

# Enzyme Substrates

## A palette of colors for immunohistochemistry

Vector Laboratories offers many enzyme substrate kits for use with peroxidase and alkaline phosphatase 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

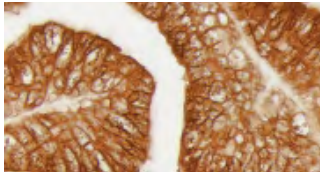
respective original substrate formulations, and the alkaline phosphatase substrate BCIP/NBT can achieve increased sensitivities with longer incubation times.

**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.

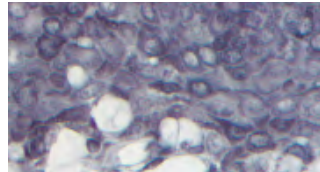
**Visualization.** Viewing options include bright field, fluorescence, dark field, electron microscopy, and spectral imaging.

**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.

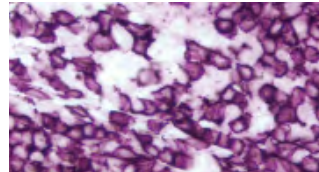
### Peroxidase Substrates



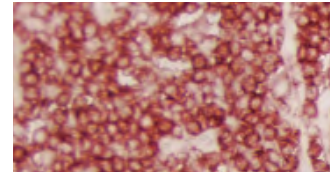
Prostate: Prostate Specific Antigen (m), ImmPRESS™ Reagent (HRP), ImmPACT™ DAB (brown).



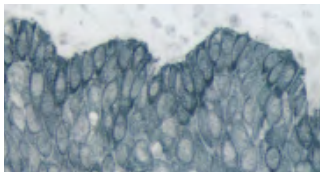
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), Vector® DAB-Ni (gray-black).



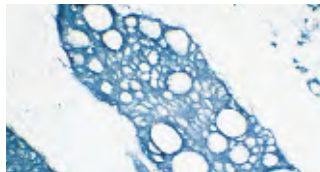
Tonsil: CD20 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ VIP (purple).



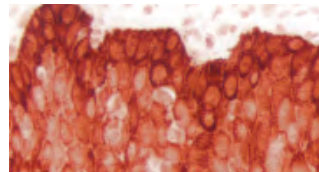
Tonsil: LCA (m), ImmPRESS™ Reagent (HRP), ImmPACT™ AMEC Red (red).



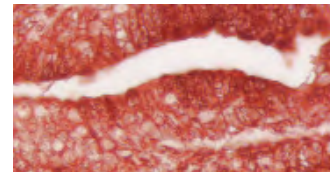
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ SG (blue-gray).



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

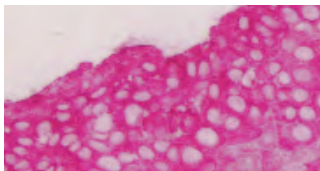


Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ NovaRED™ (red).

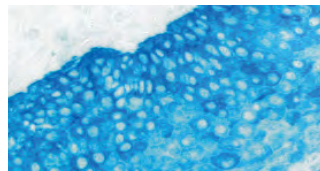


Prostate: Prostate Specific Antigen (m), ImmPRESS™ Reagent (HRP), ImmPACT™ AEC (red).

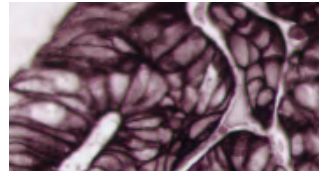
### Alkaline Phosphatase Substrates



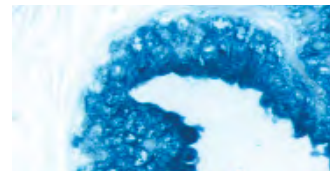
Tonsil: Cytokeratin AE1/AE3 (m), Vector® ImmPRESS™-AP Reagent, ImmPACT™ Vector® Red (magenta).



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).



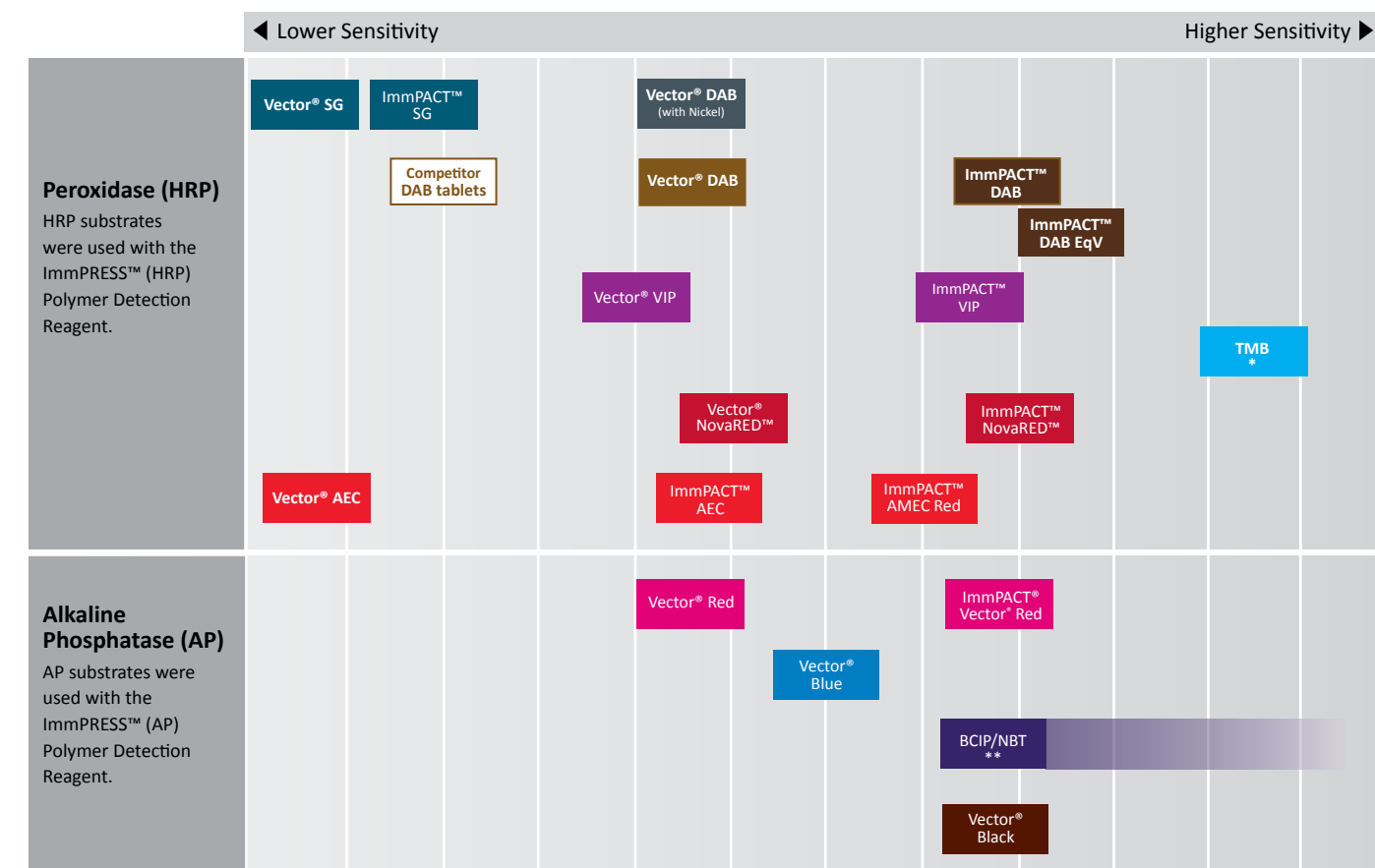
Prostate: Prostate Specific Antigen (m), VECTASTAIN® ABC-AP Kit, BCIP/NBT (indigo).

# Enzyme Substrate Properties

Substrate	Color	Catalog Number	Microscopy					Mounting	Contrast in Pigmented Tissue	Multiple Labeling	Heat Resistant*
			Bright-field	Darkfield	Electron	Fluorescence	Spectral Imaging				
<b>Peroxidase</b>											
Vector® DAB	Brown	<a href="#">SK-4100</a>	•	•	•		•	Non-aqueous or Aqueous		•	•
Vector® DAB +Ni	Gray-Black	<a href="#">SK-4100</a>	•	•	•		•	Non-aqueous		•	
ImmPACT™ DAB	Brown	<a href="#">SK-4105</a>	•	•	•		•	Non-aqueous or Aqueous		•	•
ImmPACT™ DAB EqV	Brown	<a href="#">SK-4103</a>	•	•	•		•	Non-aqueous or Aqueous		•	•
Vector® VIP	Purple	<a href="#">SK-4600</a>	•	•	•		•	Non-aqueous	•	•	
ImmPACT™ VIP	Purple	<a href="#">SK-4605</a>	•	•	•		•	Non-aqueous	•	•	
Vector® SG	Blue-Gray	<a href="#">SK-4700</a>	•	•	•		•	Non-aqueous or Aqueous	•	•	
ImmPACT™ SG	Blue-Gray	<a href="#">SK-4705</a>	•	•	•		•	Non-aqueous or Aqueous	•	•	
Vector® NovaRED™	Red	<a href="#">SK-4800</a>	•	•	•		•	Non-aqueous	•	•	
ImmPACT™ NovaRED™	Red	<a href="#">SK-4805</a>	•	•	•		•	Non-aqueous	•	•	
Vector® AEC	Red	<a href="#">SK-4200</a>	•				•	Aqueous	•	•	
ImmPACT™ AEC	Red	<a href="#">SK-4205</a>	•				•	Aqueous	•	•	
ImmPACT™ AMEC Red	Red	<a href="#">SK-4285</a>	•				•	Aqueous	•	•	
TMB	Blue	<a href="#">SK-4400</a>	•				•	Non-aqueous			
<b>Alkaline Phosphatase</b>											
Vector® Red	Magenta	<a href="#">SK-5100</a>	•			•	•	Non-aqueous or Aqueous	•	•	•
ImmPACT™ Vector® Red	Magenta	<a href="#">SK-5105</a>	•			•	•	Non-aqueous or Aqueous	•	•	•
Vector® Blue	Blue	<a href="#">SK-5300</a>	•			•	•	Non-aqueous or Aqueous	•	•	•
Vector® Black	Brown-Black	<a href="#">SK-5200</a>	•					Non-aqueous			
BCIP/NBT	Indigo	<a href="#">SK-5400</a>	•			•	•	Non-aqueous or Aqueous		•	•

\* Substrates that are designated "heat resistant" were developed on tissue then subjected to heat induced epitope retrieval (HIER) using a pressure cooker technique (stained tissue was pressure cooked for one minute in Antigen Unmasking Solution, returned to room temperature, and rinsed in buffer). Resulting sensitivity after this treatment was found to be equivalent to non-HIER treated tissue.

# 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	<a href="#">SK-4100</a>	1 Kit	300 ml
	DAB +Ni	Gray-Black	<a href="#">SK-4100</a>	1 Kit	300 ml
	ImmPACT™ DAB	Brown	<a href="#">SK-4105</a>	120 ml	120 ml
	Vector® VIP	Purple	<a href="#">SK-4600</a>	1 Kit	300 ml
	ImmPACT™ VIP	Purple	<a href="#">SK-4605</a>	120 ml	120 ml
	Vector® SG	Blue-Gray	<a href="#">SK-4700</a>	1 Kit	300 ml
	ImmPACT™ SG	Blue-Gray	<a href="#">SK-4705</a>	120 ml	120 ml
	Vector® NovaRED™	Red	<a href="#">SK-4800</a>	1 Kit	300 ml
	ImmPACT™ NovaRED™	Red	<a href="#">SK-4805</a>	120 ml	120 ml
	AEC	Red	<a href="#">SK-4200</a>	1 Kit	300 ml
	ImmPACT™ AEC	Red	<a href="#">SK-4205</a>	120 ml	120 ml
	ImmPACT™ AMEC Red	Red	<a href="#">SK-4285</a>	120 ml	120 ml
	TMB	Blue	<a href="#">SK-4400</a>	1 Kit	300 ml
	Alkaline Phosphatase	Vector® Red	Magenta	<a href="#">SK-5100</a>	1 Kit
ImmPACT™ Vector® Red		Magenta	<a href="#">SK-5105</a>	1 Kit	120 ml
Vector® Blue		Blue	<a href="#">SK-5300</a>	1 Kit	200 ml
Vector® Black		Brown-Black	<a href="#">SK-5200</a>	1 Kit	200 ml
BCIP/NBT		Indigo	<a href="#">SK-5400</a>	1 Kit	200 ml

# 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:

1. The same enzyme system with different substrates to detect each antigen.

2. 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

Recommended combinations of substrates and the recommended order in which they should be 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 & Vector® VIP (purple) SK-4605 & SK-4600	ImmPACT™ DAB, ImmPACT™ DAB EqV & Vector® DAB (brown) SK-4105, SK-4103, SK-4100	DAB-Ni (gray-black) SK-4100	ImmPACT™ NovaRED™ & Vector® NovaRED™ (red) SK-4805 & SK-4800	ImmPACT™ SG & Vector® SG (blue-gray) SK-4705 & SK-4700	ImmPACT™ AEC & Vector® 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 & Vector® VIP (purple) Cat. No. SK-4605, SK-4600	—	+	—		+	+	—	+	—	—
ImmPACT™ DAB, ImmPACT™ DAB EqV & Vector® DAB (brown) Cat. No. SK-4105, SK-4103, SK-4100	+	+	+	+		—	—	+	+	+
Vector® DAB-Ni (gray-black) Cat. No. SK-4100	+	—	—	+	+		+	—	—	—
ImmPACT™ NovaRED™ & Vector® NovaRED™ (red) Cat. No. SK-4805, SK-4800	—	+	+	—	+	+		+	—	—
ImmPACT™ SG & Vector® SG (blue-gray) Cat. No. SK-4705, SK-4700	+	—	—	+	+	—	—		+	+
ImmPACT™ AEC & Vector® 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