

# Immunohistochemistry Resource Guide

A Comprehensive Guide to Immunohistochemistry Staining



## Making IHC as easy as ABC

Helping you to reach new visualization frontiers in your research: this is our mission. Since our founding in 1976, a primary driving principle has been to develop and manufacture labeling and detection technologies that make IHC as easy as ABC.

- A. Reliable and reproducible reagents that instill trust and confidence.
- B. Simple and robust product designs that streamline workflows and allow elucidation of complex biological systems.
- C. A knowledge base of over 100 years of combined IHC experience to help you accelerate the pace of discovery.

It's as simple as that.

*Vector Laboratories empowers scientific advances with innovative proteomic and glycomic solutions. Supporting scientific industries worldwide for 45 years and counting, Vector Laboratories is a trusted manufacturing partner with unmatched technical expertise and a culture of service. Customers rely on Vector Laboratories' immunohistochemistry, immunofluorescence, glycobiology, and bioconjugation products and custom manufacturing capabilities to move science forward with impact. Vector Laboratories' market-tested product portfolio provides the critical tools researchers need to precisely visualize and study tissues and cells as well as tackle today's biggest healthcare challenges. The company's products and technologies have been cited in more than 350,000 peer-reviewed publications, and its catalog and custom products are included in laboratory Standard Operating Procedures around the world. Learn more at [vectorlabs.com](https://vectorlabs.com).*

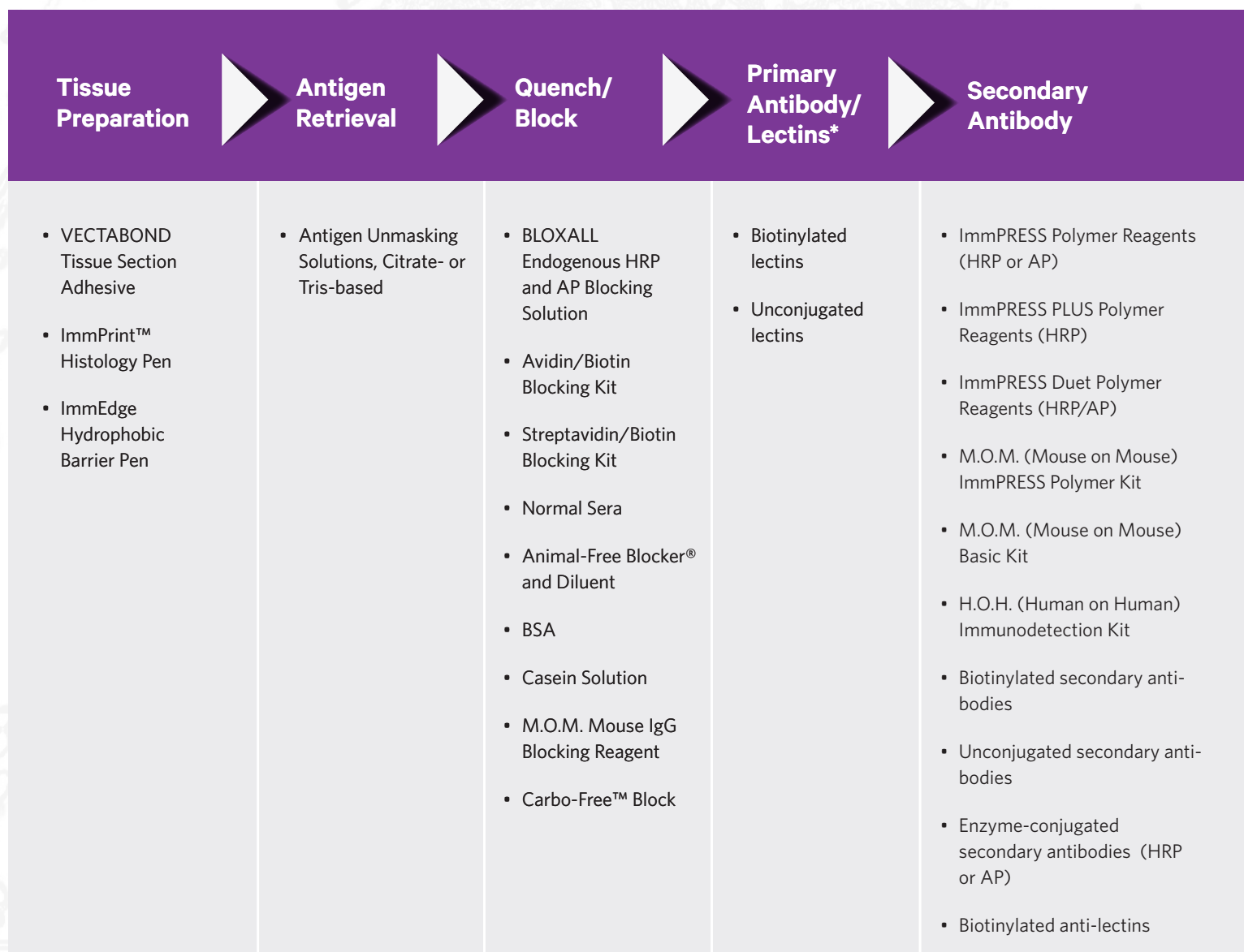
# Table of Contents

2	<b>Immunohistochemistry Introduction</b>
2	Immunohistochemistry Workflow
4	Immunohistochemistry Selection Guide
6	Pioneering in IHC Technology
8	<b>Choosing a Detection System</b>
10	Avidin-Biotin (ABC) Based Detection
	- VECTASTAIN® ABC Systems
	- VECTASTAIN ABC Kits
	- Choosing a VECTASTAIN ABC Kit
	- Customizing your VECTASTAIN ABC Kit
18	Polymer-Based Detection
	- ImmPRESS® One-Step Polymer Systems
	- ImmPRESS One-Step Double Staining Polymer Systems
	- ImmPRESS Two-Step Amplified Polymer Systems
	- Choosing an ImmPRESS Polymer Kit
	- Multiple Antigen Labeling Simplified
24	<b>Species on Species Detection (Mouse)</b>
25	M.O.M.® (Mouse on Mouse) Immunodetection Kits
26	H.O.H.™ (Human on Human) Immunodetection Kits
27	<b>Choosing an Enzyme Substrate</b>
	- Enzyme Substrates
	- Enzyme Substrate Properties
	- Enzyme Substrate Color Examples
31	<b>Multiple Antigen Labeling</b>
	- Enzyme Substrate Combinations
	- Multiple Labeling Examples
34	<b>Counterstaining</b>
	- Counterstain/Substrate Compatibility
36	<b>Blocking Background Signal</b>
	- BLOXALL® Endogenous HRP/AP Blocking Solution
	- Avidin/Biotin and Streptavidin/Biotin Blocking Reagents
	- Normal Sera and Bovine Serum Albumin (BSA)
	- Animal-Free Blocking Solutions
	- General Protein Blocking Reagents
38	<b>Secondary and Tertiary Detection Reagents</b>
	- Biotinylated and Unconjugated Secondary Antibodies
	- Enzyme Conjugated Secondary Antibodies
	- Avidin and Streptavidin Enzyme Conjugates
40	<b>Mounting Media</b>
	- VectaMount® Mounting Media
42	<b>Accessory Reagents</b>
	- VECTABOND® Reagent Tissue Section Adhesive
	- ImmEdge® Hydrophobic Barrier Pen
	- Histology Pens, Control Antibodies, and Antigen Unmasking Solutions

Cover Image: Human kidney tissue demonstrating a glomerulus (a cluster of blood vessels; brown), which is surrounded by various tubules as well as associated smooth muscle cells (red). Together these represent the 'basic filtration unit' of the kidney. Image kindly supplied by Steffen Rickelt, David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology (MIT).

# Immunohistochemistry Workflow

Vector Laboratories is your resource for premium labeling and detection products at each step of your IHC workflow.



\* For more information visit: [vectorlabs.com/lectins](http://vectorlabs.com/lectins)

HRP - Horseradish peroxidase  
AP - Alkaline phosphatase



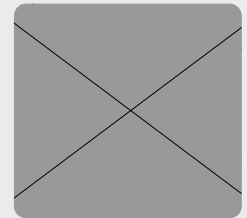


- VECTASTAIN ABC Reagents (HRP)
- VECTASTAIN Elite ABC Reagents (HRP)
- VECTASTAIN ABC-AP Reagents (AP)
- VECTASTAIN Elite ABC PLUS Kit (HRP)
- ImmPRESS Excel Amplified Staining Kits (HRP)
- M.O.M. (Mouse on Mouse) Elite Kit (HRP)
- Enzyme-conjugated avidin/streptavidin (HRP or AP)

- HRP substrates
- AP substrates/Levamisole Solution

- Hematoxylin
- Methyl Green
- Nuclear Fast Red

- VectaMount Express Mounting Medium
- VectaMount Permanent Mounting Medium
- VectaMount AQ Aqueous Mounting Medium



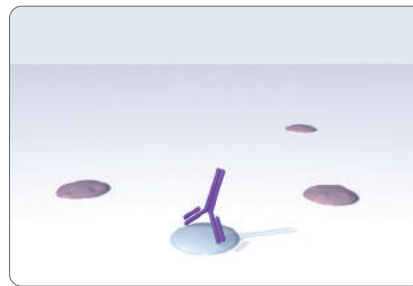
# Immunohistochemistry Selection Guide

Follow the simple steps below to choose the most appropriate labeling and detection solution for your experiment.

1

## Choose Primary Antibody

- Specific for antigen of interest
- Consider tissue species and preparation (fixation)
- Consider antigen retrieval requirements



**VECTABOND Reagent**  
(Tissue Section Adhesive)

SECONDARY DETECTION SYSTEMS

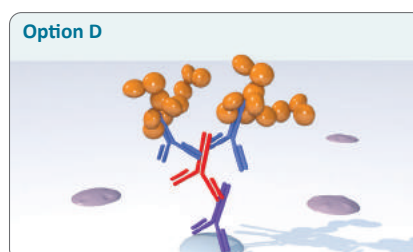
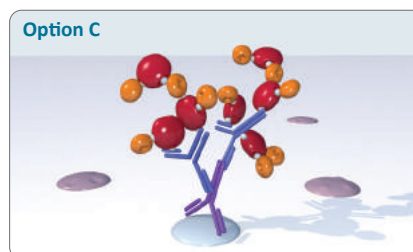
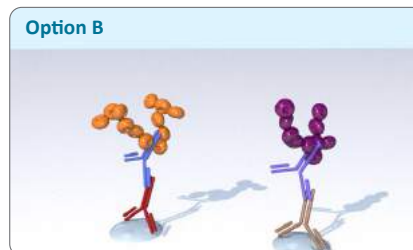
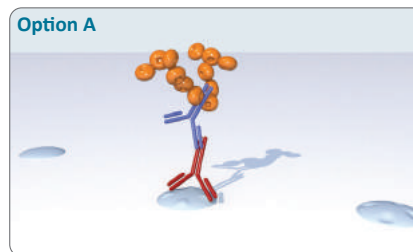
## Blocking Reagents

- Choices determined by the options selected in Steps 1-4
- BLOXALL Endogenous HRP and AP Blocking Solution
- Avidin/Biotin Blocking Kit (if using VECTASTAIN ABC system)
- Normal Sera (from the species of secondary antibody)
- M.O.M. Mouse Ig Blocking Reagent
- Animal-Free Blocker and Diluent
- BSA
- Casein Solution

2

## Choose Secondary Antibody and Tertiary Detection System

- Choose HRP or AP enzyme system
- Consider sensitivity requirements
- Consider species of primary antibody
- Consider tissue species



### One Step

**Convenient. Consistent. Ready-to-use. Non-Biotin based.**

- ImmPRESS Polymer Reagents
- ImmPRESS PLUS Polymer Kits

or



### One Step

**Dual Label - two antigen detection. Convenient. Consistent. Ready-to-use. Non-Biotin based.**

- ImmPRESS Duet Double Staining HRP/AP Polymer Detection Kit

or



### Two Step

**Economical. Biotin-based.**

- Biotinylated secondary antibody + ABC Complex (VECTASTAIN Elite ABC Kits, VECTASTAIN Elite ABC PLUS Kit)

or



### Two Step

**Highest sensitivity. Non-biotin based.**

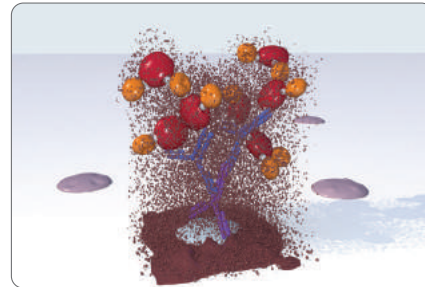
- ImmPRESS Excel Amplified Polymer Staining Systems



3

### Choose Enzyme Substrate

- Color
- Compatibility with other system reagents (counterstains, mounting media and other substrates for multiplexing)



4

### Choose Nuclear Counterstain

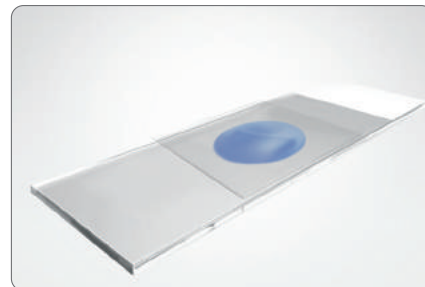
- Blue, green, or red compatibility with substrate, mounting media



5

### Choose Mounting Media

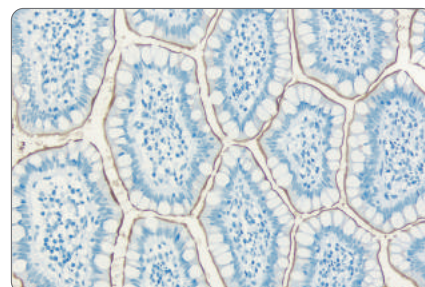
- Aqueous vs. non-aqueous
- Compatibility with substrate(s) and counterstain



6

### Visualize

- Brightfield microscope



Small bowel: CD10 (m), VECTASTAIN Elite ABC Kit, ImmPACT® DAB HRP Substrate (brown). Hematoxylin QS counterstain (blue).

### Legend



Primary antibody



Secondary antibody



Amplifier antibody



Enzyme (HRP or AP)



Biotinylated enzyme (HRP or AP)



Avidin

# Pioneering in IHC/IF Technology

Observation is one of the fundamental steps in the scientific method. However, for centuries the scientific study of tissues was limited to observations of dissections with the unaided eye (gross anatomy).

This all changed in the 17th century when Anton Van Leeuwenhoek fabricated a microscope that allowed observations of tissues at the cellular level, thus establishing the science of histology. While early researchers found it relatively simple to distinguish between the cell boundaries and subcellular compartments in plants, doing so in animal tissue presented a much greater challenge. It wasn't until the late 19th century with the introduction of dyes, such as hematoxylin that Paul Mayer used to successfully stain nuclei, that the subcellular structure of tissues became visible and the science of histochemistry emerged.

The number of available tissue dyes and stains increased during the early 20th century, as did the number of molecular families they identified. However, the ability to identify individual cellular- or tissue-specific proteins remained elusive. This changed in the mid-20th century when Dr. Albert Coons demonstrated that fluorescently labeled antibodies could be used to localize bacteria inside macrophages, thus helping to pioneer the science of immunohistochemistry (IHC). Over the next two decades our understanding of antibodies, antigens and immunology grew rapidly. However, IHC remained largely a specialized research tool used primarily in university settings. Then in the late 1960's, Dr. Stratis Avrameas and Dr. Paul Nakane independently developed methods to covalently couple the enzyme horseradish peroxidase (HRP) to antibodies. HRP in the presence of diaminobenzidine and hydrogen peroxide creates a brown precipitate at the site of the HRP-conjugated antibody. The precipitate can be visualized using an ordinary light microscope. This allowed for the IHC results to be viewed in any lab having a light microscope, with no need for expensive, complicated fluorescence instrumentation.

The use of IHC as a research tool grew dramatically over the next decade. The technique began to be used in clinical settings at large university hospitals. The HRP assay system was further improved in the early 1980's when Dr. Su-Ming Hsu showed that the high affinity of avidin for biotin could be used to increase the stability of the enzyme antibody complex and improve the sensitivity of the assay. Vector

Laboratories was instrumental in the development of the IHC field by commercializing such key technologies. The use of avidin- and biotin-based detection systems dominated the IHC market for the next two decades.

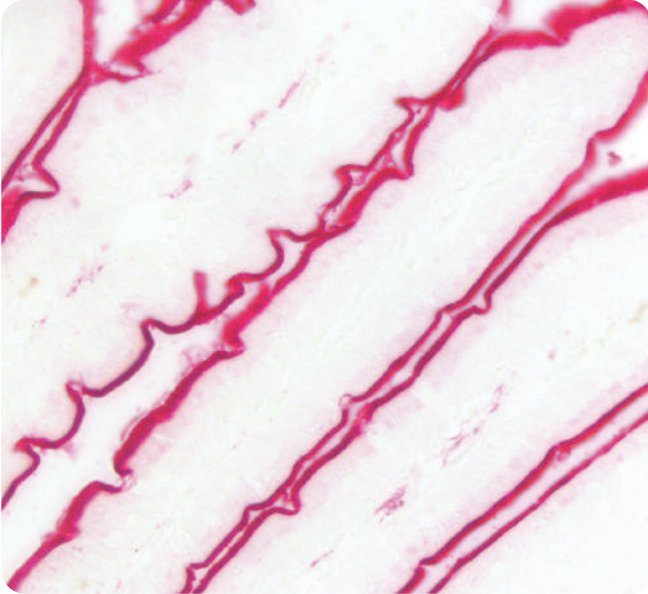
Up to this time, visualization using fluorescence microscopy was challenging due to the rapid photobleaching of fluorophores when exposed to the light of the microscope. This significantly limited the time over which a sample could be observed. In the early 1990's, VECTASHIELD® Antifade Mounting Medium was introduced by Vector Laboratories as the first commercially available mountant for fluorescence. Not only did it have no autofluorescence (in the popular visualization channels), it was also effective in preventing the photobleaching, or fading of the fluorophores. This advancement in microscopy not only made image acquisition and analysis much more convenient, it provided researchers tools to challenge the limits of fluorescence detection.

In the last decade, immunofluorescence applications have been further improved by the adaptation of new super-resolution methods. Super-resolution microscopy allows imaging at a scale smaller than 200 nm. Due to its characteristics and convenience, VECTASHIELD Mounting Medium has been found to be quite suitable for super-resolution imaging methods like stochastic optical reconstruction microscopy (STORM) and structured illumination microscopy (3D-SIM). Olivier, et al., describes VECTASHIELD Mounting Medium as a "simple yet powerful buffer for 3D-STORM".

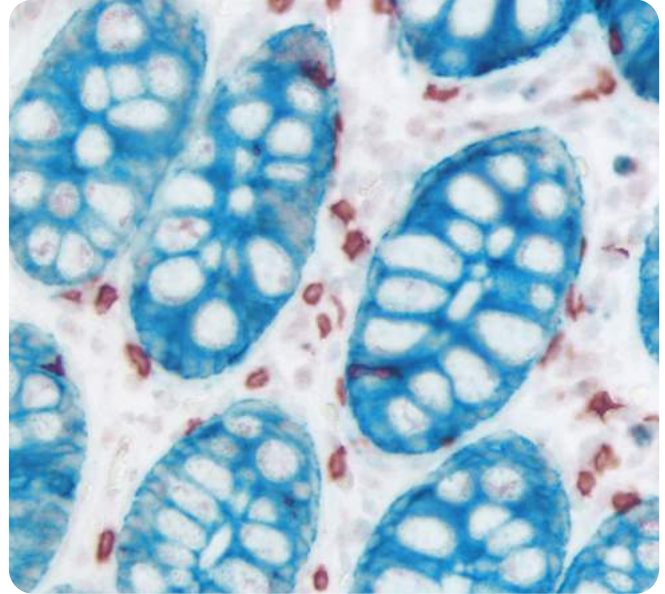
## References

- Coons AH, Creech HJ, and Jones RN "Immunological properties of an antibody containing a fluorescent group" *Proc. Soc. Exp. Biol. Med.* 47, 200-202 (1941)
- Nakane P and Pierce GB Jr "Enzyme-labeled antibodies for the light and electron microscopic localization of tissue antigens" *J. Cell. Biol.* 33, 307-318 (1967)
- Leduc E, Avrameas S, and Bouteille M "Ultrastructural localization of antibody in differentiating plasma cells" *J. Exp. Med.* 127, 109-118. (1968)
- Hsu S-M, Raine L, and Fanger H "Use of Avidin-Biotin-Peroxidase Complex (ABC) in Immunoperoxidase Techniques: A Comparison between ABC and Unlabeled Antibody (PAP) Procedures" *J. Histochem. Cytochem.* 29(4), 577-580 (1981)
- Shi SR, Key ME, and Kalra KL "Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections" *J Histochem Cytochem.* Jun, 39(6), 741-8 (1991)
- Bretschneider S, Eggeling C, and Hell SW "Breaking the Diffraction Barrier in Fluorescence Microscopy by Optical Shelving," *PRL* 98, 218103 (2007)
- Schermelleh L, Carlton PM, Haase S, Shao L, Winoto L, Kner P, Burke B, Cardoso MC, Agard DA, Gustafsson MGL, Leonhardt H, and Sedat JW "Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy," *Science* 320, 1332-1336 (2008)
- Olivier N, Keller D, Rajan VS, Gönczy P, and Manley S "Simple buffers for 3D STORM microscopy," *Biochemical Optics Express* 4, 885-899 (2013)
- Wegel, E, et al. "Imaging cellular structures in super-resolution with SIM, STED and Localisation Microscopy: A practical comparison", *Scientific Reports*, 6, 27290 (2016)
- Childs GV "History of Immunohistochemistry" *Pathobiology of Human Disease* 3775-3796 (2014)

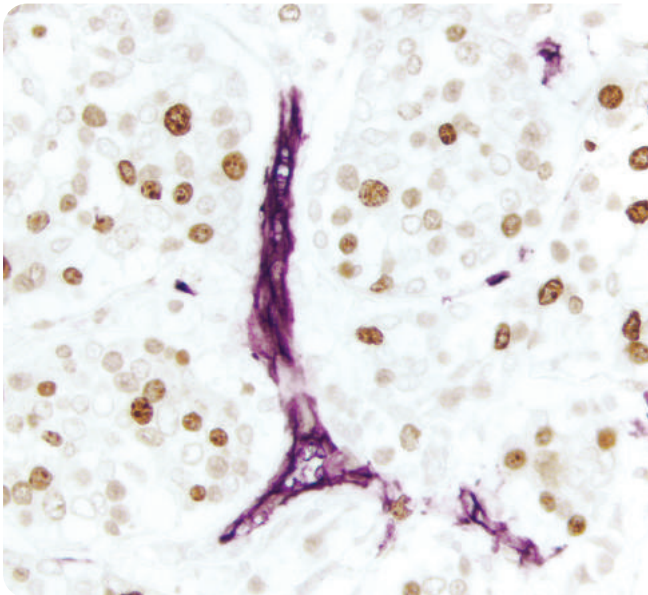




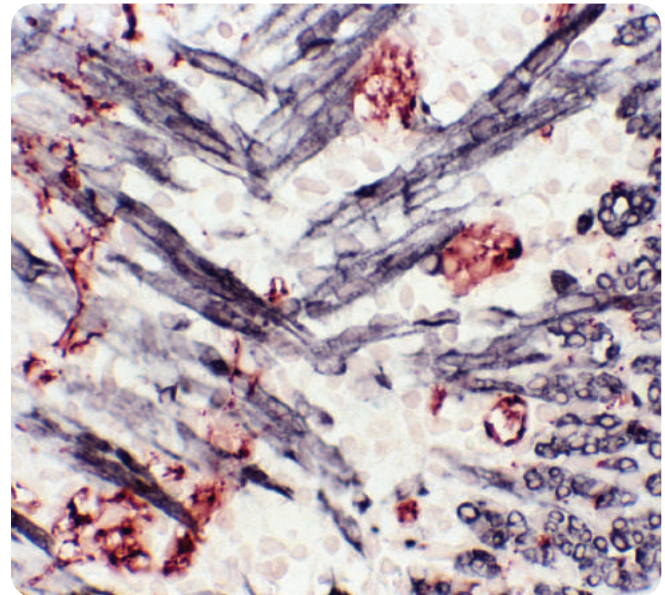
Mouse Colon: Smooth Muscle Actin (m), M.O.M. Basic Immunodetection Kit, VECTASTAIN ABC-AP Kit, Vector® Red AP Substrate (magenta).



Colon: Cytokeratin (AE1/AE3, m), ImmPRESS-AP Anti-Mouse IgG Reagent, Vector Blue AP Substrate (blue); CD3 (rb), ImmPRESS Anti-Rabbit IgG HRP Reagent, ImmPACT AMEC Red HRP Substrate (red).



Breast Carcinoma: • Ki67 (rm), ImmPRESS Reagent (HRP; Universal), Vector DAB (brown) • CD34 (m), ImmPRESS Reagent (HRP; Universal), Vector VIP (purple).



Newborn Mouse Tongue: Synapsin (mouse, M.O.M. Elite HRP Kit, Vector NovaRED HRP Substrate (red); Desmin (m), M.O.M. Elite HRP Kit, DAB-Ni HRP Substrate (black).

A grayscale microscopic image of a tissue section, showing a dense network of cells and fibers. A prominent, dark, circular feature is visible in the upper right quadrant, possibly representing a specific cell or a region of interest. The overall texture is granular and complex.

## Choosing a Detection System

### Immunohistochemistry Overview

Immunohistochemistry (IHC) is a method to detect specific target antigens (proteins) in tissue sections using antibodies. Immunocytochemistry (ICC) uses similar techniques to localize cellular proteins in cell preparations. Both IHC and ICC are powerful tools that provide insights into gene expression, spatial relationships, and biomarker identification in a wide variety of applications. These applications include basic research, assessment of normal and disease states within human and animal tissues, and assessment of plant pathology.

The target antigen, bound by the detection antibody, is visualized using either chromogenic or fluorescence detection. In chromogenic detection, the detection antibody is conjugated to an enzyme. The enzyme, usually horseradish peroxidase or alkaline phosphatase, catalyzes the conversion of its respective chromogen to a colored precipitate at the site of the antigen. This precipitate can be visualized by using brightfield microscopy. Certain chromogens can also be visualized by using electron, darkfield or fluorescence microscopy. In fluorescence detection, the detection antibody is conjugated to a fluorophore which can be visualized using fluorescence microscopy.

For the purposes of this guide IHC will be referenced for both IHC and ICC techniques.



# Comparison of Detection Systems

Choose the appropriate detection system for your experiment based on enzyme, sensitivity, cost, biotin vs. non-biotin formats, flexibility, and time considerations.

Detection System	Enzyme	Sensitivity	Cost/ Assay	Biotin- Free	Micro- polymer	Modular	Mouse Primary on Mouse Tissue	Ready-to-Use (R.T.U.) Format	Typical number of steps
<b>ImmPRESS Kits</b>									
ImmPRESS Excel Amplified HRP Polymer Kits	HRP	*****	****	.	.			.	2
ImmPRESS HRP Polymer Kits	HRP	****	***	.	.			.	1
ImmPRESS HRP PLUS Polymer Kits	HRP	****	***	.	.			.	1
ImmPRESS VR HRP Polymer Kits	HRP	****	***	.	.			.	1
ImmPRESS AP Polymer Kits	AP	****	***	.	.			.	1
ImmPRESS Duet Polymer Detection Kit	HRP/AP	****	****	.	.			.	1
<b>VECTASTAIN Kits</b>									
VECTASTAIN Elite ABC Kits	HRP	****	**			.			2
VECTASTAIN Elite ABC PLUS Kit	HRP	****	**			.			2
R.T.U. VECTASTAIN Elite Kits	HRP	****	**			.		.	2
VECTASTAIN Universal Quick Kits	HRP	****	**			.			2
R.T.U. VECTASTAIN Universal Quick Kits	HRP	****	**			.		.	2
VECTASTAIN ABC-AP Kits	AP	****	.			.			2
Original VECTASTAIN ABC Kits	HRP	***	.			.			2
<b>M.O.M. (Mouse on Mouse) Kits</b>									
M.O.M. (Mouse on Mouse) ImmPRESS Polymer Kit	HRP	***	***	.	.		.		1
M.O.M. (Mouse on Mouse) Kits	HRP	***	***			.	.		2
<b>Additional Options</b>									
Enzyme Conjugated Avidin/Streptavidin	HRP or AP	***	.			.			2
R.T.U. HRP Avidin/Streptavidin	HRP	***	.			.		.	2
Enzyme Conjugated Secondary Antibody	HRP or AP	**	.	.					1

HRP - Horseradish peroxidase

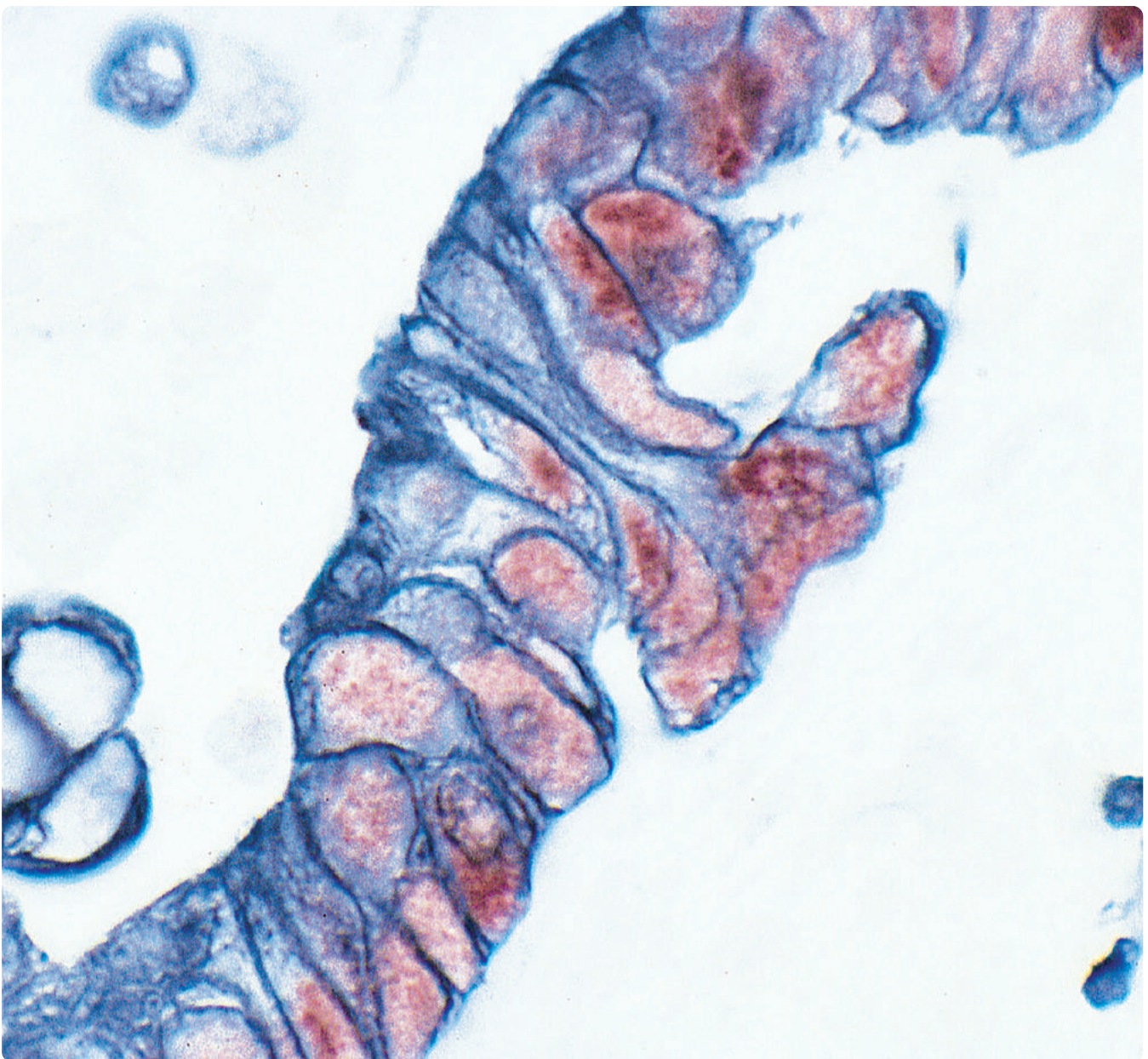
AP - Alkaline phosphatase

VR - Veterinary Reagents

## Avidin-Biotin Complex (ABC)-Based Detection

### Modular and versatile with high sensitivity and low background

ABC-based detection is one of the most widely-used methods for staining. These systems exploit the high affinity exhibited between the protein avidin and the vitamin biotin. Avidin is tetravalent, so each avidin molecule can bind up to four biotinylated conjugates. In ABC systems, avidin and biotinylated enzyme is combined to form large macromolecular complexes containing multiple enzyme molecules. These added complexes bind to any biotinylated target, such as primary or secondary antibodies, nucleic acids, lectins, and macromolecules. When the chromogenic enzyme substrate is applied, it yields a colored precipitate at the site of the reaction. The large multi-enzyme complexes amplify the signal, providing greater sensitivity.



*Tumor: • p53 (m), VECTASTAIN Elite ABC Kit, Vector NovaRED (red) • Cytokeratin (s), VECTASTAIN Elite ABC Kit, Vector SG (blue-gray).*

# VECTASTAIN ABC Systems

VECTASTAIN ABC detection systems are uniquely formulated with our Avidin DH and biotinylated enzyme conjugates to deliver enhanced signal sensitivity with low background. They are compatible with a wide range of target types, applications, and substrates. These reliable and economical VECTASTAIN ABC Systems have come to be a mainstay product in immunohistochemistry laboratories.

## Recommended applications:

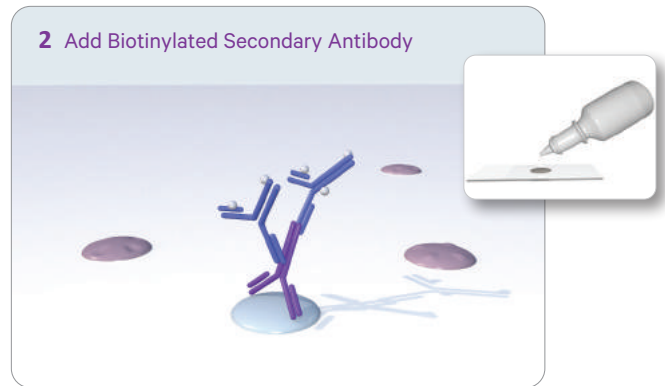
- Tissue and cell staining
- Protein and nucleic acid blotting
- *In situ* hybridization
- ELISAs
- Neuronal tracing

## Using the VECTASTAIN ABC System

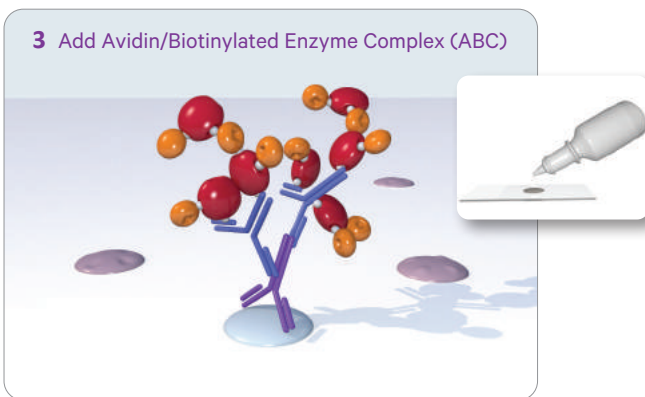
### 1 Add Primary Antibody



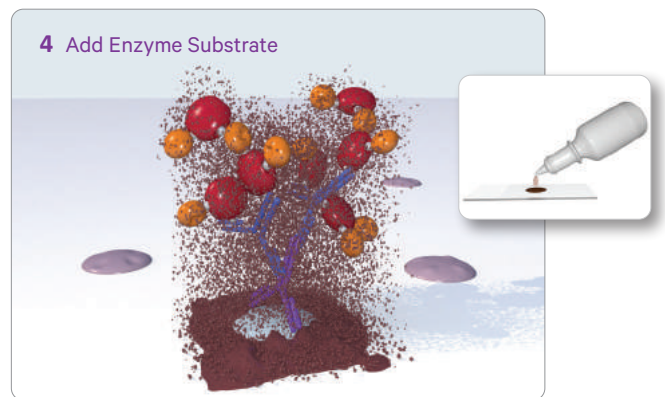
### 2 Add Biotinylated Secondary Antibody



### 3 Add Avidin/Biotinylated Enzyme Complex (ABC)



### 4 Add Enzyme Substrate





## VECTASTAIN ABC Kits

### Peroxidase-Based Kits:

Peroxidase-based detection systems are a preferred choice for many IHC applications that require sharp, distinct localization of the target antigen. VECTASTAIN ABC peroxidase systems are offered in a number of different formats, and when used in combination with our peroxidase substrates, achieve precise specific staining with negligible background interference. (For peroxidase substrates see p. 27-30).

#### VECTASTAIN Elite ABC Kits (Peroxidase)

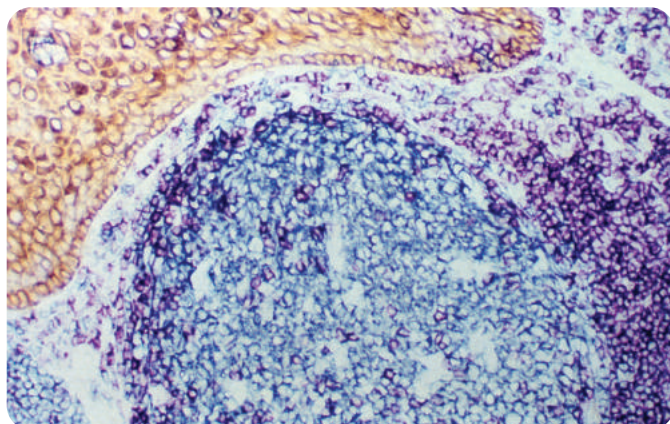
The VECTASTAIN Elite ABC complex is smaller and more uniform than conventional avidin-biotin complexes. They have greater accessibility to biotinylated targets within tissue samples. VECTASTAIN Elite ABC Kits are our most sensitive avidin-biotin based peroxidase systems.

- Highest available sensitivity, low background
- Cost effective: Higher sensitivity means lower cost per slide
- Available without (Standard Kit) or with biotinylated species-specific or universal secondary antibodies
- Available in ready-to-use formats that yield the same high sensitivity and low background as the corresponding conventional VECTASTAIN ABC Kit reagents.

#### Original VECTASTAIN ABC Kit (Peroxidase)

Our original avidin-biotin ABC complex formulation.

- Good sensitivity, low background
- Available with or without biotinylated species-specific secondary antibody
- Economical



Tonsil: • Multi-Cytokeratin (m), VECTASTAIN Elite ABC Kit, Vector DAB (brown) • CD3 (m), VECTASTAIN Elite ABC Kit, Vector VIP (purple) • CD20 (m), VECTASTAIN Elite ABC Kit, Vector SG (blue-gray).

#### VECTASTAIN Elite ABC HRP Kits

- > Standard (Elite ABC Reagent only)
- > Rabbit IgG
- > Mouse IgG
- > Human IgG
- > Rat IgG
- > Goat IgG
- > Sheep IgG
- > Universal
- > Universal PLUS\*
- > R.T.U. VECTASTAIN Elite ABC Reagent
- > R.T.U. VECTASTAIN Elite ABC Kit, Universal

\* The Universal PLUS kit also includes enzyme quench solution and HRP substrate.

#### VECTASTAIN ABC HRP Kits

- > Standard (ABC Reagent only)
- > Rabbit IgG
- > Mouse IgG
- > Mouse IgM
- > Rat IgG
- > Goat IgG
- > Guinea Pig IgG

*Note: Species-specific kits are selected corresponding to the species in which the primary antibody is raised.*

## VECTASTAIN Elite ABC Universal PLUS Kit (Peroxidase)

This addition to our portfolio includes essential IHC workflow components in prediluted, ready-to-use formats. This kit will reduce optimization times for assay development and avoid using mismatched reagents from different vendors.

- Reduce time-consuming optimization requirements
- Convenient ready-to-use solutions
- Streamline the workflow with matched reagents
- Increase reliability and reproducibility between assays

## VECTASTAIN Universal Quick Kits (Peroxidase)

With VECTASTAIN Universal Quick Kits, you can quickly detect primary antibodies made in mouse, rabbit, or goat. These kits rely on a proprietary preformed peroxidase-streptavidin complex to achieve outstanding sensitivity with short incubation times.

- Rapid protocol: Staining in less than 20 minutes following primary antibody incubation. Working solutions can be used immediately after dilution.
- High sensitivity, low background
- Biotinylated Universal Pan-Specific secondary antibody recognizes mouse, rabbit, and goat primary antibodies, as well as those from related species such as rat, bovine, and sheep. (Do not use to stain rat, mouse or other rodent, rabbit, goat, bovine, or sheep tissue due to potential reactivity with endogenous IgG.)
- Available in concentrate or ready-to-use format

## Alkaline Phosphatase-Based Kits:

Alkaline phosphatase-based detection kits are a good alternative to using peroxidase-based reagents in specimens that exhibit problematic levels of endogenous peroxidase activity. Alkaline phosphatase systems also provide additional substrate colors that can be used in single label assays or used in combination with peroxidase substrates for multiplex experiments. The VECTASTAIN ABC-AP kits are offered as species-specific, universal (anti-mouse/rabbit IgG) or standard formats.

### VECTASTAIN Quick HRP Kits

- R.T.U. VECTASTAIN Quick Kit, Universal
- VECTASTAIN Quick Kit, Universal

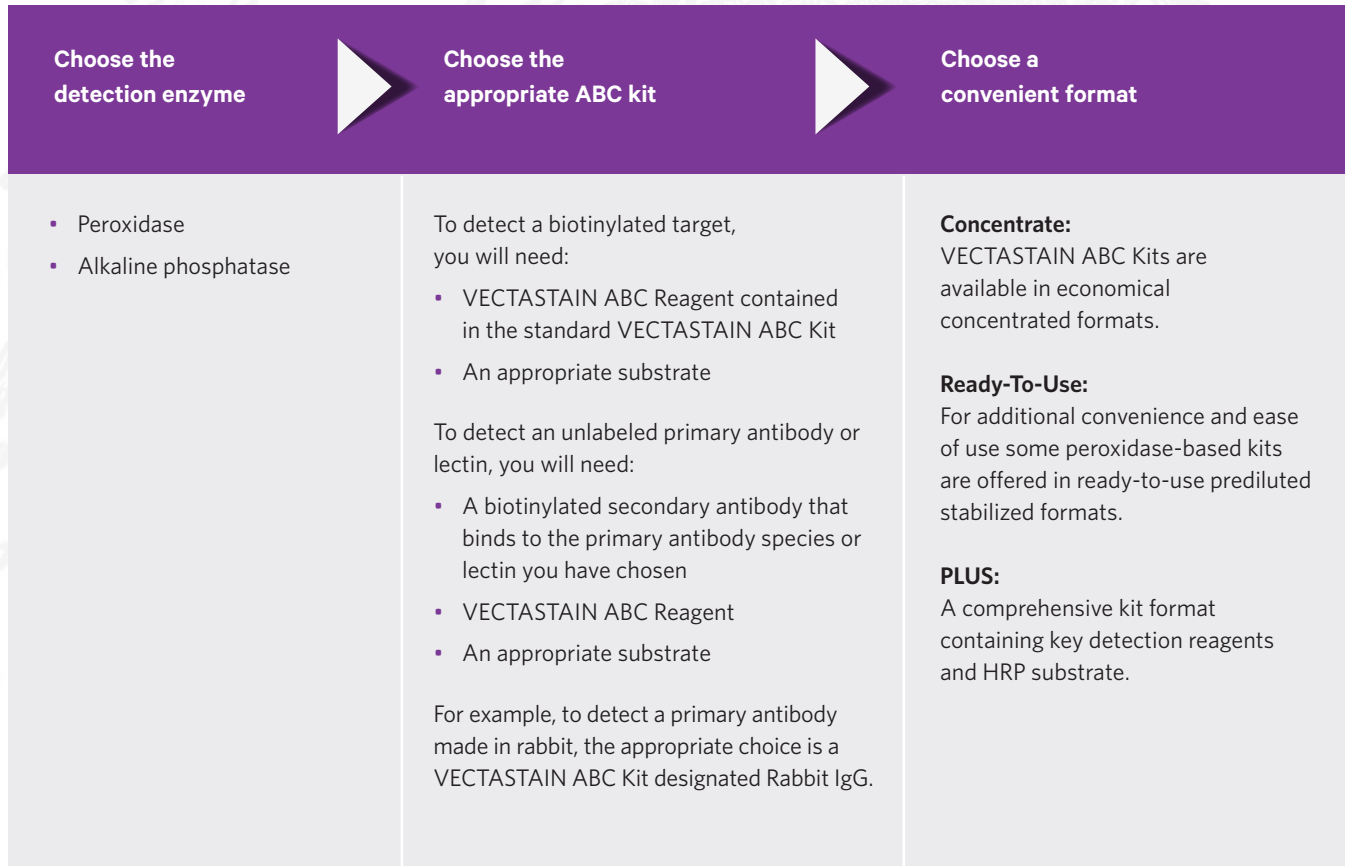
### VECTASTAIN ABC AP Kits

- Standard (ABC Reagent only)
- Rabbit IgG
- Mouse IgG
- Rat IgG
- Universal

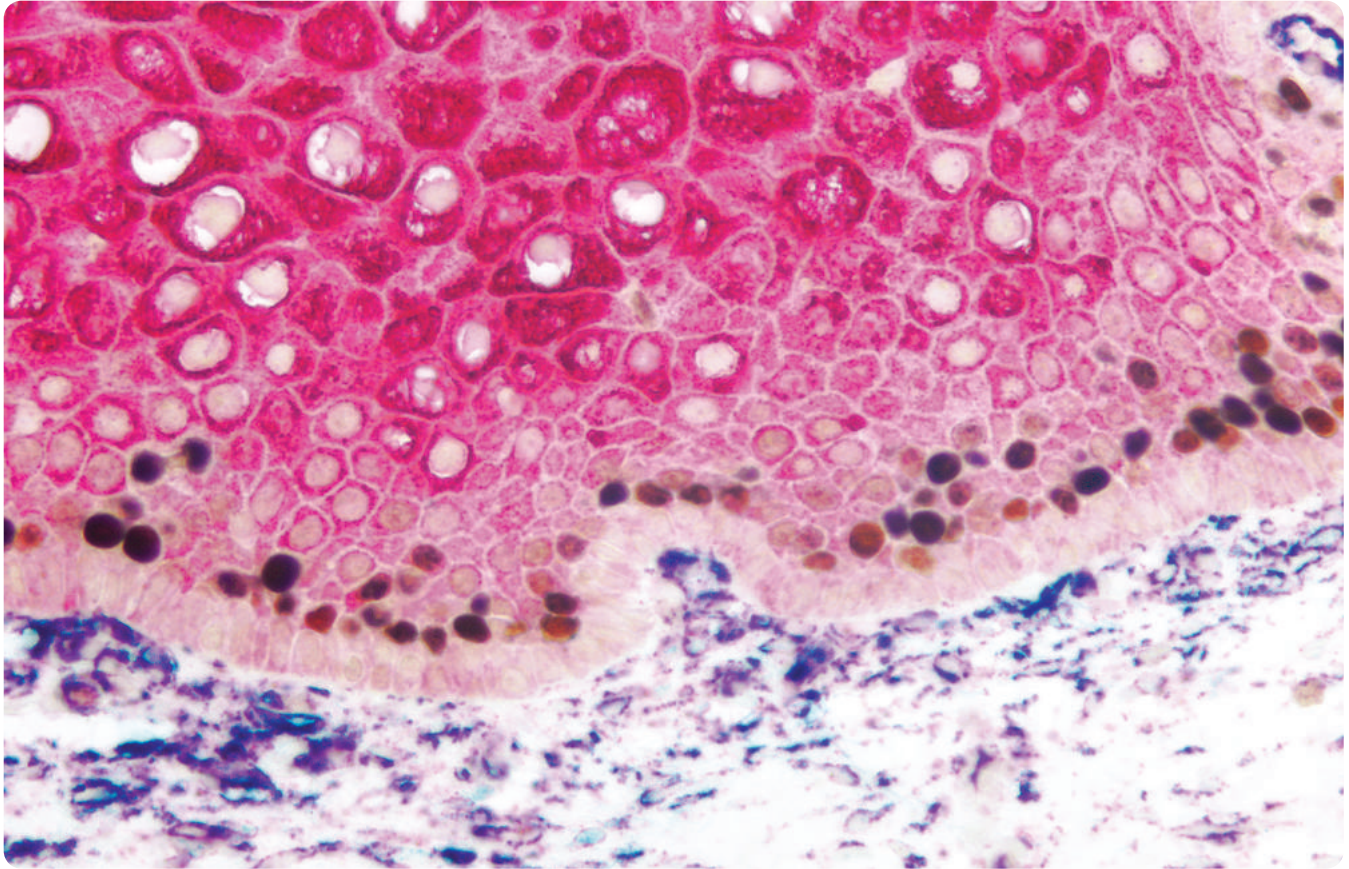
Product	Elite (Peroxidase)	Elite® PLUS (Peroxidase)	Original (Peroxidase)	Quick (Peroxidase)	Alkaline Phosphatase
VECTASTAIN® ABC Kit, Standard	PK-6100		PK-4000		AK-5000
VECTASTAIN® ABC Kit, Rabbit IgG	PK-6101		PK-4001		AK-5001
VECTASTAIN® ABC Kit, Mouse IgG	PK-6102		PK-4002		AK-5002
VECTASTAIN® ABC Kit, Mouse IgM			PK-4010		
VECTASTAIN® ABC Kit, Human IgG	PK-6103				
VECTASTAIN® ABC Kit, Rat IgG	PK-6104		PK-4004		AK-5004
VECTASTAIN® ABC Kit, Goat IgG	PK-6105		PK-4005		
VECTASTAIN® ABC Kit, Sheep IgG	PK-6106				
VECTASTAIN® ABC Kit, Guinea Pig IgG			PK-4007		
VECTASTAIN® ABC Kit, Universal	PK-6200	PK-8200			AK-5200
R.T.U. VECTASTAIN® ABC Reagent	PK-7100				
R.T.U. VECTASTAIN® ABC Kit, Universal	PK-7200				
R.T.U. VECTASTAIN® Universal Quick Kit				PK-7800	
VECTASTAIN® Universal Quick Kit (concentrate)				PK-8800	

## Avidin-Biotin Complex (ABC)-Based Detection

# Choosing a VECTASTAIN ABC Kit







*Tumor: • Ki67 (m), VECTASTAIN Elite ABC Kit, Vector DAB (brown) • CD34 (m), VECTASTAIN ABC-AP Kit, Vector Blue (blue) • Cytokeratin AE1/AE3 (m), VECTASTAIN ABC-AP Kit, Vector Red (red).*

## Consider Species Cross-Reactivity

When choosing the optimal detection system for your application, it is important to consider not only the species of the primary antibody but also the species of the tissue under examination. If the species of the primary antibody and the species of the tissue are closely related (for example, rat and mouse), the biotinylated secondary antibody may cross-react with endogenous IgG in the tissue section. This can lead to background staining.

The following options may minimize background staining in these instances:

- › Directly label the primary antibody with biotin (SP-1200 or SP-1210) and detect it using the VECTASTAIN Elite ABC Kit (Standard, PK-6100), followed by an HRP Substrate.

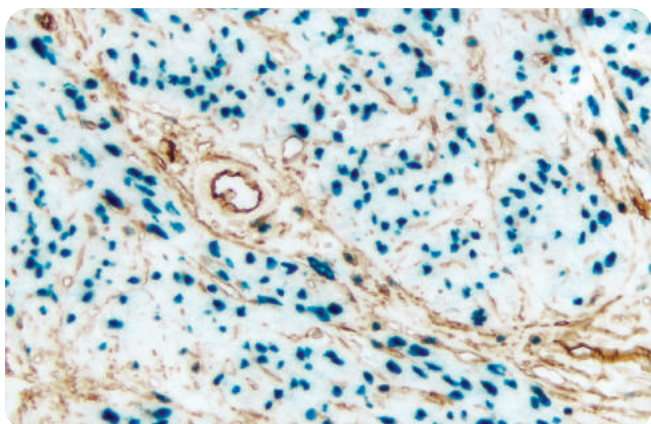
- › Use a biotinylated secondary antibody specifically adsorbed to remove cross-reacting antibodies of closely-related species (e.g., biotinylated anti-mouse IgG, rat adsorbed).
- › Use the M.O.M. (Mouse on Mouse) Immunodetection System for applications of mouse primary antibodies on mouse tissue (p. 24-25).

## Substrates

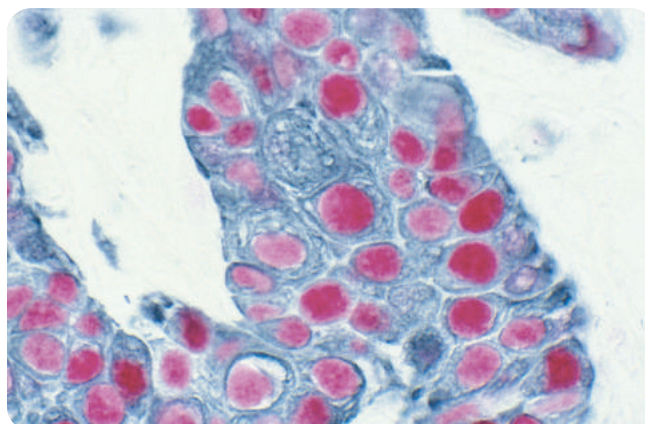
After choosing the VECTASTAIN ABC Kit for your application, select a substrate that matches the enzyme system of the kit (p. 27-30).



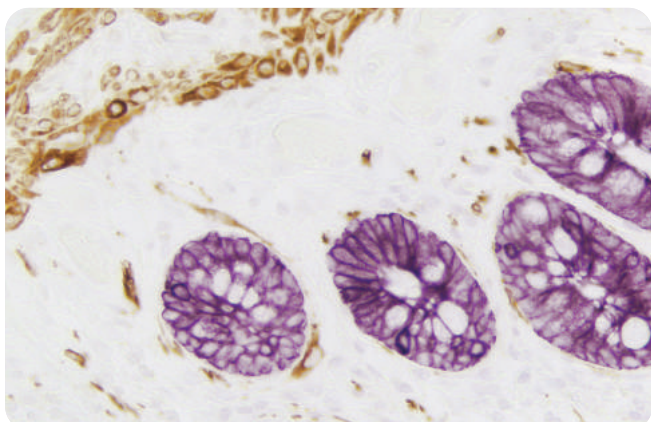
## Avidin-Biotin Complex (ABC)-Based Detection



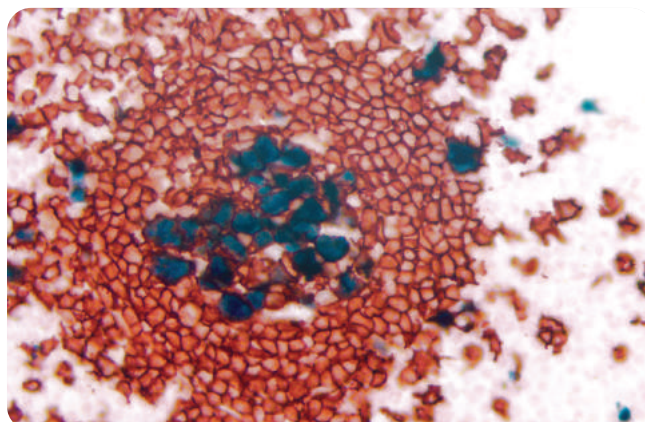
Endometrium: • Progesterone Receptor (m), VECTASTAIN Universal ABC- AP Kit, Vector Blue AP Substrate (blue) • CD34 (m), VECTASTAIN Universal Elite ABC Kit, Vector DAB HRP Substrate (brown).



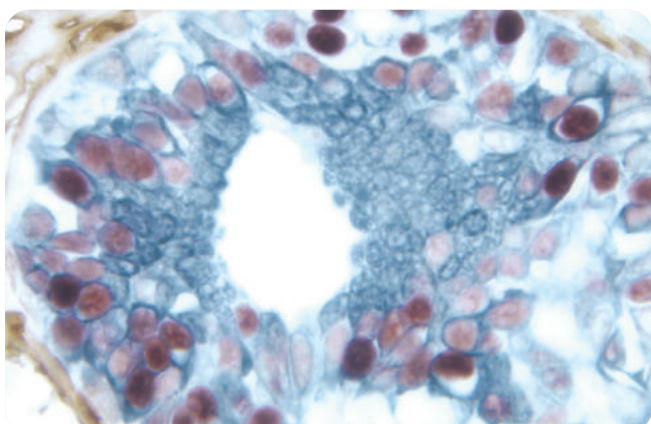
Tumor: • p53 protein (m), VECTASTAIN ABC-AP Kit, Vector Red AP Substrate (red) • Pan-Cytokeratin (sheep), VECTASTAIN Elite ABC Kit, Vector SG HRP Substrate (blue/gray).



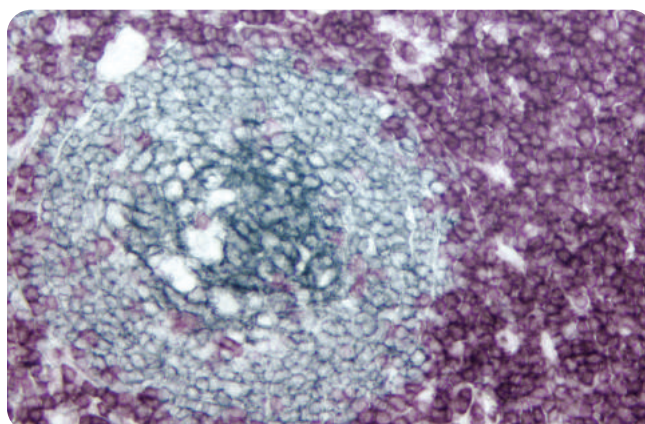
Intestine: • Desmin (m), VECTASTAIN Elite ABC Kit, ImmPACT DAB (brown) substrate. • Cytokeratin (m), VECTASTAIN Elite ABC Kit, Vector VIP (purple) substrate.



Tonsil: • Cyclin A (m), VECTASTAIN Universal ABC-AP Kit, Vector Blue AP Substrate (blue) • CD20 (m), VECTASTAIN Universal Elite ABC Kit, Vector NovaRED® HRP Substrate (red).



Breast Carcinoma: • Estrogen Receptor (m); VECTASTAIN Elite ABC Kit, Vector NovaRED substrate (red) • CD34 (m), VECTASTAIN Elite ABC Kit, DAB substrate (brown) • Cytokeratin 8/18 (m), VECTASTAIN Elite ABC Kit, Vector SG substrate (blue/gray).



Tonsil: • CD3 (m), VECTASTAIN Universal Elite ABC Kit, Vector VIP substrate (purple) • CD20 (m), VECTASTAIN Universal Elite ABC Kit, Vector SG substrate (blue/gray).

# Customizing your VECTASTAIN ABC Kit

If a VECTASTAIN ABC system is not available with a biotinylated secondary antibody of your required specificity, you can custom-build the exact kit that you require. All of our biotinylated, affinity-purified secondary antibodies (p. 38-39) are designed for use with VECTASTAIN ABC Standard Kits and the appropriate blocking serum. Our mix-and-match kit components allow you to both design a custom kit to suit your needs and to use kit components interchangeably. The reagents can be purchased individually, allowing you to combine them to suit your specific needs.

For example, to make a VECTASTAIN Elite ABC Kit for use with a mouse IgG primary antibody on rat tissues:

1. Choose the VECTASTAIN ABC Kit that contains the desired detection enzyme but with no secondary antibody (e.g. VECTASTAIN Elite ABC Kit, Standard).
2. Choose the biotinylated secondary antibody (e.g., biotinylated horse anti-mouse IgG, rat adsorbed).
3. Choose the blocking solution. We recommend a serum from the same species as the secondary antibody. (In our example, normal horse serum). Alternatively, select our animal-free blocking reagents for multiple antigen labeling (multiplex) IHC applications where antibodies from different species and a variety of detection systems are used on the same tissue section.

## 1 Choose Standard VECTASTAIN ABC Kit with the appropriate detection enzyme

Enzyme	Product	Catalog Number
Peroxidase	VECTASTAIN® Elite ABC Kit	PK-6100
Peroxidase	R.T.U. VECTASTAIN® Elite® ABC Reagent	PK-7100
Peroxidase	VECTASTAIN® ABC Kit	PK-4000
Alkaline Phosphatase	VECTASTAIN ABC-AP Kit	AK-5000

## 2 Choose the biotinylated secondary antibody\*

Product	Concentrate	R.T.U.†
Anti-Goat IgG (H+L) made in rabbit, biotinylated	BA-5000	
Anti-Goat IgG (H+L) made in horse, biotinylated	BA-9500	BP-9500
Anti-Human IgG (H+L) made in goat, biotinylated	BA-3000	
Anti-Mouse IgG (H+L) made in horse, biotinylated	BA-2000	BP-2000
Anti-Mouse IgG (H+L) made in horse, rat adsorbed, biotinylated	BA-2001	
Anti-Mouse IgG (H+L) made in goat, biotinylated	BA-9200	BP-9200
Anti-Mouse IgM (H+L) $\mu$ chain specific, made in goat, biotinylated	BA-2020	
Anti-Rabbit IgG (H+L) made in goat, biotinylated	BA-1000	BP-9100
Anti-Rabbit IgG (H+L) made in horse, biotinylated	BA-1100	BP-1100
Anti-Rat IgG (H+L) made in rabbit, biotinylated	BA-4000	
Anti-Rat IgG (H+L) made in rabbit, mouse adsorbed, biotinylated	BA-4001	
Anti-Rat IgG (H+L) made in goat, biotinylated	BA-9400	BP-9400
Anti-Rat IgG (H+L) made in goat, mouse adsorbed, biotinylated	BA-9401	
Universal Anti-Mouse/Rabbit IgG (H+L) made in horse, biotinylated	BA-1400	BP-1400
Universal Pan-Specific Anti-Mouse/Rabbit/Goat IgG (H+L) made in horse, biotinylated	BA-1300	

## 3 Choose the blocking solution

Product	Concentrate	R.T.U.†
Normal Goat Serum	S-1000	S-1012
Normal Rabbit Serum	S-5000	
Normal Horse Serum	S-2000	S-2012
Animal-Free Blocker® and Diluent	SP-5030	SP-5035

\* For a complete list of all biotinylated secondary antibodies please visit our website.

† Ready-to-use, prediluted stabilized solutions.



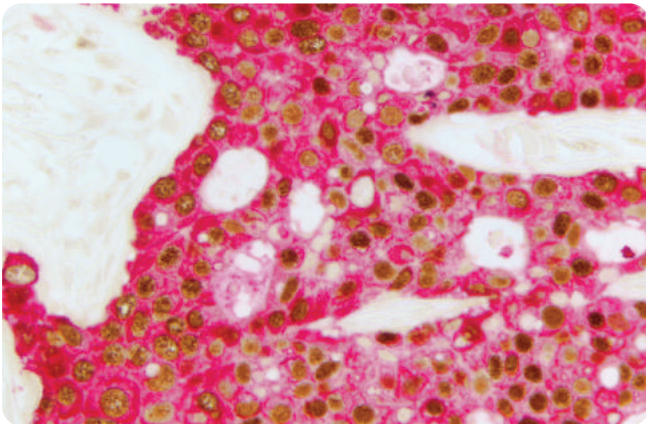
# Polymer-Based Detection

**Non-biotin micropolymer-based detection for greater signal, low background, and superior access to epitopes**

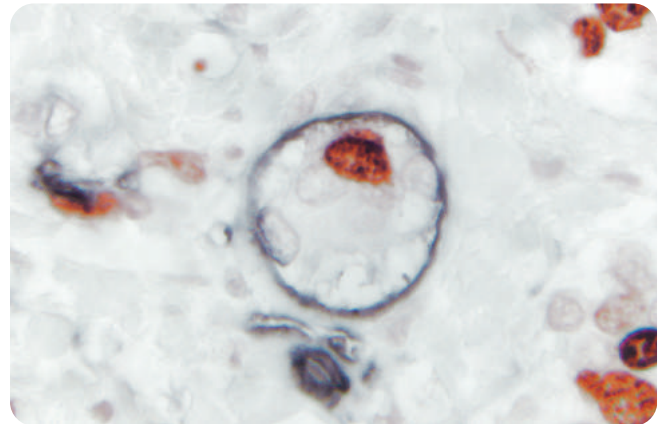
Polymer-based reagents are a more recent introduction into IHC detection methodology than traditional avidin and biotin conjugates, such as ABC kit formats. Polymers offer distinct advantages over these traditional methods particularly for applications such as multiple antigen labeling (multiplexing) on the same tissue section, or in instances where detectable levels of endogenous biotin may be problematic.

Polymer-based systems essentially consist of an integrated polymer of active enzyme and secondary antibody that binds to a primary antibody target. This integrated format introduces significantly more enzyme at the site of localization, thereby generating a greater reaction with the subsequent chromogen, compared with a secondary antibody directly conjugated with enzyme. Additionally, use of a one-step polymer method shortens the IHC procedure by avoiding the two-step biotinylated secondary antibody and ABC reagent that are required for standard avidin-biotin systems.

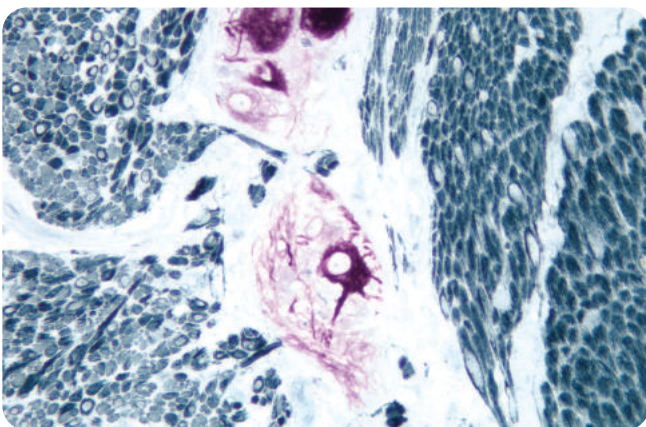
Polymer-based systems were initially introduced consisting of dextran or similar molecules that had inherent issues in some tissues due to their large size. Our ImmPRESS polymer systems have been highly refined and consist of micropolymers that penetrate more easily into thicker sections, avoid steric hindrance concerns, and provide defined, specific binding to the primary antibody.



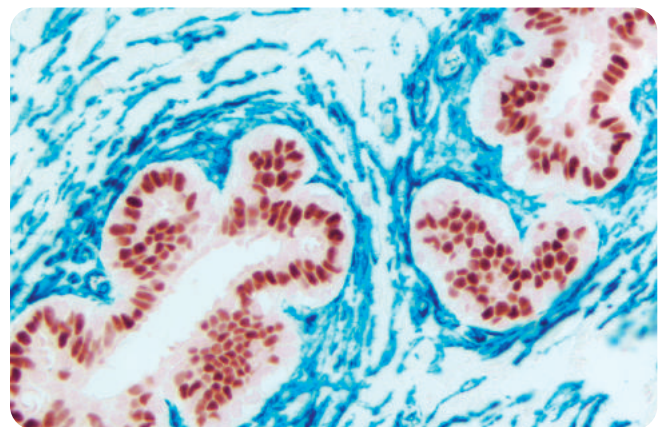
*Breast Carcinoma: • Estrogen Receptor (m), ImmPRESS Reagent (HRP; Universal), Vector DAB (brown) • Cytokeratin AE1/AE3 (m), VECTASTAIN ABC-AP Kit (Universal), Vector Red (magenta).*



*Breast Carcinoma: • Estrogen Receptor (m), ImmPRESS Reagent (HRP; Universal), Vector NovaRED HRP Substrate (red) • CD34 (m), ImmPRESS Reagent (HRP; Universal), Vector DAB+Ni HRP Substrate (gray-black).*



*Small Bowel: • Neurofilament 200 kDa (m), ImmPRESS Reagent (HRP) Anti-Mouse IgG, Vector VIP (purple) • Desmin (m), ImmPRESS Reagent (HRP) Anti-Mouse IgG, Vector SG (blue-gray).*



*Breast Carcinoma: • Estrogen Receptor (m), ImmPRESS Reagent (HRP; Universal), Vector NovaRED (red) • CD34 (m), VECTASTAIN ABC-AP Kit (Universal), Vector Blue (blue).*

# ImmPRESS One-Step Polymer Systems

(Single Antigen Detection)

## ImmPRESS Polymer Detection Kits (Peroxidase or Alkaline Phosphatase)

ImmPRESS Polymer Kits consist of unique micropolymers of highly active peroxidase or alkaline phosphatase enzyme attached to highly cross-adsorbed, affinity-purified secondary antibodies. This micropolymer conjugation technology allows a higher density of enzymes per antibody to bind to the target with minimal steric interference. The ImmPRESS Polymer Kits produce outstanding immunohistochemistry and immunocytochemistry results due to increased target accessibility, binding specificity, and signal intensity along with low background staining.

- High sensitivity and very low background for crisp, strong staining
- Ready-to-use, one-step detection system – no mixing or titering
- Includes prediluted blocking serum
- Shorter assay time
- Non-biotin based
- Excellent resolution
- Especially suited for nuclear and membrane antigens
- Ideal for multiple antigen labeling (p. 23, 31-33)

## ImmPRESS-HRP PLUS Polymer Kits (Peroxidase)

ImmPRESS HRP PLUS polymer kits are comprehensive kits that include additional key IHC workflow components compared with non-PLUS ImmPRESS HRP polymer kits. The ImmPRESS HRP PLUS polymer kits reduce optimization requirements and streamline the assay with matched reagents.

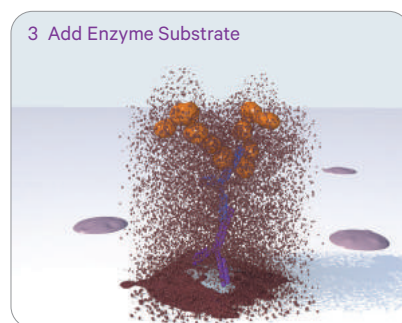
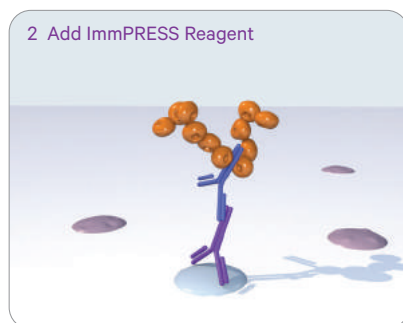
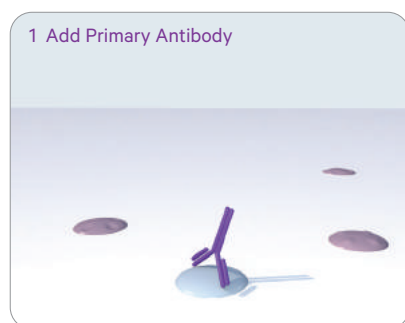
Each ImmPRESS PLUS polymer Kit includes the following:

- › BLOXALL Endogenous Enzyme Blocking Solution
- › 2.5% Normal Serum
- › ImmPRESS HRP polymer reagent
- › ImmPACT DAB EqV Substrate (Chromogen and Diluent)

## ImmPRESS-VR Polymer Kits (Peroxidase)

ImmPRESS VR (Veterinary Reagents) Kits are available additionally cross-adsorbed to ensure minimal cross-reactivity against endogenous tissue elements in animal species commonly used for diagnostics and research-based animal models (bovine, goat, sheep, swine, horse, cat, dog, rabbit, rat, mouse).

## Using the ImmPRESS Polymer Kits



### ImmPRESS-HRP Polymer Kits

- › Anti-Rabbit IgG
- › Anti-Mouse IgG
- › Anti-Mouse IgG, Rat Adsorbed
- › Anti-Rat IgG
- › Anti-Rat IgG, Mouse Adsorbed
- › Anti-Goat IgG
- › Universal Antibody, Anti-Rabbit/Mouse IgG

### ImmPRESS-HRP PLUS Polymer Kits

- › Anti-Rabbit IgG
- › Anti-Mouse IgG
- › Universal Antibody, Anti-Rabbit/Mouse IgG

### ImmPRESS-AP Polymer Kits

- › Anti-Rabbit IgG
- › Anti-Mouse IgG
- › Anti-Rat IgG
- › Anti-Rat IgG, Mouse Adsorbed
- › Anti-Goat IgG

### ImmPRESS-VR Polymer Kits

- › Anti-Rabbit IgG
- › Anti-Mouse IgG

# ImmPRESS One-Step Double Staining Polymer Systems (Double Antigen Detection)

## ImmPRESS Duet Double Staining Polymer Kits (Peroxidase/Alkaline Phosphatase)

ImmPRESS Duet Double Staining Polymer Kits enable fast, well-defined localization and visualization of two different target antigens on the same tissue section. Utilizing our unique ImmPRESS micropolymer chemistry, we independently conjugate highly-active horseradish peroxidase (HRP) and alkaline phosphatase (AP) enzyme polymers to affinity-purified, highly cross-adsorbed anti-mouse IgG and anti-rabbit IgG secondary antibodies. These reagents are then blended at optimized dilutions to provide a stable pre-diluted, ready-to-use HRP/AP formulation that will detect mouse and rabbit primary antibodies, and facilitate a time-saving, simplified, one-step double label detection protocol. For maximum sensitivity and staining contrast between target antigens, ImmPACT DAB EqV (HRP, brown) and ImmPACT Vector Red (AP, magenta) substrates are included to complete this easy to use staining kit.

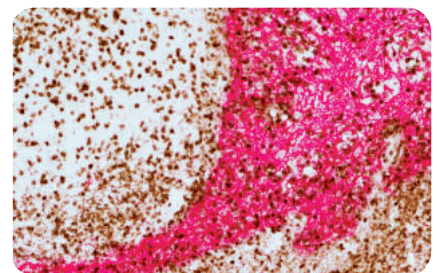
The ImmPRESS Duet Double Staining Polymer Kit is intended for use on non-rodent tissue specimens. Reliable and reproducible results are obtained on tissue sections where the two target antigens do not overlap (co-localize) in the same structure of the same cell, but rather are expressed in different cell compartments or different cell types.

Each ImmPRESS Duet Double Staining Polymer Kit includes the following:

- › BLOXALL Endogenous Enzyme Blocking Solution
- › 2.5% Normal Horse Serum
- › ImmPRESS Duet HRP/AP Polymer Reagent (mixture of Anti-Rabbit IgG and Anti-Mouse IgG)
- › ImmPACT DAB EqV Substrate (HRP, brown)
- › ImmPACT Vector Red Substrate (AP, magenta)

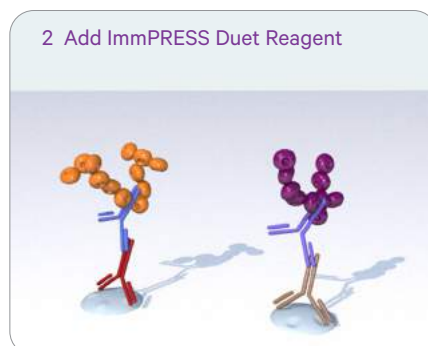
### ImmPRESS Duet Double Staining Polymer Kits

- › Anti-Rabbit IgG (HRP-brown), Anti-Mouse IgG (AP-magenta)
- › Anti-Mouse IgG (HRP-brown), Anti-Rabbit IgG (AP-magenta)



Human tonsil (paraffin section) stained for CD3 (DAB, brown) and AE1/AE3 cytokeratin (Vector Red, magenta) using ImmPRESS Duet Kit (MP-7714).

## Using the ImmPRESS One-Step Double Staining Polymer Systems



\* Primary antibodies may be applied separately or mixed together.

† Enzyme substrates are applied sequentially. Refer to instructions for recommended procedure.



# ImmPRESS Two-Step Amplified Polymer Systems

## (Single Antigen Detection)

### ImmPRESS-HRP Excel Amplified Polymer Staining Kits (Peroxidase)

ImmPRESS Excel Amplified Peroxidase (HRP) Polymer Staining Systems are complete staining kits that capitalize on all the advantages of the ImmPRESS HRP Polymer System technology and offer additional sensitivity and convenience.

This system employs a ready-to-use (R.T.U.) Amplifier Antibody, followed by an ImmPRESS Excel HRP Polymer Reagent. These reagents are affinity-purified and extensively cross-adsorbed to ensure high sensitivity and low background. The included ImmPACT DAB EqV (equal volume) substrate produces a crisp, dark brown reaction product with excellent sensitivity that is characteristic of the ImmPRESS/ImmPACT combination.

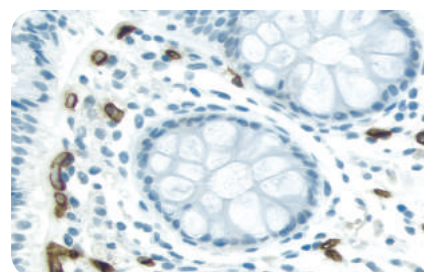
The ImmPRESS Excel Amplified HRP Kits are recommended for applications that require detection of weakly-expressed antigens, in cases of unknown expression levels such as gene knock-in studies, or in determining up-regulation of a given target. This straightforward kit format yields reliable, consistent results and saves time in trying to establish optimal titrations with concentrated detection reagents.

Each ImmPRESS Excel Amplified Staining Kit includes the following:

- › BLOXALL Endogenous Enzyme Blocking Solution
- › 2.5% Normal Horse Serum
- › Amplifier Antibody (goat anti-rabbit IgG or goat anti-mouse IgG)
- › ImmPRESS Excel Polymer Detection Reagent (horse anti-goat IgG)
- › ImmPACT DAB EqV Substrate (Chromogen and Buffer)

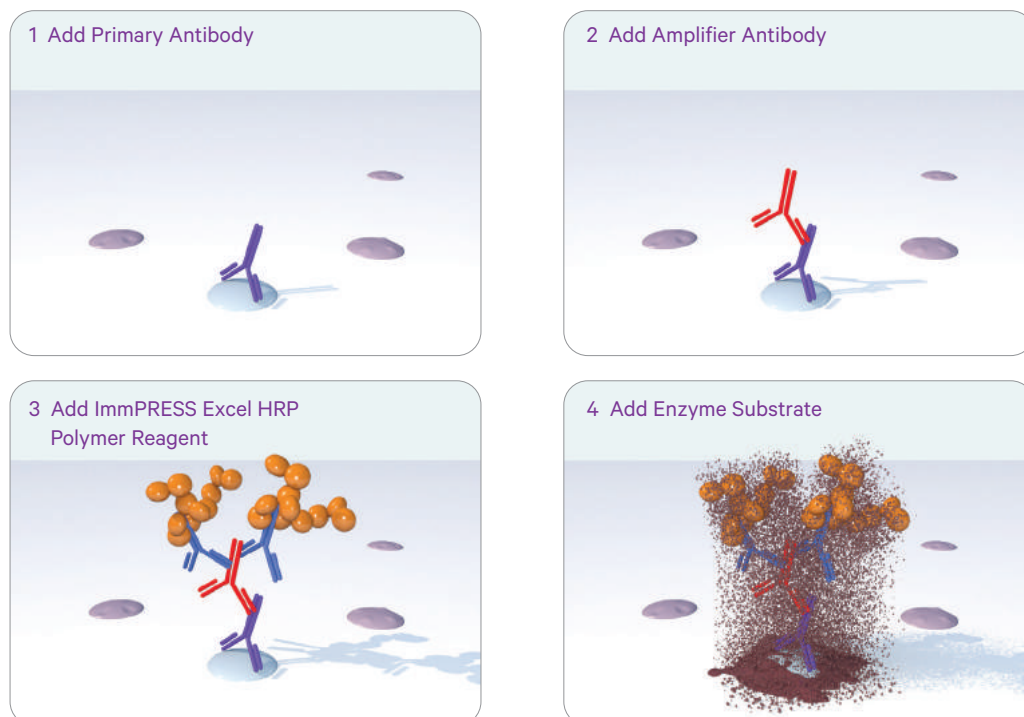
#### ImmPRESS-HRP Excel Amplified Polymer Kits

- > Anti-Rabbit IgG
- > Anti-Mouse IgG

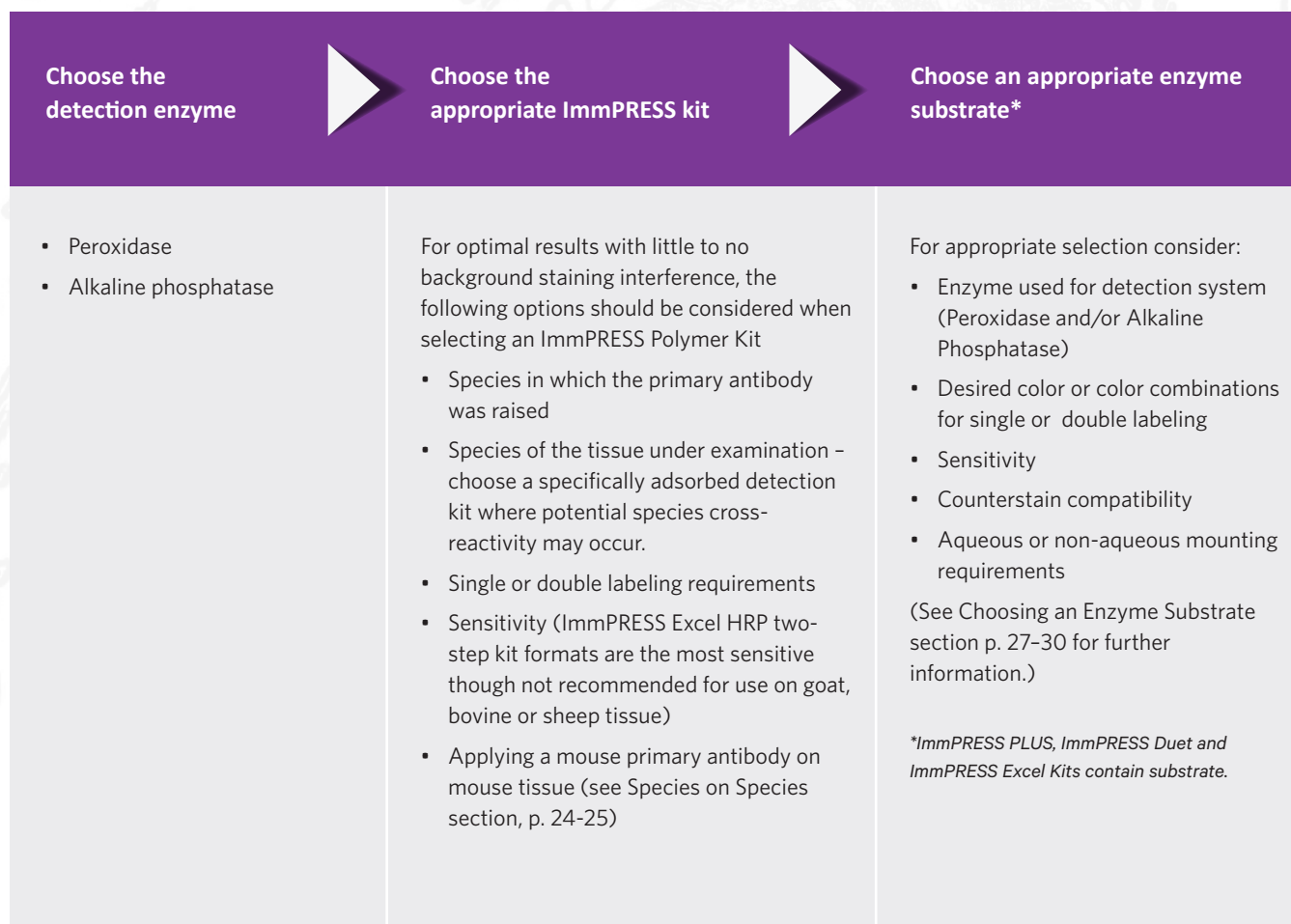


Colon: Anti-CD34 (m), ImmPRESS Excel Amplified Anti-Mouse IgG Staining Kit, ImmPACT DAB EqV (brown), Hematoxylin QS counterstain (blue).

### Using the ImmPRESS Two-Step Amplified Polymer Systems



## Choosing an ImmPRESS Polymer Kit



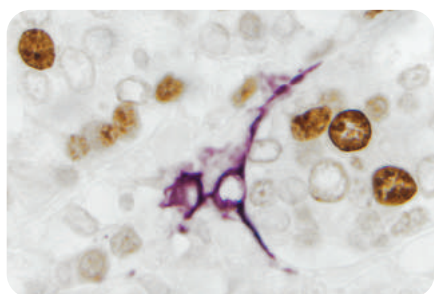
Product	Peroxidase (HRP)	Peroxidase PLUS* (HRP)	Veterinary Reagents (HRP)	Excel Amplified* (HRP)	Alkaline Phosphatase (AP)	Duet Double Staining* (HRP & AP)
ImmPRESS® Anti-Rabbit IgG Kit (made in horse)	MP-7401	MP-7801	MP-6401	MP-7601	MP-5401	
ImmPRESS® Anti-Rabbit IgG Kit (made in goat)	MP-7451					
ImmPRESS® Anti-Mouse IgG Kit (made in horse)	MP-7402	MP-7802	MP-6402	MP-7602	MP-5402	
ImmPRESS® Anti-Mouse IgG Kit (made in goat)	MP-7452					
ImmPRESS Anti-Mouse IgG, Rat Adsorbed, Kit (made in horse)	MP-7422					
ImmPRESS® Anti-Rat IgG Kit (made in goat)	MP-7404				MP-5404	
ImmPRESS® Anti-Rat IgG, Mouse Adsorbed, Kit (made in goat)	MP-7444				MP-5444	
ImmPRESS® Anti-Goat IgG Kit (made in horse)	MP-7405				MP-5405	
ImmPRESS® Universal Antibody Kit Anti-Rabbit/Mouse Kit (made in horse)	MP-7500	MP-7800				
ImmPRESS® Duet Anti-Rabbit (HRP, Brown) Anti-Mouse (AP, magenta)						MP-7714
ImmPRESS® Duet Anti-Mouse (HRP, Brown) Anti-Rabbit (AP, magenta)						MP-7724

\* BLOXALL blocking solution and substrate included.

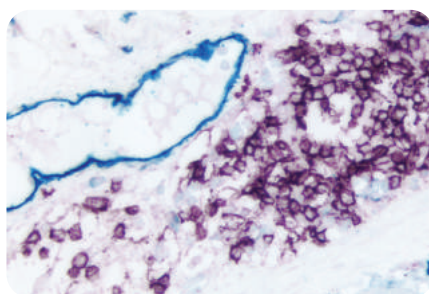
# Multiple Antigen Labeling Simplified

A key advantage of the ImmPRESS Polymer Reagent is that it significantly shortens staining times for multiple antigen labeling (multiplexing).

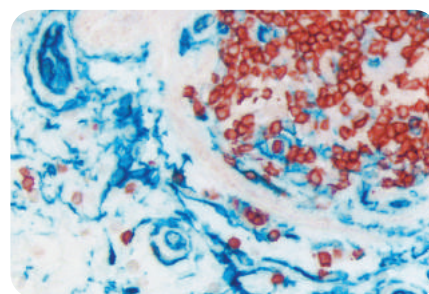
- Fewer steps than conventional protocols decrease slide handling.
- Eliminates the need for avidin/biotin blocking steps in samples with endogenous biotin.



Breast Carcinoma: • Ki67 (rm), ImmPRESS Reagent (HRP; Universal), Vector DAB (brown) • CD34, ImmPRESS Reagent (HRP; Universal), Vector VIP (purple).



Colon: • M2A antigen (m), VECTASTAIN ABC-AP Kit (Universal), Vector Blue (blue) • CD20 (m), ImmPRESS Reagent (HRP; Universal), Vector VIP (purple).

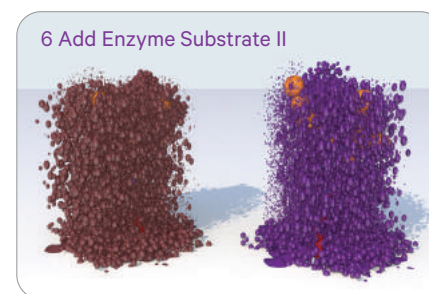
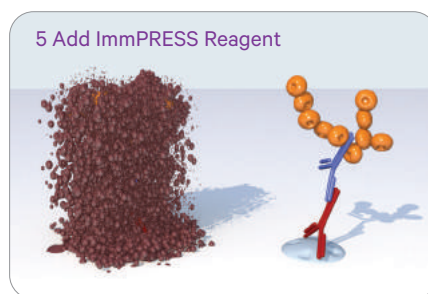
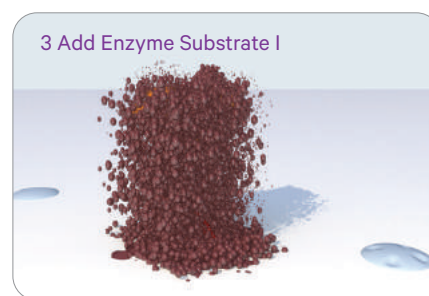
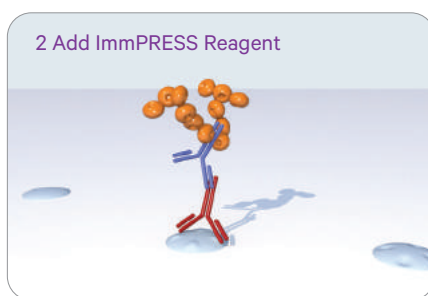


Colon: • CD3 (rm), ImmPRESS Reagent (HRP) Anti-Rabbit IgG, ImmPACT AMEC Red • CD34 (m), ImmPRESS-AP Anti-Mouse IgG Reagent, Vector Blue (blue).

## Kit Selection Considerations

- For the detection of two antigens on the same section (i.e., human/primate species) using the combination of a mouse and a rabbit primary antibody, we recommend using an ImmPRESS Duet Double Staining Polymer Kit (p. 20).
- For detection of two or more antigens on non-human/primate tissue sections and/or if using a mouse or rabbit primary antibody in combination with a goat or rat primary antibody, we recommend selecting individual species-specific ImmPRESS kits (p. 19) that meet your assay criteria, and applying them sequentially as indicated in the diagram below.

## Using the ImmPRESS Polymer Kits for multiple antigen labeling



For additional information on multiple antigen labeling please see our [IHC Multiplexing Resource Guide](#).



A microscopic image of tissue, likely a cross-section of an organ, showing various cellular structures and patterns. A white rectangular overlay is positioned in the center of the image, containing text. The background image is in grayscale and shows a dense network of cells and fibers.

## Species on Species Detection

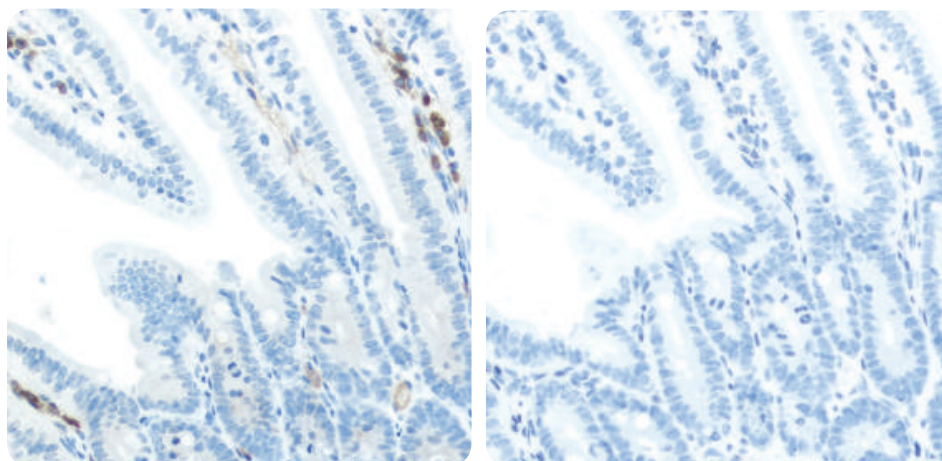
Solutions when your primary antibody is the same species as your specimen.

When a primary antibody is the same species as the specimen, the secondary antibody cannot distinguish between the endogenous immunoglobulins and the primary antibody. This can result in high background staining that obscures antigen-specific staining. [Mouse on Mouse detection](#) is especially important because of the vast number of primary antibodies made in mouse and the wide use of mice in model systems, xenografts, and other applications. [Human on Human detection](#) is suitable for tissue cross-reactivity (TCR) assessment of potential therapeutic human antibodies.

# M.O.M. (Mouse on Mouse) Immunodetection Kits

The Vector M.O.M. Immunodetection kits are specifically designed to localize mouse primary antibodies on mouse tissue while avoiding background staining. These M.O.M. Kits contain our proprietary M.O.M. Mouse IgG Blocking Reagent. M.O.M. Kits are available based on either avidin-biotin technology (M.O.M. Elite Peroxidase Kit, Fluorescein Kit, or Basic Kit) or polymer technology (M.O.M. ImmPRESS HRP Polymer Kit). Use the M.O.M. Immunodetection systems to introduce two or more different labels using a multiple antigen labeling protocol. You can detect several mouse primary antibodies on the same tissue section, regardless of the species of the tissue. Excellent staining results for a once difficult application have now become routine with the Vector Laboratories' M.O.M. System.

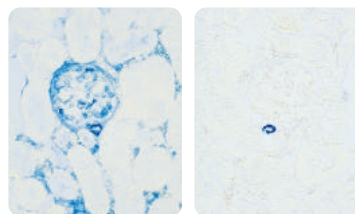
- Significantly reduces endogenous mouse Ig staining when using mouse primary antibodies on mouse tissue
- Simple protocols
- Eliminates tedious calculations
- Eliminates primary antibody prebinding steps
- Clear, crisp, specific staining of antigens of interest
- Compatible with fluorescent or enzyme-based detection
- Available with or without enzyme or fluorochrome



Mouse intestine stained with standard anti-mouse IgG polymer system (left) and Vector M.O.M. ImmPRESS HRP Polymer Kit (right). Brown signal indicates IgG background staining. (Both: No primary antibody, Vector DAB stain, hematoxylin counterstain.)

## Recommended applications:

- Studies in genetically engineered mice
- Transgenic and knock-out models
- Mouse xenograft tissue
- Normal mouse tissue



Sections of mouse kidney stained with mouse antibody against smooth muscle actin using VECTASTAIN ABC-AP Kit and Vector Blue substrate. Using standard biotinylated anti-mouse antibody and normal blocking serum, confusing background is seen (left). With the Vector M.O.M. Basic Kit, clean background and specific staining is achieved (right).

Product	Catalog Number
M.O.M.® Elite® Immunodetection Kit, Peroxidase	PK-2200
M.O.M.® Immunodetection Kit, Fluorescein	FMK-2201
M.O.M.® Immunodetection Kit, Basic	BMK-2202
M.O.M.® ImmPRESS HRP Polymer Kit, Peroxidase	MP-2400
M.O.M.® Blocking Reagent	MKB-2213
M.O.M.® Biotinylated Anti-Mouse IgG Reagent*	MKB-2225
M.O.M.® ImmPRESS Polymer Reagent, Anti-Mouse IgG, Peroxidase	MPX-2402

\* This reagent must be used with the M.O.M. Blocking Reagent (MKB-2213). It is not intended to be a stand-alone reagent for mouse on mouse applications.

# H.O.H. (Human on Human) Immunodetection Kit

The H.O.H. Immunodetection Kit is intended to detect human (or humanized) antibodies on frozen or paraffin embedded human tissue sections.

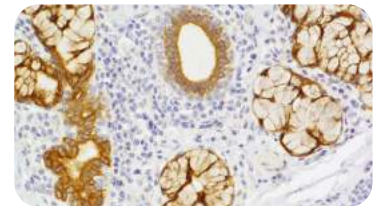
The inability of a secondary antibody to distinguish between a primary antibody produced in humans and the endogenous human immunoglobulins present in human tissue results in high background staining, which obscures specific staining. This problem can be eliminated by using the H.O.H. Kit and the result is clear, crisp, specific staining of the antigens of interest.

This kit employs a straightforward two-step primary antibody preparation followed by standard IHC assay detection procedures. Once the human primary antibody solution has been prepared, assay time is approximately 90 minutes.

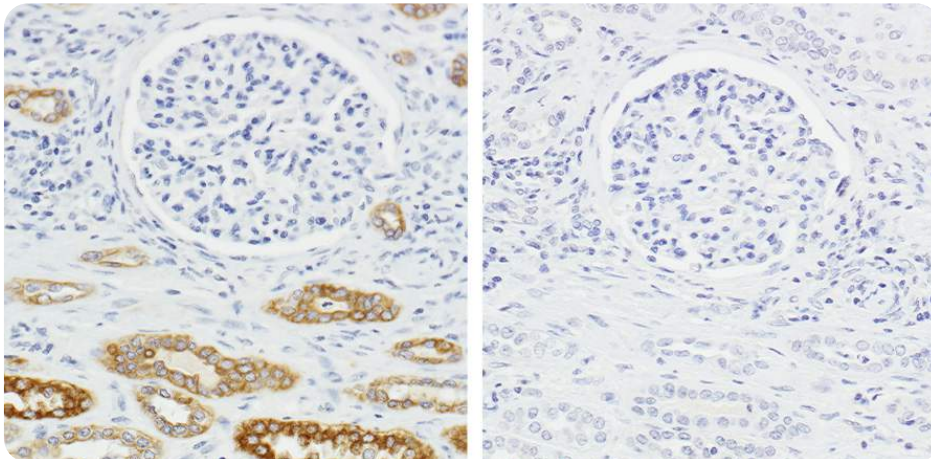
- Essentially background-free results even on frozen lymphoid tissue sections
- A simplified IHC procedure for H.O.H. detection
- Minimal optimization requirements
- A comprehensive kit format with matched volume reagents
- Kit includes the DAB substrate

## Recommended applications:

- Normal human tissue
- Tissue cross-reactivity assessment



Positive staining (brown) for cytokeratin using the H.O.H. Kit. Note strong specific epithelial staining and no confounding background interference. Hematoxylin counterstain (blue).



Left image: Serial sections of human kidney (FFPE) showing strong, specific staining using human anti-cytokeratin primary antibody detected with HOH-3000 (brown regions). Right image: Negative control showing an absence of staining (no background).

Product	Catalog Number
H.O. H.™ (Human on Human) Immunodetection Kit	HOH-3000



## Choosing an Enzyme Substrate

Vector Laboratories enzyme substrates produce a range of sensitivities across a broad palette of colors.

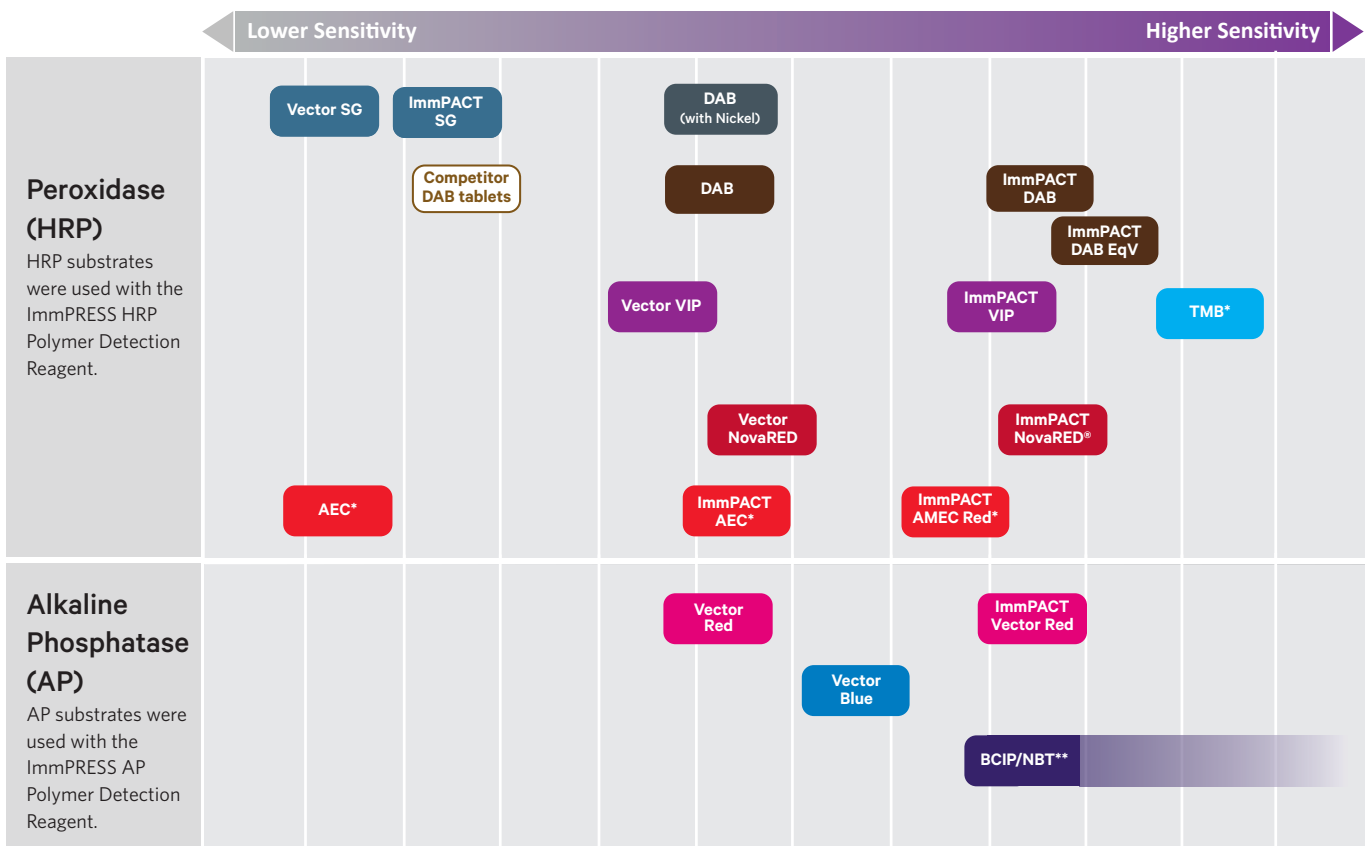
Consider the following factors when choosing a substrate to match the enzyme in your detection system and your application.

- **Sensitivity.** Substrates differ in sensitivity. Some may increase in sensitivity with longer incubation times.
- **Color.** Color contrast is essential in multiple antigen labeling applications, in pigmented tissues such as melanomas, and in counterstained tissues. Where performance is equal, color choices might also depend on personal preference.
- **Visualization.** Visualization 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 with an IHC protocol, followed by ISH detection that includes a heat denaturation step. In multiple antigen labeling procedures requiring additional applications of heat-induced epitope retrieval (HIER), apply the heat-resistant substrate first.

# Enzyme Substrates

We offer researchers an array of both conventional and unique enzyme substrates that produce a broad range of colors. Our reagents require no dissolving of powders or tablets and are provided in convenient dropper bottles which are safe and easy to handle.




















## Relative sensitivity of substrates in IHC



\* AEC & AMEC substrates must be aqueously mounted \*\* Longer incubation times increase sensitivity

# Enzyme Substrate Properties

See also Enzyme Substrate Combinations for multiple antigen labeling (p. 31-33) and Counterstain/Substrate Compatibility (p. 35).

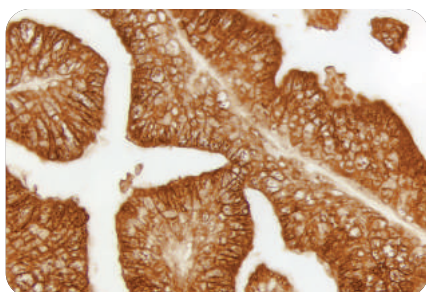
Substrate	Color	Catalog Number	Microscopy					Mounting	Contrast in Pigmented Tissue	Multiple Labeling	Heat Resistant*
			Bright-field	Darkfield	Electron	Fluorescence	Spectral Imaging				
<b>Peroxidase</b>											
Vector® DAB	 Brown	SK-4100	•	•	•		•	Non-aqueous or Aqueous		•	•
Vector® DAB +Ni	 Gray-Black	SK-4100	•	•	•		•	Non-aqueous		•	
ImmPACT® DAB	 Brown	SK-4105	•	•	•		•	Non-aqueous or Aqueous		•	•
ImmPACT® DAB EqV	 Brown	SK-4103	•	•	•		•	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	•	•	
Vector® AEC	 Red	SK-4200	•				•	Aqueous	•	•	
ImmPACT® AEC	 Red	SK-4205	•				•	Aqueous	•	•	
ImmPACT® AMEC Red	 Red	SK-4285	•				•	Aqueous	•	•	
TMB	 Blue	SK-4400	•				•	Non-aqueous			
<b>Alkaline Phosphatase</b>											
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		•	•

\* 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 1 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.

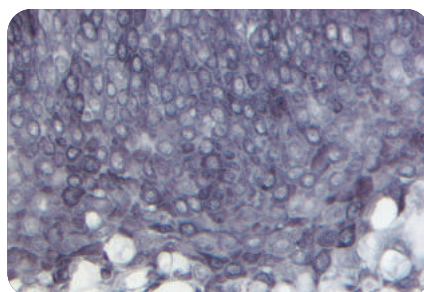


# Enzyme Substrate Color Examples

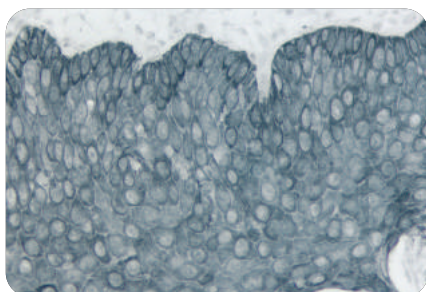
## Peroxidase Substrates



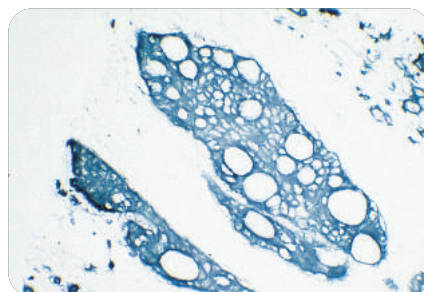
Prostate: Prostate Specific Antigen (m), ImmPRESS Reagent (HRP), ImmPACT DAB (brown).



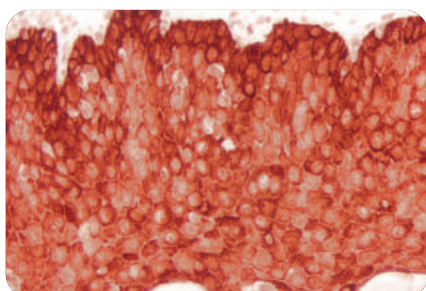
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS Reagent (HRP), Vector DAB-Ni (gray-black).



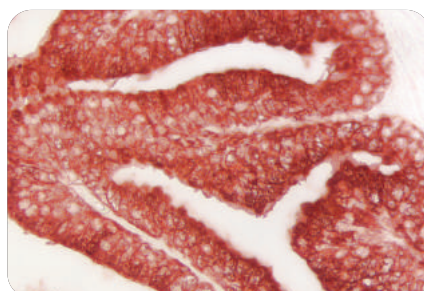
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS Reagent (HRP), ImmPACT SG (blue-gray).



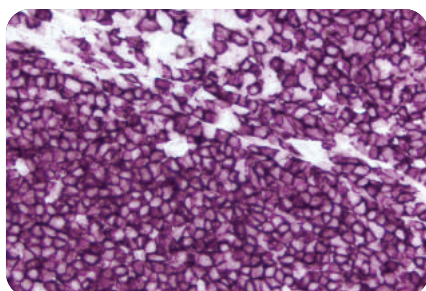
Tumor: Cytokeratin (s), VECTASTAIN Elite ABC Kit, TMB (blue).



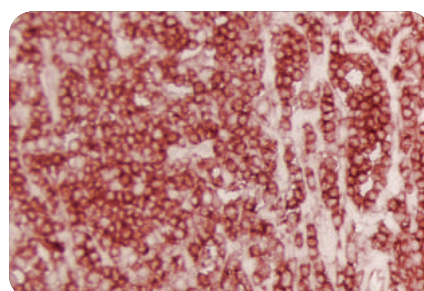
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS Reagent (HRP), ImmPACT NovaRED (red).



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

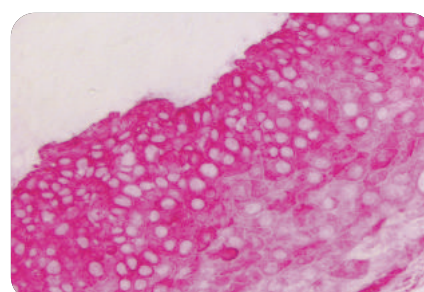


Tonsil: CD20 (m), ImmPRESS Reagent (HRP), ImmPACT VIP (purple).

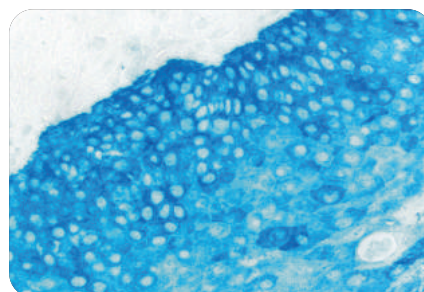


Tonsil: LCA (m), ImmPRESS Reagent (HRP), ImmPACT AMEC Red (red).

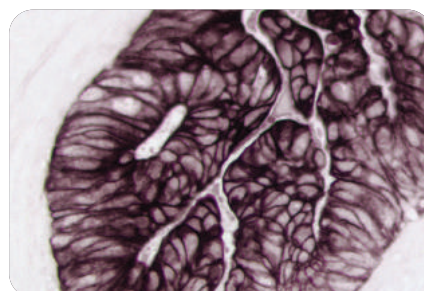
## Alkaline Phosphatase Substrates



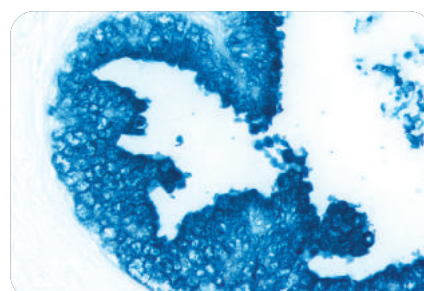
Tonsil: Cytokeratin AE1/AE3 (m), Vector ImmPRESS-AP Reagent, ImmPACT Vector Red (magenta).



Tonsil: Cytokeratin AE1/AE3 (m), 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).

A grayscale microscopic image of a tissue section, likely a glandular structure, with a prominent dark, circular area in the center. The surrounding tissue shows a complex, cellular pattern with various sized cells and structures.

## Multiple Antigen Labeling

Localization of two or more antigens on the same tissue section is a powerful research tool that can provide valuable insights into cellular biochemistry, protein-protein interactions, and spatial relationships of biomarkers.

Our detection systems and enzyme substrates have been developed and rigorously tested to deliver the high sensitivity, low background, and extreme clarity that is required to differentiate multiple epitopes simultaneously. You can choose to use the same enzyme system with different substrates or different enzyme systems and their respective substrates.

For a detailed description of these applications, protocols, and additional images please visit our website or request a free copy of our guide, [IHC Multiplexing Resource Guide](#).



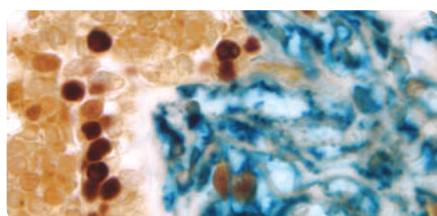
# Enzyme Substrate Combinations

Recommended combinations of substrates and the recommended order in which they should be used.

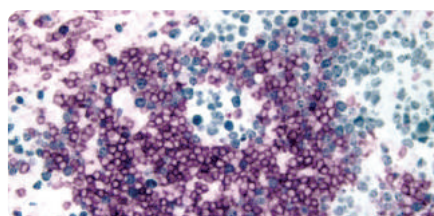
Second Substrate \ First Substrate		Alkaline Phosphatase			Peroxidase					
		ImmPACT Vector Red & Vector Red (magenta) SK-5105 & SK-5100	Vector Blue (blue) SK-5300	BCIP/NBT (indigo) SK-5400	ImmPACT VIP & Vector VIP (purple) SK-4605 & SK-4600	ImmPACT DAB, ImmPACT DAB EqV & DAB (brown) SK-4105, SK-4103, SK-4100	DAB-Ni (gray-black) SK-4100	ImmPACT NovaRED & Vector NovaRED (red) SK-4805 & SK-4800	ImmPACT SG & SG (blue-gray) SK-4705 & SK-4700	ImmPACT AEC, ImmPACT AMEC Red & AEC (red) SK-4205, SK-4285, SK-4200
Alkaline Phosphatase	ImmPACT Vector Red & Vector Red (magenta) SK-5105 & SK-5100		-	-	-	+	+	-	+	-
	Vector Blue (blue) SK-5300	+		-	+	+	+	+	+	+
	BCIP/NBT (indigo) SK-5400	+	-		+	+	+	+	+	+
Peroxidase	ImmPACT VIP & Vector VIP (purple) SK-4605, SK-4600	-	+	-		+	+	-	+	-
	ImmPACT DAB, ImmPACT DAB EqV & 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 & SG (blue-gray) SK-4705, SK-4700	+	-	-	+	+	-	-		+
	ImmPACT AEC, ImmPACT AMEC Red & AEC (red) SK-4205, SK-4285, SK-4200	-	-	-	-	+	-	-	+	

+ Indicates good contrast

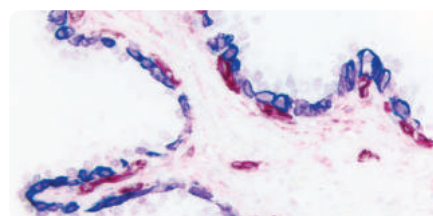
- Indicates incompatibility of substrates for various reasons



Breast Carcinoma: • Estrogen Receptor (m), VECTASTAIN Elite ABC Kit, Vector NovaRED HRP substrate (red) • CD34 (m), VECTASTAIN ABC-AP Kit, Vector Blue AP Substrate (blue) • Cytokeratin 8/18 (m), VECTASTAIN Elite ABC Kit, Vector DAB HRP Substrate (brown).



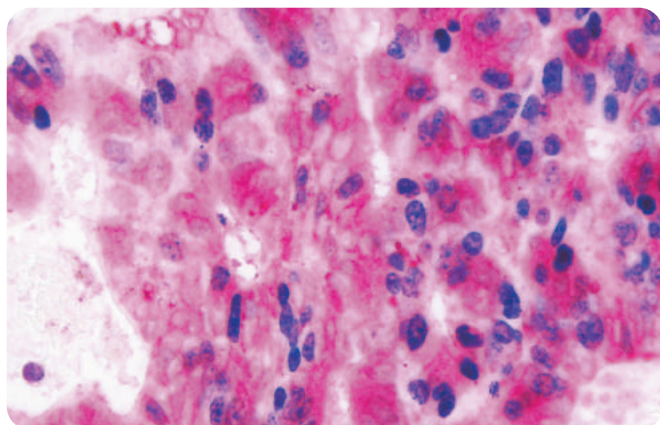
Tonsil: • CD3 (m), ImmPRESS Anti-Mouse IgG Reagent, Vector VIP HRP Substrate (purple) • Ki67 (m), ImmPRESS Anti-Mouse IgG Reagent, Vector SG HRP Substrate (blue/gray).



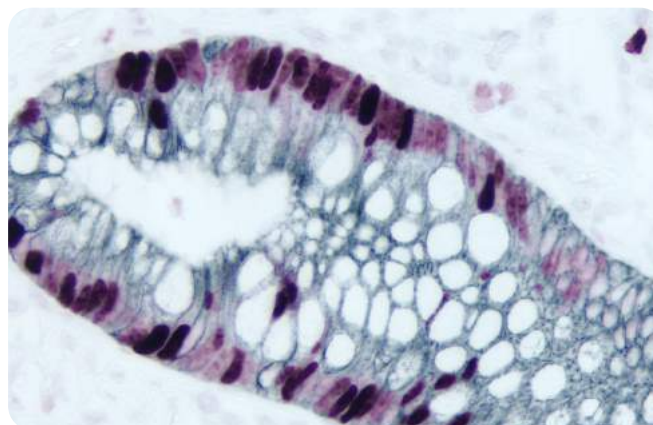
Prostate: • Cytokeratin 5 (m), VECTASTAIN Universal ABC-AP Kit, Vector Blue AP Substrate (blue) • CD34 (m), VECTASTAIN Universal ABC-AP Kit, Vector Red AP Substrate (red).



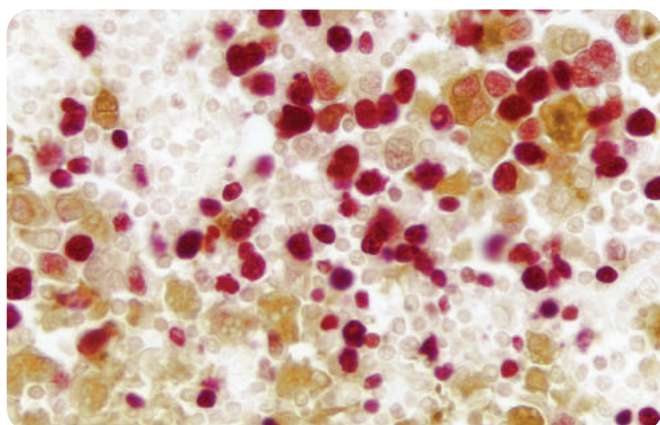
## Multiple Labeling Examples



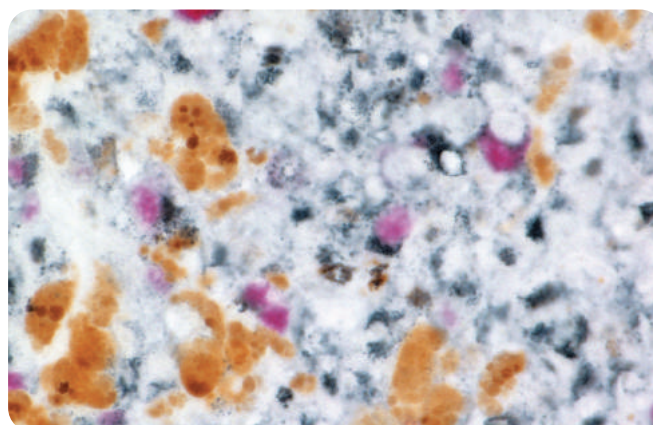
Colon Cancer: • Ki67 (rm), ImmPRESS-AP Anti-Rabbit IgG Reagent, Vector Blue AP Substrate (blue) • Cox2 (rm), ImmPRESS-AP Anti-Rabbit IgG Reagent, ImmPACT Vector Red AP Substrate (red).



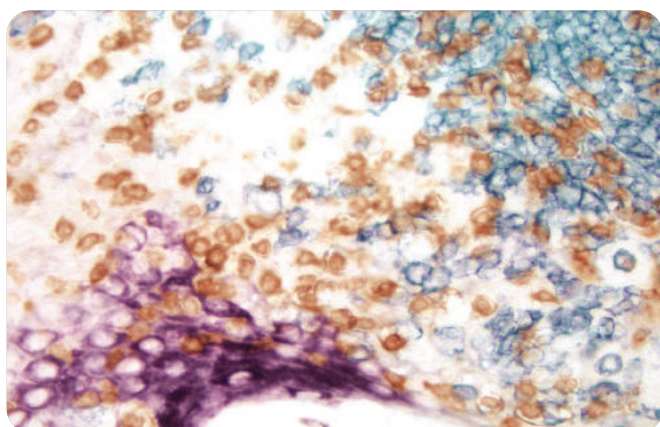
Colon: • Ki67 (rm), ImmPRESS Universal (Anti-Mouse/Anti-Rabbit IgG) HRP Reagent, Vector VIP (purple) • Cytokeratin (m), ImmPRESS Universal (Anti-Mouse/Anti-Rabbit IgG) HRP Reagent, Vector SG HRP Substrate (gray).



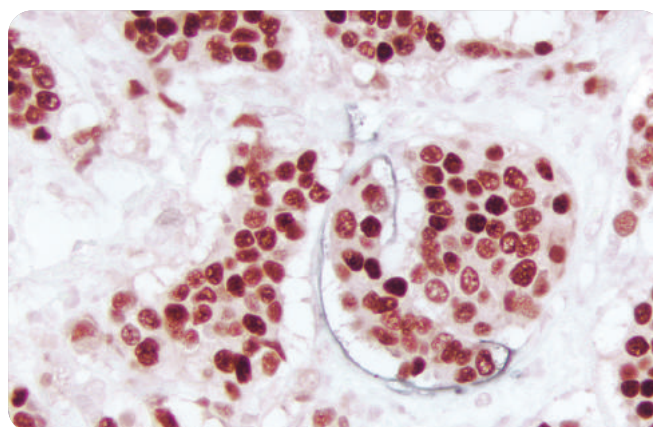
Lymph Node: Ki67 (m), VECTASTAIN Universal ABC-AP Kit, Vector Red AP Substrate (red); Multi-cytokeratin (m), VECTASTAIN Universal Elite ABC Kit, DAB HRP Substrate (brown).



Melanoma: • Cyclin A (m), ImmPRESS-AP Anti-Mouse IgG, ImmPACT Vector Red AP Substrate (magenta) • Melanoma Marker (m) ImmPRESS HRP Anti-Mouse IgG, Vector SG HRP Substrate (gray). Note contrast of double stain with the brown pigments in the tissue.



Tonsil: • CD3 (m), VECTASTAIN Elite ABC Kit (Universal), Vector DAB (brown) • CD20 (m), VECTASTAIN Elite ABC Kit (Universal), Vector SG (blue-gray) • Multi-Cytokeratin (m), VECTASTAIN Elite ABC Kit (Universal), Vector VIP (purple).



Breast Carcinoma: • Estrogen Receptor (rm), ImmPRESS Universal Reagent, Vector NovaRED HRP Substrate (red) • M2A Antigen (m), ImmPRESS Universal HRP Reagent, Vector DAB+Ni HRP Substrate (gray/black).



# Counterstaining

A counterstain introduces color to specific cellular structures to provide contrast to the colored enzyme substrate. Counterstaining aids in visualization and target localization, facilitating interpretation of morphology and cell structure within the tissue section. Our nuclear counterstains are packaged as convenient, ready-to-use solutions for use on individual slides or in staining dishes.

## Hematoxylin (blue)

- Based on Gill's III formulation
- Progressive stain formula. The intensity can be adjusted to optimize results for either manual or automated systems
- Excellent color contrast with most commonly used peroxidase and alkaline phosphatase substrates
- Suitable for use with non-aqueous and aqueous mounting media
- Alcohol- and mercury-free

## Hematoxylin QS (blue)

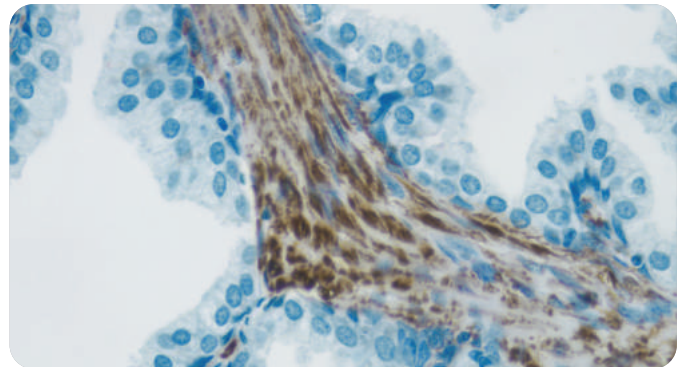
- Modification of Mayer's hematoxylin developed especially for immunocytochemistry
- Ready-to-use without filtration or 'blueing' step
- Stains in less than 45 seconds
- Excellent color contrast with most commonly used peroxidase and alkaline phosphatase substrates
- Suitable for use with non-aqueous and aqueous mounting media
- Mercury-free

## Methyl Green (light green)

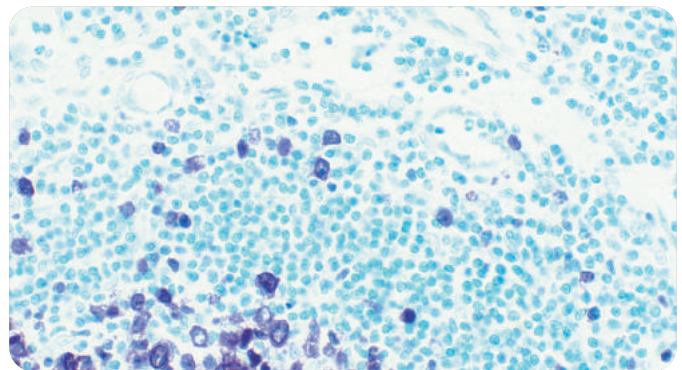
- Superior formulation of methyl green suitable for use with a wide range of enzyme substrates
- Simple, two-step procedure
- Excellent alternative in multiple antigen labeling when hematoxylin obscures the substrate colors
- Suitable for use with non-aqueous mounting media

## Nuclear Fast Red (pink)

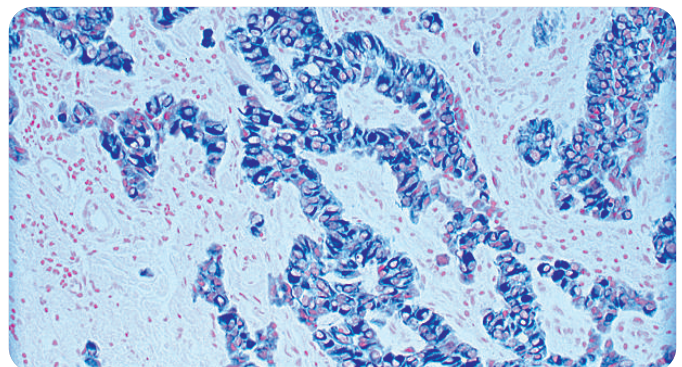
- Fast one-step protocol
- Excellent alternative in multiple antigen labeling when hematoxylin obscures the substrate colors
- Good contrast with a variety of substrates
- Suitable for use with non-aqueous mounting media



*Tumor tissue section showing specific cytoplasmic cell staining (DAB, brown) with Hematoxylin QS counterstain (blue).*



*Tonsil section showing specific cell staining (Vector VIP, purple) with Methyl Green counterstain (green).*



*Tumor tissue section showing specific cytoplasmic cell staining (SG, blue/gray) with Nuclear Fast Red counterstain (red).*

## Counterstain/Substrate Compatibility Table

This table is designed as a reference to determine the optimal counterstain/substrate combination for your application. Considerations should be given to tissue type, antigen unmasking protocol and other detection parameters to achieve the desired staining intensity.

Substrate	Catalog Number	Vector Hematoxylin and Hematoxylin QS H-3401 and H-3404	Vector Methyl Green H-3402	Vector Nuclear Fast Red H-3403
ImmPACT DAB (brown)	SK-4105	Excellent Contrast	Excellent Contrast	Fair Contrast
ImmPACT DAB EqV	SK-4103	Excellent Contrast	Excellent Contrast	Fair Contrast
DAB (brown)	SK-4100	Excellent Contrast	Excellent Contrast	Fair Contrast
DAB-Ni (gray-black)	SK-4100	Excellent Contrast	Fair Contrast *	Good Contrast
ImmPACT AEC (red)	SK-4205	Excellent Contrast	Counterstain Incompatibility **	Color Incompatibility
ImmPACT AMEC Red (red)	SK-4285	Excellent Contrast	Counterstain Incompatibility **	Color Incompatibility
AEC (red)	SK-4200	Excellent Contrast	Counterstain Incompatibility **	Color Incompatibility
TMB (blue)	SK-4400	Color Incompatibility	Counterstain Incompatibility	Excellent Contrast
ImmPACT VIP (purple)	SK-4605	Fair Contrast	Excellent Contrast	Poor Contrast
Vector VIP (purple)	SK-4600	Fair Contrast	Excellent Contrast	Poor Contrast
ImmPACT SG (blue-gray)	SK-4705	Poor Contrast	Good Contrast	Excellent Contrast
SG (blue-gray)	SK-4700	Poor Contrast	Good Contrast	Excellent Contrast
ImmPACT NovaRED (red)	SK-4805	Excellent Contrast	Excellent Contrast ***	Color Incompatibility
Vector NovaRED (red)	SK-4800	Excellent Contrast	Excellent Contrast ***	Color Incompatibility
ImmPACT Vector Red (magenta)	SK-5105	Excellent Contrast	Excellent Contrast	Color Incompatibility
Vector Red (magenta)	SK-5100	Excellent Contrast	Excellent Contrast	Color Incompatibility
Vector Black (black)	SK-5200	Excellent Contrast	Excellent Contrast *	Excellent Contrast
Vector Blue (blue)	SK-5300	Color Incompatibility	Good Contrast	Excellent Contrast
BCIP/NBT (indigo)	SK-5400	Color Incompatibility	Excellent Contrast *	Excellent Contrast

\* This substrate shows a slight decrease in sensitivity following the methyl green protocol. This decrease can be minimized by reducing the heat incubation and acetone rinse times in the methyl green protocol.

\*\* Substrate dissolves in acetone wash.

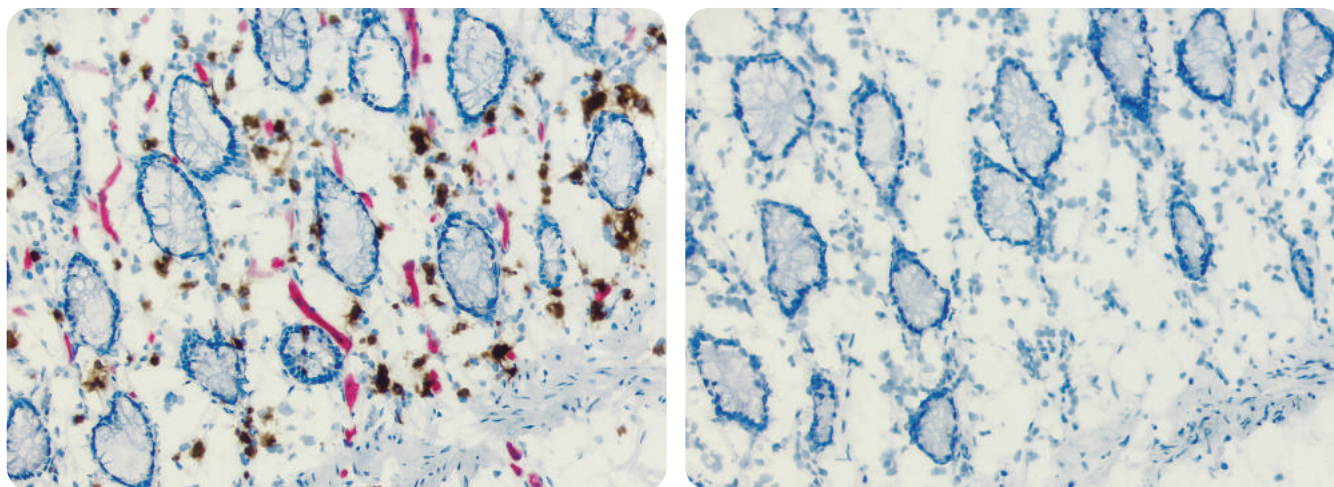
\*\*\* A slight color change in ImmPACT NovaRED and Vector NovaRED reaction product may be seen using methyl green.

Product	Mountants	Catalog Number
Hematoxylin	Non-aqueous and Aqueous	H-3401
Hematoxylin QS	Non-aqueous and Aqueous	H-3404
Methyl Green	Non-aqueous	H-3402
Nuclear Fast Red	Non-aqueous	H-3403



# Blocking Background Signal

Blocking agents minimize background signal from endogenous enzyme activity, biotin, and non-specific binding of tissue elements (charged particles, macromolecules, Fc receptors) with detection reagents.



*Endogenous alkaline phosphatase (AP) and peroxidase (HRP) activities in frozen, acetone-fixed intestine revealed with Vector Red AP Substrate (magenta) and ImmPACT DAB HRP Substrate (brown), (left). Same substrates used on BLOXALL Solution-treated tissue (right). BLOXALL Blocking Solution completely eliminates both endogenous enzyme activities.*

## BLOXALL Endogenous HRP/AP Blocking Solution

Tissues may contain endogenous peroxidase, pseudoperoxidase, and/or alkaline phosphatase activity that will produce background staining. BLOXALL Endogenous HRP/AP Blocking Solution inactivates each of these enzymes in one step.

- Compatible with formalin-fixed, paraffin-embedded tissue sections, frozen tissue sections, and cell preparations
- Ready-to-use in a convenient dropper bottle
- More effective than conventional blocking methods
- Brief 10-minute incubation

## Levamisole Solution

Levamisole Solution specifically inhibits endogenous alkaline phosphatase activity.

- Can be added to the alkaline phosphatase substrate solution
- Does not inhibit the isoenzyme used for the VECTASTAIN ABC-AP reagents, ImmPRESS- AP Reagents and other alkaline phosphatase conjugates
- Ready-to-use in a convenient dropper bottle

## Avidin/Biotin Blocking Kit

The Avidin/Biotin Blocking Kit blocks all endogenous biotin, biotin receptors, and avidin binding sites present in tissues to prevent non-specific binding of avidin or biotinylated reagents with avidin-biotin detection systems. Ready-to-use in a convenient dropper bottle.

## Streptavidin/Biotin Blocking Kit

Streptavidin/Biotin Blocking Kit blocks all endogenous biotin, biotin receptors, and streptavidin binding sites present in tissues to prevent non-specific binding of streptavidin or biotinylated reagents with biotin/streptavidin detection systems. Ready-to-use in a convenient dropper bottle.

## Normal Sera

Our Normal Sera are pooled samples collected from healthy adult animals. The serum is heat-treated and centrifuged to remove precipitates and then filtered. Each serum is tested with the appropriate antibody to check for possible cross-reactivities. The sera can be used to block non-specific binding or as an antibody diluent.

## 2.5% Normal Sera

Our 2.5% Normal Sera are pooled samples collected from healthy adult animals.

- Heat-treated and centrifuged to remove precipitates and then filtered
- Tested for cross-reactivities
- Can be used for blocking non-specific binding or as an antibody diluent

## Bovine Serum Albumin (BSA)

Immunohistochemical Grade.

- Can be used as a diluent or a blocking agent
- Free of impurities present in other grades of BSA, which can introduce artifacts or increase background staining in IHC staining, ELISAs, or blots

## 10x Casein Solution

10x Casein Solution is a general blocking agent for IHC, nucleic acid blotting, protein blotting, and other applications.

## Carbo-Free Blocking Solution

Carbo-Free Blocking Solution is a protein-based agent that is essentially free of glycoproteins. It is ideal for applications using lectins in which glycoprotein contamination could generate background staining or false positive results.

- Can be used to block non-specific binding or as an antibody diluent

## R.T.U. Animal-Free Blocker and Diluent

This plant protein-derived product is a universal antibody diluent and blocking reagent intended for cell- and tissue-based IHC and IF applications. This ready-to-use solution can be used as an alternative to normal sera, BSA, casein and non-fat dry milk.

R.T.U. Animal-Free Block and Diluent is supplied without any sodium azide. It can therefore be used with both peroxidase and alkaline phosphatase antibody conjugates and all secondary detection reagents including polymer systems and avidin/ biotin reagents that incorporate these enzymes. This makes the blocking solution especially convenient in multiple antigen labeling IHC applications in which antibodies from different species and a variety of detection systems are used on the same tissue section.

R.T.U. Animal-Free Block and Diluent is a unique formulation different from our concentrated (5x) animal-free blocker. It has been designed with optimized conditions and neutral pH specifically for IHC and IF methods.

## Animal-Free Blocker (5x concentrate solution)

Animal-Free Blocker is a plant-derived blocking agent and diluent for IHC, nucleic acid blotting, protein blotting, and other applications. This reagent contains no animal-derived protein and can be used as an alternative to sera, BSA, casein, or non-fat dry milk.

Product	Catalog Number
BLOXALL® Endogenous Blocking Solution	SP-6000
Levamisole Solution	SP-5000
Avidin/Biotin Blocking Kit	SP-2001
Streptavidin/Biotin Blocking Kit	SP-2002
Normal Goat Serum	S-1000
Normal Horse Serum	S-2000
Normal Chicken Serum	S-3000
Normal Swine Serum	S-4000
Normal Rabbit Serum	S-5000
2.5% Normal Goat Serum	S-1012
2.5% Normal Horse Serum	S-2012
Bovine Serum Albumin (BSA)	SP-5050
10x Casein Solution	SP-5020
Carbo-Free™ Blocking Solution	SP-5040
R.T.U. Animal-Free Blocker® and Diluent	SP-5035
Animal-Free Blocker®	SP-5030

# Secondary and Tertiary Detection Reagents

Our secondary antibodies are prepared by hyper-immunizing animals in a manner that produces high-affinity antibodies. These are then purified by an affinity chromatography procedure designed to remove any low-affinity antibodies. Cross-reactivities that can interfere with specific labeling are removed by solid-phase adsorption techniques. The final product is then subjected to rigorous quality-control assays including immunodiffusion, solid-phase enzyme immunoassays, gel electrophoresis, solid-phase binding assays, and IHC tissue staining. These unconjugated antibodies are used to generate our enzyme conjugated and biotinylated secondary antibodies.

## Biotinylated and Unconjugated Secondary Antibodies

Our high-affinity, purified, biotinylated and unconjugated secondary antibodies are manufactured under controlled conditions to retain maximum specificity and affinity. Our secondary antibodies are subjected to rigorous quality control assays and can be used for tissue and cell staining, ELISAs, and blotting.

Secondary Antibodies	Biotinylated					Unconjugated		
	Host Species (Concentrate)			Host Species (R.T.U.)†		Host Species (Concentrate)		
	Goat	Rabbit	Horse	Goat	Horse	Goat	Rabbit	Horse
Anti-Cat IgG (H+L)	BA-9000							
Anti-Chicken IgG (H+L)	BA-9010							
Anti-Goat IgG (H+L)		BA-5000						
Anti-Goat IgG (H+L)			BA-9500		BP-9500		AI-5000	
Anti-Guinea Pig IgG (H+L)	BA-7000							
Anti-Hamster IgG (H+L)	BA-9100					AI-9100		
Anti-Horse IgG (H+L)	BA-8000							
Anti-Mouse IgG (H+L)			BA-2000		BP-2000	AI-9200		
Anti-Mouse IgG (H+L), rat adsorbed			BA-2001					
Anti-Mouse IgG (H+L)	BA-9200			BP-9200				AI-2000
Anti-Mouse IgM (H+L), mu chain specific	BA-2020							
Anti-Rabbit IgG (H+L)	BA-1000			BP-9100		AI-1000		
Anti-Rabbit IgG (H+L)			BA-1100		BP-1100			
Anti-Rat IgG (H+L)		BA-4000						
Anti-Rat IgG (H+L), mouse adsorbed		BA-4001					AI-4001	
Anti-Rat IgG (H+L)	BA-9400			BP-9400				
Anti-Rat IgG (H+L), mouse adsorbed	BA-9401							
Anti-Sheep IgG (H+L)		BA-6000						
Universal Anti-Mouse/Rabbit IgG (H+L)			BA-1400		BP-1400			
Universal Pan-Specific Anti-Mouse/Rabbit/Goat IgG (H+L)			BA-1300					

† Ready-to-use, prediluted stabilized solutions



Anti-Human Secondary Antibodies	Biotinylated	Unconjugated
	Host Species (Concentrate)	Host Species (Concentrate)
	Goat	Goat
Anti-Human IgG (H+L)	BA-3000	AI-3000
Anti-Human IgE, ε (Epsilon) chain specific	BA-3040	
Anti-Human IgG, γ (Gamma) chain specific	BA-3080	AI-3080
Anti-Human IgM, μ (Mu) chain specific	BA-3020	AI-3020
Anti-Human κ (Kappa) Chain, kappa chain specific	BA-3060	AI-3060

### Enzyme Conjugated Secondary Antibodies

Our high-affinity, purified antibodies are cross-linked with alkaline phosphatase (AP) or horseradish peroxidase (HRP) of the highest specificity. Our conjugation method ensures the maximum preservation of enzyme activity and antibody specificity. Recommended applications include tissue staining, ELISAs, and blotting.

Product	Catalog Number
<b>Peroxidase</b>	
Anti-Mouse IgG (H+L) made in horse Peroxidase labeled	PI-2000
Anti-Rabbit IgG (H+L) made in goat Peroxidase labeled	PI-1000
Anti-Human IgG (H+L) made in goat Peroxidase labeled	PI-3000
Anti-Goat IgG (H+L) made in horse Peroxidase labeled	PI-9500

### Avidin and Streptavidin Enzyme Conjugates

Our enzyme-conjugated avidin and streptavidin are suitable for use in solid-phase assays, tissue/cell staining systems, and blotting. The conjugates are produced in optimized ratios with enzymes of the highest specific activity. Covalent linkages are specifically chosen to provide stable, highly active conjugates.

Product	Catalog Number
<b>Alkaline Phosphatase</b>	
Alkaline Phosphatase Streptavidin	SA-5100
Alkaline Phosphatase Avidin D	A-2100
<b>Peroxidase</b>	
Horseradish Peroxidase Streptavidin, concentrate	SA-5004
Horseradish Peroxidase Streptavidin, R.T.U.	SA-5704
Horseradish Peroxidase Avidin D, concentrate	A-2004

# Mounting Media

## VectaMount Express Mounting Medium

VectaMount Express is a non-aqueous clearing and mounting medium enabling the rapid mounting of cell and tissue specimens following IHC staining. This novel formulation is engineered to enable mounting directly following staining, saving time by eliminating the need for extensive ethanol and clearing washes prior to coverslipping. Just stain your slides as per your usual workflow, briefly wash in isopropyl alcohol, and coverslip.

- Non-aqueous clearing and mounting medium for IHC-stained slides
- Eliminates the need for extensive ethanol washes and solvent-based clearing agents (e.g., xylene)
- Rapid drying formula for fast visualization of stained samples
- Compatible with HRP and AP enzyme substrates
- Preserves staining for at least 18 months at room temperature
- Refractive index of 1.49

## VectaMount Permanent Mounting Medium

VectaMount Mounting Medium is an optically clear formula for permanently preserving histochemical stains or precipitable enzyme substrates in tissue sections or cell preparations.

- Permanent non-aqueous mounting
- Low hazard
- Compatible with horseradish peroxidase, alkaline phosphatase, and glucose oxidase substrates
- Refractive index: 1.49 when dry

## VectaMount AQ Aqueous Mounting Medium

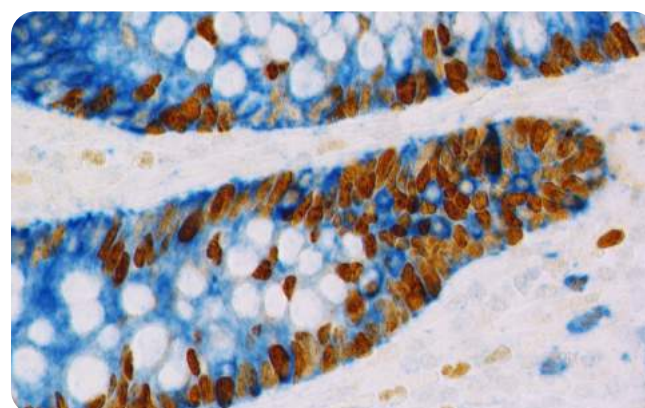
VectaMount AQ Aqueous Mounting Medium preserves the color and clarity of enzyme substrates whose reaction products are soluble in alcohol or other organic solvents. Stained and mounted sections can be stored in a slide box at room temperature for at least two years without fading.

- Hard-setting
- Simple to use, requires no mixing

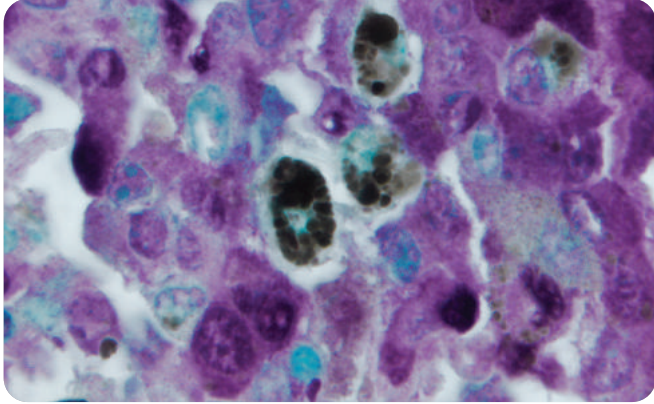
Product	Catalog Number
VectaMount® Express Mounting Medium	H-5700
VectaMount® Permanent Mounting Medium	H-5000
VectaMount® AQ Aqueous Mounting Medium	H-5501

## Mounting Media/Substrate Compatibility

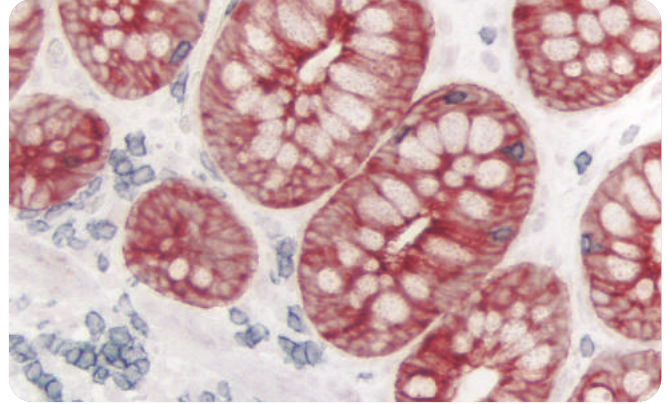
Substrate	VectaMount Express Mounting Medium	VectaMount Permanent Mounting Medium	VectaMount AQ Aqueous Mounting Medium
<b>Peroxidase</b>			
DAB	•	•	•
DAB-Ni	•	•	
ImmPACT DAB	•	•	•
ImmPACT DAB EqV	•	•	•
Vector VIP	•	•	
ImmPACT VIP	•	•	
Vector NovaRED	•	•	
ImmPACT NovaRED	•	•	
Vector SG	•	•	•
ImmPACT SG	•	•	•
AEC			•
ImmPACT AEC			•
ImmPACT AMEC Red			•
TMB		•	
<b>Alkaline Phosphatase</b>			
Vector Red	•	•	•
ImmPACT Vector Red	•	•	•
Vector Blue	•	•	•
Vector Black		•	
BCIP/NBT	•	•	•



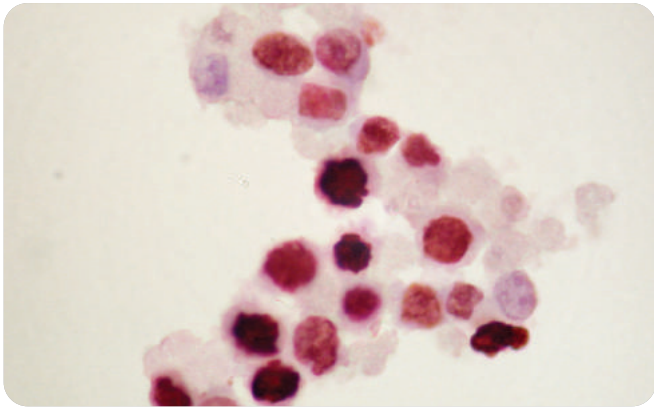
Human Colon – double label: Mouse Anti-Ki67, ImmPRESS-HRP Anti-Mouse IgG, ImmPACT DAB EqV and Rabbit Anti-Cytokeratin ImmPRESS-AP Anti-Rabbit IgG, Vector Blue. Mounted in VectaMount Express Mounting Media.



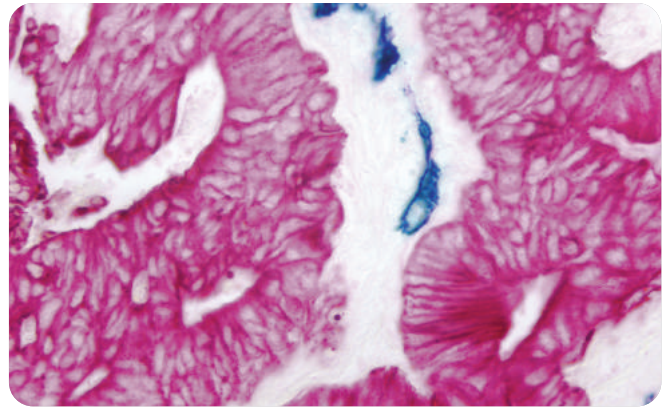
Melanoma: S100 (rp), VECTASTAIN Elite ABC Kit, Vector VIP (purple), Vector Methyl Green counterstain (green). Note color contrast with brown pigments in tissue.



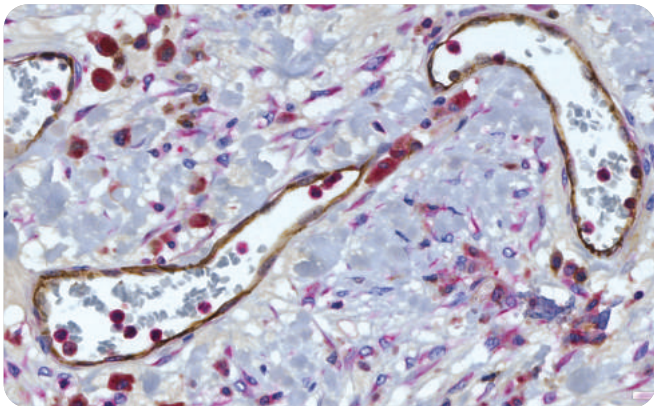
Colon: • CD3 (rm), ImmPRESS Reagent (HRP) Anti-Rabbit IgG, ImmPACT SG (blue-gray) • Cytokeratin AE1/AE3 (m), ImmPRESS Reagent (HRP) Anti-Mouse IgG, ImmPACT AMEC Red (red).



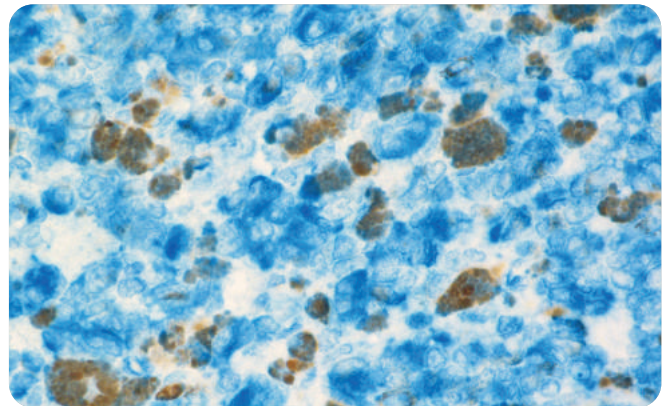
Cytospin of EBV+ cell line: Epstein-Barr virus nuclear antigen 1 (EBNA-1; rat), ImmPRESS Reagent (HRP) Anti-Rat IgG, ImmPACT NovaRED (red). (Image courtesy of Dr. GM Reynolds, Centre for Liver Research, University of Birmingham, U.K.)



Tumor: • CD34 (m), VECTASTAIN Universal ABC-AP Kit, Vector Blue AP Substrate (blue) • Cytokeratin 8/18 (m), VECTASTAIN Universal ABC-AP Kit, Vector Red AP Substrate (red).



Breast carcinoma: • CD31 (m), ImmPRESS Anti-Mouse IgG HRP Reagent, ImmPACT DAB EqV HRP Substrate (brown) • MRC1 (r), ImmPRESS- AP Anti-Rabbit IgG Reagent, ImmPACT Vector Red AP Substrate (magenta). Image courtesy of Richard Allen, part of the Academic Unit of Inflammation and Tumour Targeting headed by Professor Claire Lewis.



Melanoma: Vimentin (rm), ImmPRESS-AP Anti-Rabbit IgG Reagent, Vector Blue AP Substrate (blue). Note color contrast with brown pigments in tissue.



# Accessory Reagents

## VECTABOND Reagent Tissue Section Adhesive

VECTABOND Reagent chemically modifies the surface of glass to form a highly adherent charged surface. This charge significantly increases the adherence of both frozen and paraffin-embedded tissue sections and cell preparations to glass microscope slides and coverslips. Tissue sections will remain attached even when subjected to the most extreme conditions, such as high-temperature antigen retrieval and *in situ* hybridization. VECTABOND Reagent treated slides can be stored indefinitely.

## ImmEdge Hydrophobic Barrier Pen

The ImmEdge Pen is a hydrophobic barrier (PAP) pen for immunohistochemistry and *in situ* hybridization. It provides a water-repellent barrier that keeps reagents localized on tissue specimens and prevents mixing of reagents when multiple sections are mounted on the same slide.

- Heat-stable
- Insoluble in alcohol and acetone
- Stable for use with buffers with and without detergent (Tween 20™, Triton™ X-100, etc.)
- Completely removed by all commonly used xylene and xylene-substitute clearing agents
- Contains no ozone-depleting solvents
- Compatible with both enzyme- and fluorescence-based detection systems



## ImmPrint Histology Pen

The ImmPrint Histology Pen is a permanent marking pen designed for writing on glass microscope slides, tissue cassettes, and most hard surfaces. Unlike other pens commonly used for histology, the ImmPrint Pen has a smooth writing tip that resists drying out.

- High-density, fast-drying, black ink
- Resistant to most organic solvents encountered in histological applications

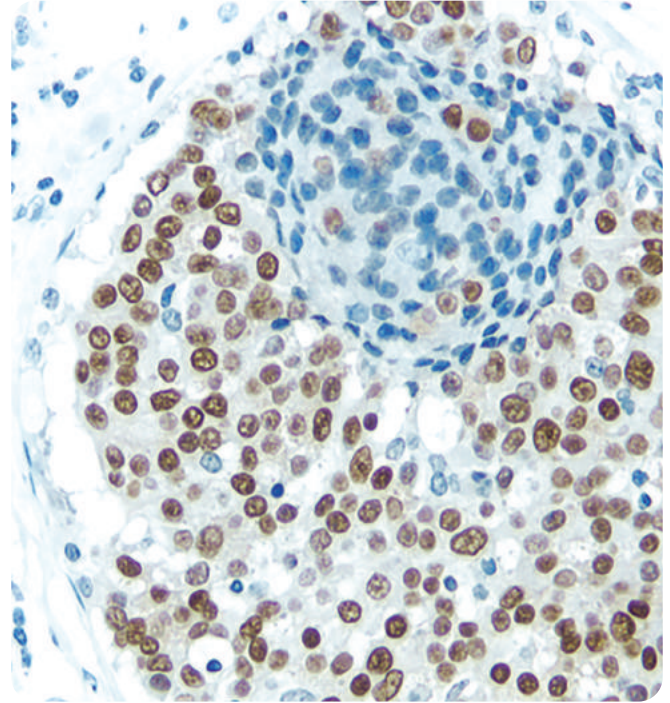
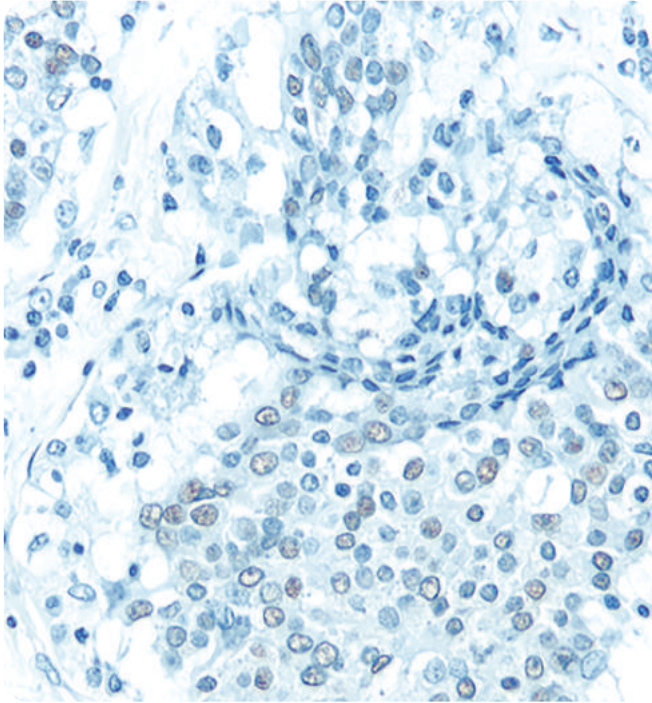
## Control Antibodies

These antibodies are IgG preparations for use as controls for primary antibodies made in rabbit, mouse, rat, or goat. Each has been purified from pooled serum of healthy adult animals and contain a spectrum of the IgG subclasses. When applied appropriately, these controls will help determine whether the primary antibody staining signal is specific for the antigen or whether staining is the result of non-specific adsorption of primary antibody to tissue sites.

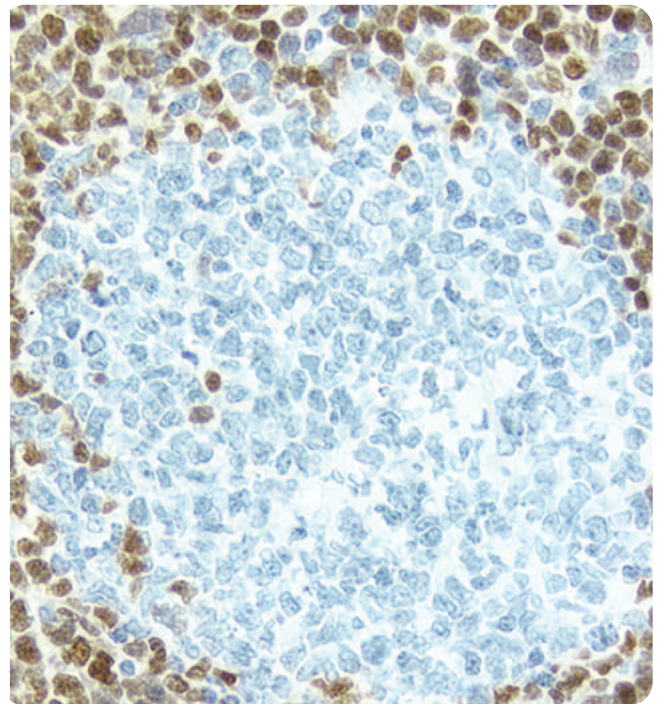
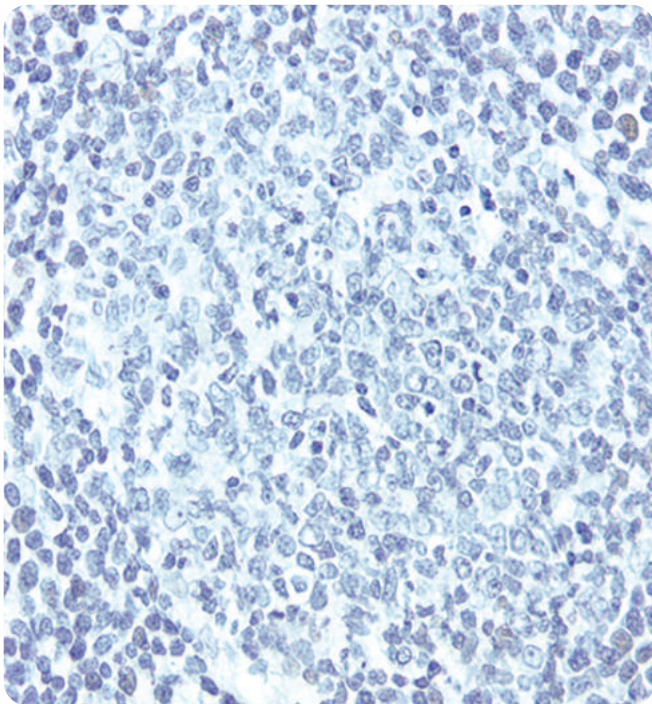
## Antigen Unmasking Solutions

Our Antigen Unmasking Solutions are highly effective at revealing antigens in formalin-fixed, paraffin-embedded tissue sections when used in combination with a high temperature treatment procedure. We offer two formulations of Antigen Unmasking Solution: Citrate-based solution (pH 6.0) and Tris-based solution (pH 9.0), each supplied as 100X concentrated stocks.

Product	Catalog Number
VECTABOND® Reagent (Tissue Section Adhesive)	SP-1800
ImmEdge® Hydrophobic Barrier PAP Pen	H-4000
ImmPrint™ Histology Pen	H-6100
<b>Control Antibodies</b>	
Rabbit IgG	I-1000
Mouse IgG	I-2000
Rat IgG	I-4000
Goat IgG	I-5000
<b>Antigen Unmasking Solutions</b>	
Citrate-based (100X) (pH 6.0)	H-3300
Tris-based (100X) (pH 9.0)	H-3301



Breast Carcinoma: Without (left panel) and with (right panel) Citrate-based Antigen Unmasking Solution, Estrogen receptor (m), ImmPRESS Anti-Rabbit IgG Kit, DAB (brown) substrate. Hematoxylin QS (blue) counterstain.



Lymph Node: Without (left panel) and with (right panel) TRIS-based Antigen Unmasking Solution, Cyclin D1 (rm), ImmPRESS Anti-Rabbit IgG Kit, DAB (brown) substrate. Hematoxylin QS (blue) counterstain.



# Contact Details

## Ordering Information

Order online at [vectorlabs.com](https://vectorlabs.com)

Orders may also be placed by email, telephone, or mail. Please include the following with each order:

- Product name and catalog number
- Unit size and quantity
- Billing and shipping addresses
- Purchase order number
- Name, phone number, address and email address of person placing order

Orders using VISA, Mastercard, or American Express are accepted and processed immediately. Telephone orders over \$1000 may require written confirmation. A confirmation should be boldly marked "Confirming Order. Do Not Duplicate". Duplicate shipments due to incorrectly marked confirming orders cannot be returned for credit. No returned product will be accepted or credited without prior authorization from Vector Laboratories.

Please contact us to discuss discounts for custom or large orders.

## Payment/shipping terms

For non-credit card orders, our payment terms are net 30 days from date of invoice, title and risk of loss transfer Ex Works (Incoterms 2010) Seller's location, freight prepaid and added unless shipped on Buyer's account (FedEx, UPS, DHL). Buyers are required to submit a credit application before credit terms are extended. Orders placed before 3 pm Pacific Time on Monday through Friday (excluding holidays) are usually processed the same day they are received. Unless requested otherwise,



## USA Headquarters:

Vector Laboratories, Inc.  
6737 Mowry Avenue  
Newark, CA 94560  
Tel: +1 (650) 697-3600  
Ordering and Technical Service (USA): +1 (800) 227-6666  
Customer Service: [vector@vectorlabs.com](mailto:vector@vectorlabs.com)  
Technical Support: [technical@vectorlabs.com](mailto:technical@vectorlabs.com)  
International Inquiries: [techintl@vectorlabs.com](mailto:techintl@vectorlabs.com)

Although provided in a highly purified form, our products are not intended for clinical diagnosis or drug use, nor have they been packaged under sterile conditions. All products listed in this catalog are for research purposes only. The listing of any product in this catalog does not imply the absence of a patent covering its use, does not constitute license under any existing or pending patent, nor is it intended or implied as a recommendation for the use of such products in infringement of any patent. The responsibility for determining the existence of such patents rests solely with the user.

Animal-Free Blocker, BLOXALL, Carbo-Free, Elite, H.O.H., ImmEdge, ImmPACT, ImmPACT NovaRED, ImmPRESS, ImmPRINT, M.O.M., VECTABOND, VectaMount, VECTASHIELD, VECTASTAIN, Vector, and Vector NovaRED are trademarks of Vector Laboratories, Inc. All other trademarks cited herein are the property of their respective owners.

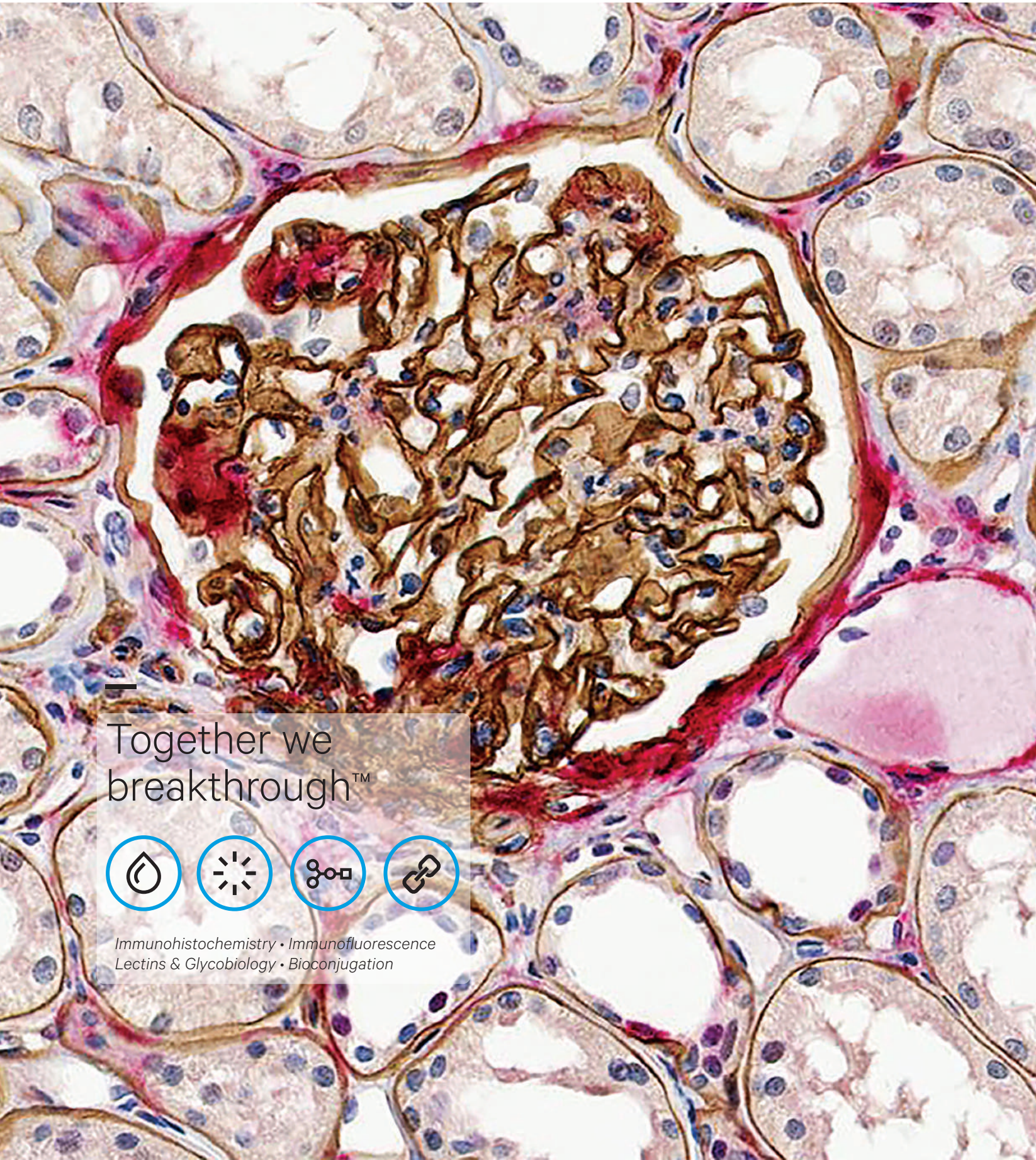
For more information please visit  
[vectorlabs.com](https://vectorlabs.com)

©2022 Vector Laboratories. All rights reserved.  
For research use only. Not intended for animal or human therapeutic or diagnostic use.  
LIT3001.Rev00









—  
Together we  
breakthrough™



*Immunohistochemistry • Immunofluorescence  
Lectins & Glycobiology • Bioconjugation*