

Enzyme Substrates

Vector Laboratories offers many enzyme substrate kits for use with peroxidase, alkaline phosphatase, and glucose oxidase detection systems.

All Vector Laboratories substrate kit reagents are supplied in convenient dropper bottles promoting ease of handling of chromogens and eliminating wait times for mixing and dissolving powders or tablets. Optimal diluents are also included with all ImmPACT™ substrates.

Choose a substrate that matches the enzyme in your detection system. Choosing the optimal substrate for your application will depend on several considerations:

Sensitivity. Substrates provide varying degrees of sensitivities (see sensitivity comparison chart). For example, ImmPACT™ substrates are about 2-4 times more sensitive than the

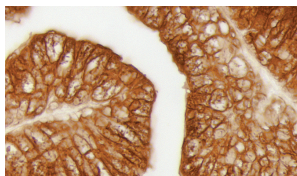
respective original substrate formulations, and the alkaline phosphatase substrate BCIP/NBT can achieve increased sensitivities with longer incubation times.

Color. Color contrast is essential in multiple antigen labeling applications, in pigmented tissues such as melanomas, and in counterstained tissues. Color choices also depend on personal preference.

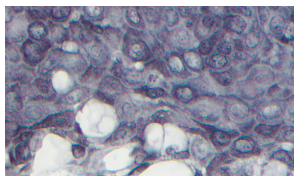
Visualization. Viewing options include brightfield, fluorescence, darkfield, electron microscopy, and spectral imaging.

Heat resistance. For IHC/ISH double labeling applications, the heat resistant substrate is applied first in IHC, followed by ISH detection that includes a heat denaturation step.

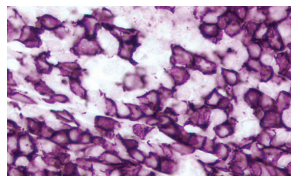
Peroxidase Substrates



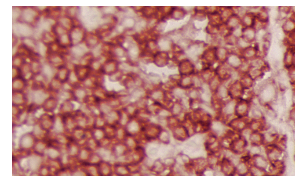
Prostate: Prostate Specific Antigen (m), ImmPRESS™ Reagent (HRP), ImmPACT™ DAB (brown).



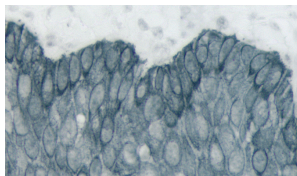
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), Vector® DAB-Ni (gray-black).



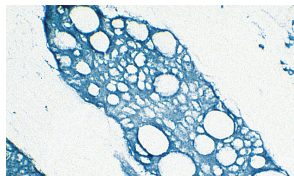
Tonsil: CD20 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ VIP (purple).



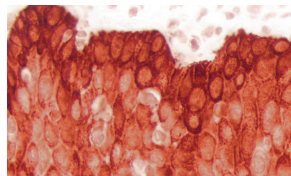
Tonsil: LCA (m), ImmPRESS™ Reagent (HRP), ImmPACT™ AMEC Red (red).



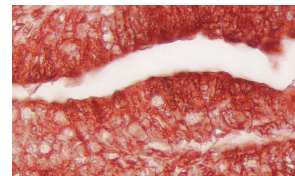
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ SG (blue-gray).



Tumor: Cytokeratin (s), VECTASTAIN® Elite® ABC Kit, TMB (blue).

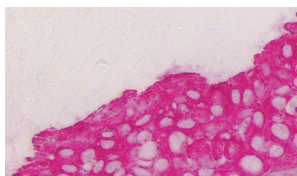


Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ NovaRED™ (red).

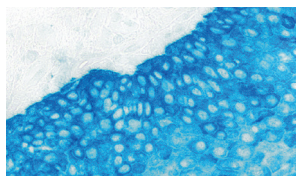


Prostate: Prostate Specific Antigen (m), ImmPRESS™ Reagent (HRP), ImmPACT™ AEC (red).

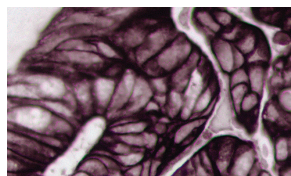
Alkaline Phosphatase Substrates



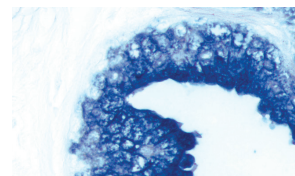
Tonsil: Cytokeratin AE1/AE3 (m), Vector® ImmPRESS™-AP Reagent, ImmPACT™ Vector® Red (magenta).



Tonsil: Cytokeratin AE1/AE3 (m), Vector® ImmPRESS™-AP Reagent, Vector® Blue (blue).



Colon Carcinoma: Pan-Cytokeratin (m), VECTASTAIN® ABC-AP Kit, Vector® Black (brown-black).

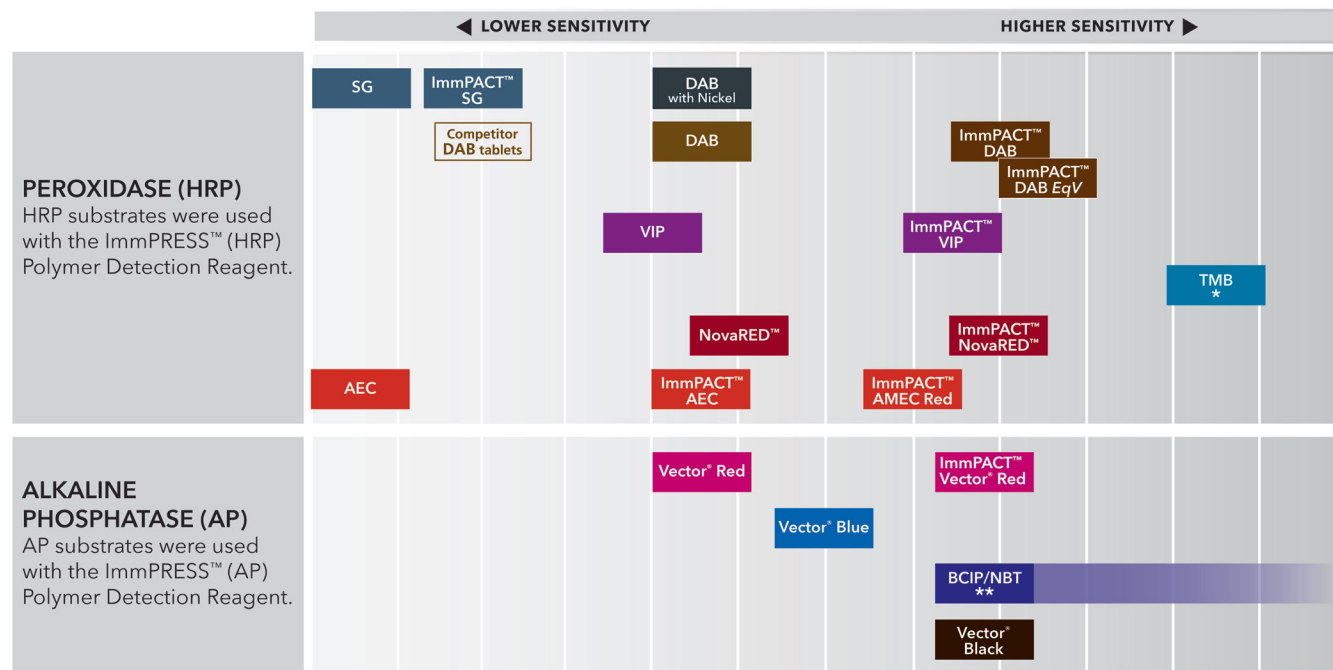


Prostate: Prostate Specific Antigen (m), VECTASTAIN® ABC-AP Kit, BCIP/NBT (indigo).

Enzyme Substrate Properties

Substrate	Color	Catalog Number	Microscopy					Mounting	Contrast in Pigmented Tissue	Multiple Labeling	Heat Resistant
			Bright-field	Darkfield	Electron	Fluorescence	Spectral Imaging				
Peroxidase											
DAB	Brown	SK-4100	●	●	●		●	Non-aqueous or Aqueous		●	●
DAB +Ni	Gray-Black	SK-4100	●	●	●		●	Non-aqueous		●	
ImmPACT™ DAB	Brown	SK-4105	●	●	●		●	Non-aqueous or Aqueous		●	●
Vector® VIP	Purple	SK-4600	●	●	●		●	Non-aqueous	●	●	
ImmPACT™ VIP	Purple	SK-4605	●	●	●		●	Non-aqueous	●	●	
Vector® SG	Blue-Gray	SK-4700	●	●	●		●	Non-aqueous or Aqueous		●	
ImmPACT™ SG	Blue-Gray	SK-4705	●	●	●		●	Non-aqueous or Aqueous		●	
Vector® NovaRED™	Red	SK-4800	●	●	●		●	Non-aqueous	●	●	
ImmPACT™ NovaRED™	Red	SK-4805	●	●	●		●	Non-aqueous	●	●	
AEC	Red	SK-4200	●				●	Aqueous	●	●	
ImmPACT™ AEC	Red	SK-4205	●				●	Aqueous	●	●	
ImmPACT™ AMEC <i>Red</i>	Red	SK-4285	●				●	Aqueous	●	●	
TMB	Blue	SK-4400	●				●	Non-aqueous			
Alkaline Phosphatase											
Vector® Red	Magenta	SK-5100	●				●	Non-aqueous or Aqueous	●	●	●
ImmPACT™ Vector® Red	Magenta	SK-5105	●				●	Non-aqueous or Aqueous	●	●	●
Vector® Blue	Blue	SK-5300	●				●	Non-aqueous or Aqueous	●	●	●
Vector® Black	Brown-Black	SK-5200	●					Non-aqueous			
BCIP/NBT	Indigo	SK-5400	●				●	Non-aqueous or Aqueous		●	●
Glucose Oxidase											
GO Kit I (NBT)	Indigo	SK-3100	●					Non-aqueous or Aqueous			

Relative Sensitivity of Substrates in Immunohistochemistry



* Reaction product deposition not discreet and can be variable

** Longer incubation times increase sensitivity

Enzyme Substrate Kits

Enzyme	Substrate	Color	Catalog Number	Unit Size	Working Solution
Peroxidase	DAB	Brown	SK-4100	1 Kit	300 ml
	DAB +Ni	Gray-Black	SK-4100	1 Kit	300 ml
	ImmPACT™ DAB	Brown	SK-4105	120 ml	120 ml
	Vector® VIP	Purple	SK-4600	1 Kit	300 ml
	ImmPACT™ VIP	Purple	SK-4605	120 ml	120 ml
	Vector® SG	Blue-Gray	SK-4700	1 Kit	300 ml
	ImmPACT™ SG	Blue-Gray	SK-4705	120 ml	120 ml
	Vector® NovaRED™	Red	SK-4800	1 Kit	300 ml
	ImmPACT™ NovaRED™	Red	SK-4805	120 ml	120 ml
	AEC	Red	SK-4200	1 Kit	300 ml
	ImmPACT™ AEC	Red	SK-4205	120 ml	120 ml
	ImmPACT™ AMEC <i>Red</i>	Red	SK-4285	120 ml	120 ml
	TMB	Blue	SK-4400	1 Kit	300 ml
Alkaline Phosphatase	Vector® Red	Magenta	SK-5100	1 Kit	200 ml
	ImmPACT™ Vector® Red	Magenta	SK-5105	1 Kit	120 ml
	Vector® Blue	Blue	SK-5300	1 Kit	200 ml
	Vector® Black	Brown-Black	SK-5200	1 Kit	200 ml
	BCIP/NBT	Indigo	SK-5400	1 Kit	200 ml
Glucose Oxidase	GO Kit I (NBT)	Indigo	SK-3100	1 Kit	300 ml
	GO Kit II (TNBT)	Black	SK-3200	1 Kit	300 ml



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Multiple Antigen Labeling

Vector Laboratories is a leader in multiple antigen labeling applications because of our wide range of very sensitive and very low background detection reagents, our proprietary enzyme substrates, and our rigorously tested protocols. These detection systems and unique enzyme substrates offer an extensive choice of color combinations for multiple labeling using either:

- The same enzyme system with different substrates to detect each antigen, *or*
- Different enzyme systems and their substrates to detect each antigen

The order of labeling, use of control sections, and additional blocking steps may be important to obtain optimal results. For a detailed description of these applications, protocols, and additional images, please visit our website or request a free copy of our brochure, "Discovery Through Color: A Guide to Multiple Antigen Labeling".

Enzyme Substrate Combinations

This table shows the substrates that are recommended for multiple antigen labeling and the order in which they are optimally used.

Second Substrate / First Substrate	ImmPACT™ Vector® Red & Vector® Red (magenta) SK-5105 & SK-5100	Vector® Blue (blue) SK-5300	BCIP/NBT (indigo) SK-5400	ImmPACT™ VIP & VIP (purple) SK-4605 & SK-4600	ImmPACT™ DAB (brown) SK-4105 & SK-4100	DAB-Ni (gray-black) SK-4100	ImmPACT™ NovaRED™ & NovaRED™ (red) SK-4805 & SK-4800	ImmPACT™ SG & SG (blue-gray) SK-4705 & SK-4700	ImmPACT™ AEC & AEC (red) SK-4205 & SK-4200	ImmPACT™ AMEC Red (red) SK-4285
ImmPACT™ Vector® Red & Vector® Red (magenta) Cat. No. SK-5105 & SK-5100		–	–	–	+	+	–	+	–	–
Vector® Blue (blue) Cat. No. SK-5300	+		–	+	+	+	+	+	+	+
BCIP/NBT (indigo) Cat. No. SK-5400	+	–		+	+	+	+	+	+	+
ImmPACT™ VIP & VIP (purple) Cat. No. SK-4605 & SK-4600	–	+	–		+	+	–	+	–	–
ImmPACT™ DAB & DAB (brown) Cat. No. SK-4105 & SK-4100	+	+	+	+		–	–	+	+	+
DAB-Ni (gray-black) Cat. No. SK-4100	+	–	–	+	+		+	–	–	–
ImmPACT™ NovaRED™ & NovaRED™ (red) Cat. No. SK-4805 & SK-4800	–	+	+	–	+	+		+	–	–
ImmPACT™ SG & SG (blue-gray) Cat. No. SK-4705 & SK-4700	+	–	–	+	+	–	–		+	+
ImmPACT™ AEC & AEC (red) Cat. No. SK-4205 & SK-4200	–	–	–	–	+	–	–	+		–
ImmPACT™ AMEC Red (red) SK-4285	–	–	–	–	+	–	–	+	–	

Key: Alkaline Phosphatase

Peroxidase

+ Indicates good contrast

– Indicates incompatibility of substrates for various reasons