during a 16 hour collection, thus the volume is occasionally not enough for assaying proteins by current methods. In addition, turbidity (OD 450 nm) readings of individual test tubes using a spectrophotometer may be cumbersome. Chondrex's Rat Urinary Protein Assay Kit, using a turbidity method in 96-well flat bottom micro-titer plates, offers a solution for assaying a large number of rat urine samples and for calculating results automatically.

Protein concentrations in urine samples can be determined by both turbidity and Bradford assay methods. However, turbidity assay method using 3% sulfosalicylic acid dihydrate is more convenient than the Bradford assay method for assaying urine protein concentration in a large number of samples, because of the wide range of dose response curve and the turbidity stability for hours. Importantly, regardless of which assay method is used, bovine serum albumin (BSA) cannot be used as a standard. For example, the dose response curve of BSA significantly differs from the dose response curve of serum proteins using turbidity assay, and the OD value of globulins by Bradford assay is only 70% of the value of BSA. Therefore, a standard protein solution prepared from normal rat serum is ideal for assaying urinary protein levels instead of using BSA regardless of the assay method.

Catalog #	Description	Size
9040	Rat Urinary Protein Assay Kit	2 x 96-well plates
90401	Rat Urinary Protein Assay Standard	1.5 mL x 4 mg/mL

Standard Curve of Rat Urinary Protein Assay

$$y = 0.0312x^2 + 0.2104x$$



Allergenic IgE Monoclonal Antibodies

IgE Assay Kit

Anti-OVA Assay Kits

Arthrogen-CIA® Arthritogenic Monoclonal Antibodies

Collagen (Type I, II, IX, and XI) for CIA and Others

Anti-Collagen Antibody Assay Kits

Anti-Collagen Antibody Subtype Assay Kits

Collagen Detection Kits

Collagen Immunostaining Kits and Reagents

Anti-Collagen Antibodies

Collagenase Assay Kits and Reagents

Rat Nephritogenic Monoclonal Antibodies

Rat Urinary Protein Assay Kit

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