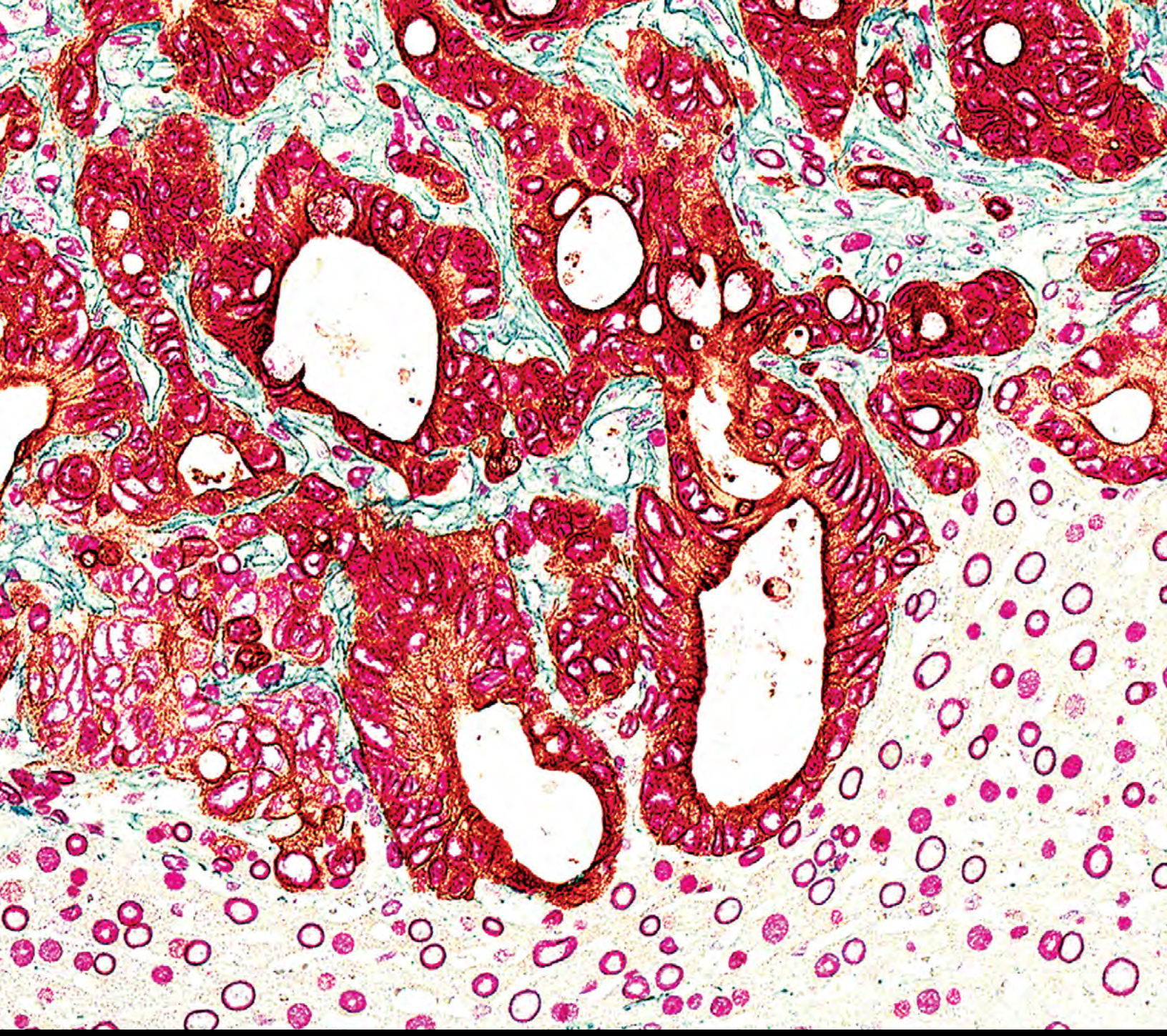


Immunohistochemistry Guide

VECTOR LABORATORIES

part of **Maravai LifeSciences**

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.

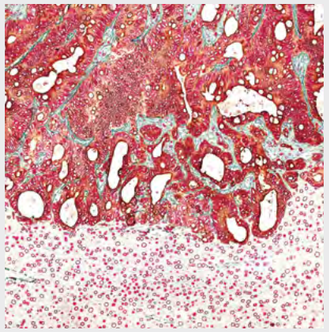
C. A knowledge base of over 100 years of combined IHC experience to help you accelerate the pace of discovery.

It's as simple as that.

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

Table of Contents

Vector Laboratories was founded on a growing portfolio of purified lectins and lectin conjugates that helped to pioneer lectin histochemistry. These products remain a key component of our business today. In the early 1980s, we leveraged our expertise in histochemistry to revolutionize the field of IHC with the commercialization of antibody-based avidin-biotin reagents and the introduction of the VECTASTAIN® ABC system. This system enabled routine laboratory use of IHC with any standard brightfield microscope. Following the success of the ABC kits, Vector Laboratories has continued to introduce many novel and innovative products to support research endeavors for cell and tissue antigen visualization. These include the ImmPRESS™ micropolymer reagents, Mouse on Mouse (M.O.M.™) detection systems, unique ImmPACT™ enzyme substrates, and VECTASHIELD® antifade mounting media for fluorescent applications.



Front cover: Mouse liver section in which human colorectal cancer-derived organoids have spread. This liver metastasis shows the invading tumor front (keratin-20, brown tumor cell) growing from the top of the images towards the bottom, embedded in increasing surrounding connective tissue (fibronectin, green fibers). All nuclei of human tumor cells and the mouse hepatocytes in the lower part of the image were stained with a nuclear membrane marker (lamin A, red round shapes). Slight green staining in the lower part of the image shows connective tissue that surrounds blood vessels in normal liver tissue. (Image provided by Dr. Steffen Rickelt and Dr. Jatin Roper, Massachusetts Institute of Technology, USA).

Immunohistochemistry Workflow

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

Tissue Preparation	Antigen Retrieval	Quench/ Block	Primary Antibody/ Lectins*	Secondary Antibody	Tertiary Reagent	Substrate/ Chromogen	Counterstain	Coverslip/ Mount	Visualize
• VECTABOND™ Tissue Section Adhesive • ImmPrint™ Histology Pen • ImmEdge™ Hydrophobic Barrier Pen	• Antigen Unmasking Solutions, Citrate- or Tris-based	• BLOXALL™ Endogenous HRP and AP Blocking Solution • Avidin/Biotin Blocking Kit • Streptavidin/Biotin Blocking Kit • Normal Sera • Animal-Free Block and Diluent • BSA • Casein Solution • Vector® M.O.M.™ Mouse Ig Blocking Reagent • Carbo-Free Block	• Biotinylated lectins • Unconjugated lectins	• ImmPRESS™ Polymer Reagents (HRP or AP) • Mouse on Mouse (M.O.M.™) ImmPRESS™ Polymer Kit • Mouse on Mouse (M.O.M.™) Basic Kit • Biotinylated secondary antibodies • Unconjugated secondary antibodies • Enzyme-conjugated secondary antibodies (HRP or AP) • Biotinylated anti-lectins	• VECTASTAIN® ABC complex reagents • ImmPRESS™ Excel Amplified Staining Kits • Mouse on Mouse (M.O.M.™) Elite® Kit (HRP) • Enzyme-conjugated avidin/streptavidin (HRP or AP)	• HRP substrates • AP substrates/ Levamisole Solution	• Hematoxylin • Methyl Green • Nuclear Fast Red	• VectaMount™ Mounting Medium • VectaMount™ AQ Mounting Medium	

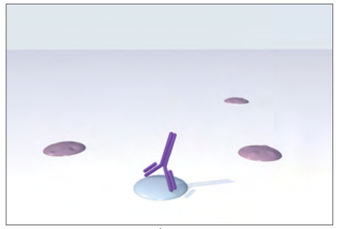
* For more information visit: vectorlabs.com/lectins

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)

Blocking Reagents

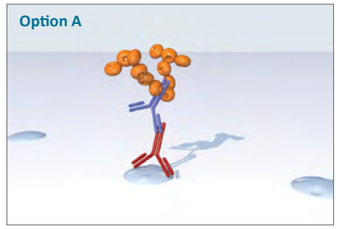
- Choices determined by the options selected in Steps 1-4
- BLOXALL™ Endogenous HRP and AP Blocking Solution
- Avidin/Biotin Blocking Kit (if using VECTASTAIN® ABC system)
- Normal Sera (from the species of secondary antibody)
- M.O.M.™ Mouse Ig Blocking Reagent
- Animal-Free Block and Diluent
- BSA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

SECONDARY DETECTION SYSTEMS

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

Option A

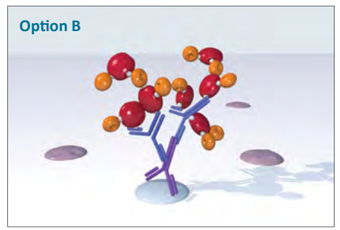


One Step
Convenient. Consistent. Ready-to-use. Non-Biotin based.

- ImmPRESS™ Polymer Reagents

or

Option B

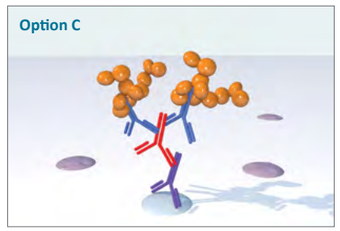


Two Step
Economical. Biotin-based.

- Biotinylated secondary antibody + ABC Complex (VECTASTAIN® Elite® ABC Kit)

or

Option C

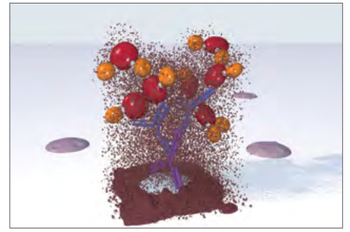


Two Step
Highest sensitivity. Non-biotin based.

- ImmPRESS™ *Excel* Amplified Polymer Staining Systems

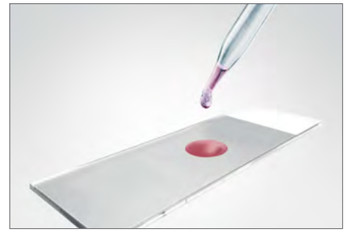
3 Choose Enzyme Substrate

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



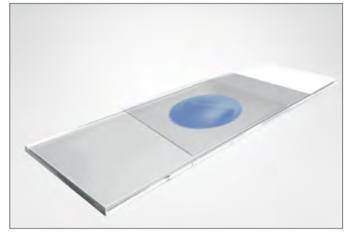
4 Choose Nuclear Counterstain

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



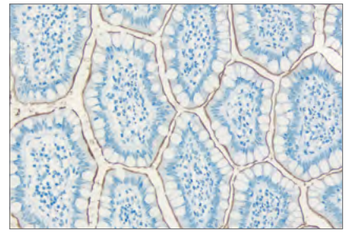
5 Choose Mounting Media

- 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 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-labeled 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.

During this time Dr. Shan-Rong Shi introduced "antigen retrieval" for formaldehyde-fixed tissues. This technique allowed IHC to be readily performed on formalin-fixed, paraffin-embedded tissues, greatly increasing clinical utility of IHC. However, in addition to improving antigenicity in tissue sections, antigen retrieval also exposed numerous sites of endogenous biotin that were previously undetected. This required steps to be added to IHC staining protocols to block endogenous biotin in biotin-containing specimens. In clinical settings in particular, antibody detection strategies returned to non-biotin HRP systems to avoid confusion resulting from endogenous biotin. However, the choice was not clear-cut, as avidin-biotin detection systems offered greater sensitivity than the previous peroxidase-labeled antibody systems.

This dilemma was finally resolved in the mid-2000s by the emergence of biotin-free polymer/multimer detection systems that offered similar sensitivity to avidin-biotin detection systems. Although early polymer-based systems suffered from background and tissue penetration problems, today's systems deliver performance comparable to the best avidin-biotin detection systems.

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)

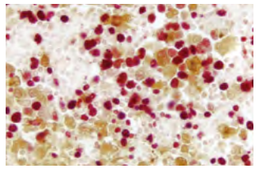
Childs GV "History of Immunohistochemistry" *Pathobiology of Human Disease.* 3775-3796 (2014)

1941
Albert Coons demonstrated that fluorescently labeled antibodies can be used to localize bacteria inside macrophages (immunohistochemistry).

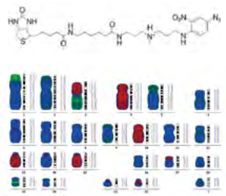
1966
Stratis Avrameas and Paul Nakane independently developed methods to covalently couple the enzyme horseradish peroxidase (HRP) to antibodies

1980
Vector Labs first to commercialize avidin-biotin enzyme complex (VECTASTAIN® ABC kits)

1981
S.M. Hsu published that the high affinity of avidin for biotin could be used to increase the stability of the enzyme antibody complex



1986
Vector Labs launched PHOTOPROBE® Biotin

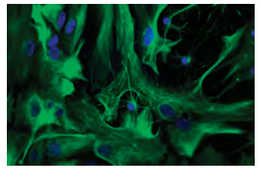


1991
S.R. Shi developed the antigen retrieval process for formaldehyde fixed tissues

1993
Vector Labs introduced first antifade mounting media for fluorescence (VECTASHIELD® Mounting Media)

1999
Vector Labs introduced a next-generation PAP pen conforming with environmental regulations (ImmEdge™ Pen)

2004
Vector Labs expanded antifade mounting media with hardening formulation (VECTASHIELD® HardSet™ Mounting Media)

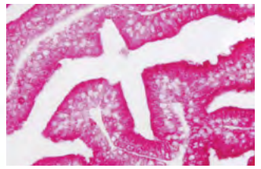


2005
Vector Labs developed detection reagents enabling greater access to antigens within tissues and improving multiple antigen labeling (ImmPRESS™ enzyme polymer)

2007
Vector Labs introduced proprietary substrates enabling multiplexing IHC (ImmPACT™ HRP substrates)

2011
Vector Labs introduced BLOXALL® Endogenous HRP and AP Blocking Solution

2014
Vector Labs introduced additional proprietary AP substrate (ImmPACT™ Vector® Red substrate)



2016
Strategic investment by Maravai LifeSciences

maravai LifeSciences

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.

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

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.

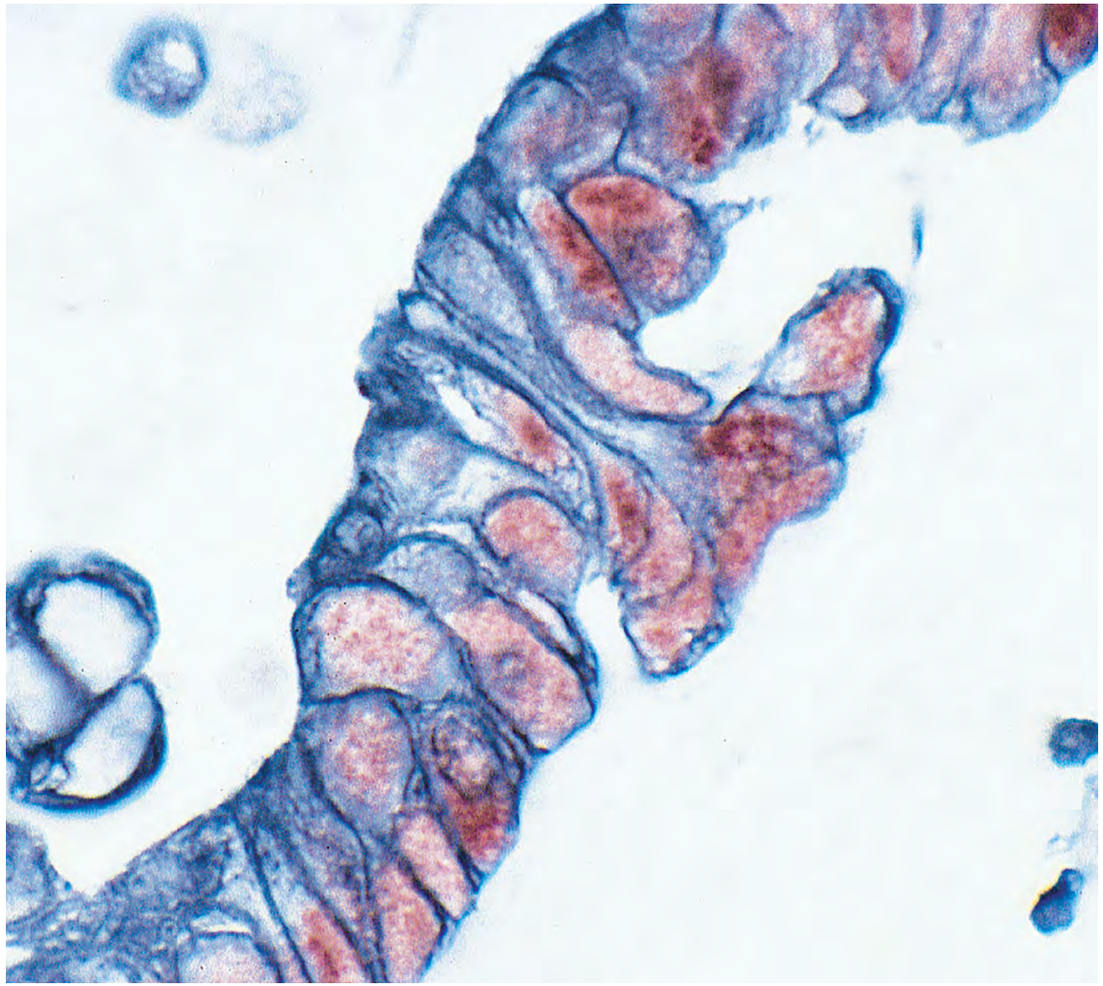
Detection System	Enzyme	Sensitivity	Cost/ Assay	Biotin-Free	Micro-polymer	Modular	Mouse Primary on Mouse Tissue	Ready-to-Use (R.T.U.) Format	Typical number of steps
ImmPRESS™ Kits									
ImmPRESS™ Excel Amplified HRP Polymer Kits	HRP	••••••	••••	•	•			•	2
ImmPRESS™ HRP Polymer Kits	HRP	•••••	•••	•	•			•	1
ImmPRESS™ VR HRP Polymer Kits	HRP	•••••	•••	•	•			•	1
ImmPRESS™ AP Polymer Kits	AP	•••••	•••	•	•			•	1
VECTASTAIN® Kits									
VECTASTAIN® Elite® ABC Kits	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
Mouse on Mouse (M.O.M.™) Kits									
Mouse on Mouse (M.O.M.™) ImmPRESS™ Polymer Kit	HRP	•••	•••	•	•		•		1
Mouse on Mouse (M.O.M.™) Kits	HRP	•••	•••			•	•		2
Additional Options									
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).

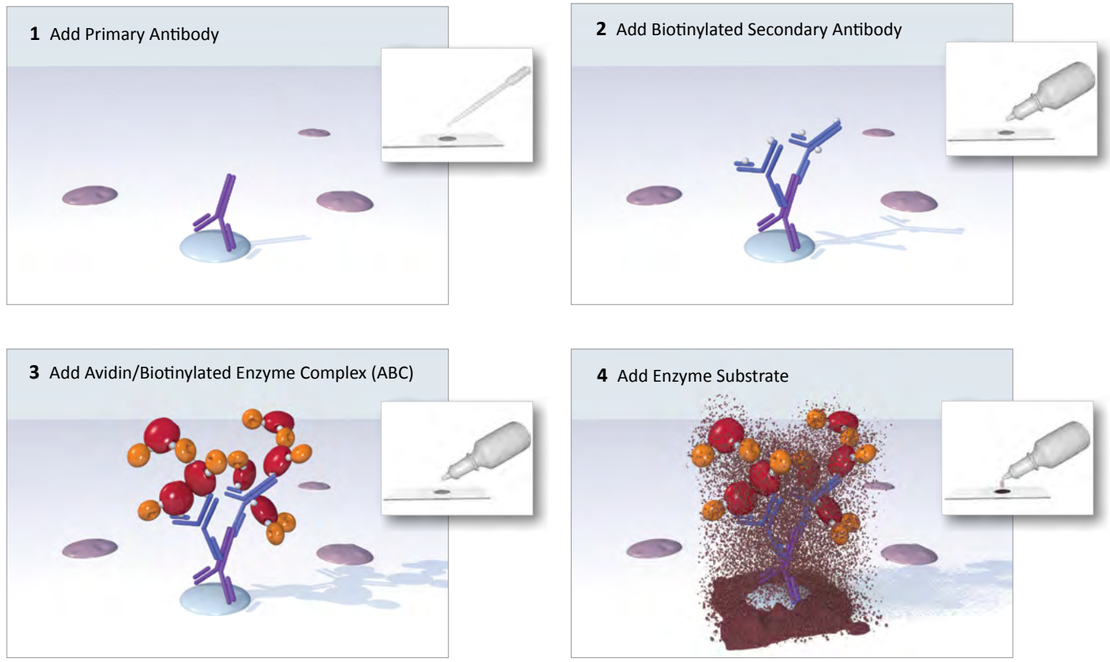
VECTASTAIN® ABC Systems

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



VECTASTAIN® ABC Kits

Peroxidase-Based Kits:

Peroxidase substrates produce sharp, dense precipitates with crisp localization. In conjunction with the high sensitivity and low background of VECTASTAIN® ABC Systems, peroxidase-based detection systems are a preferred choice for many applications. (For peroxidase substrates see p. 26-29.)

VECTASTAIN® Elite® ABC System (Peroxidase)

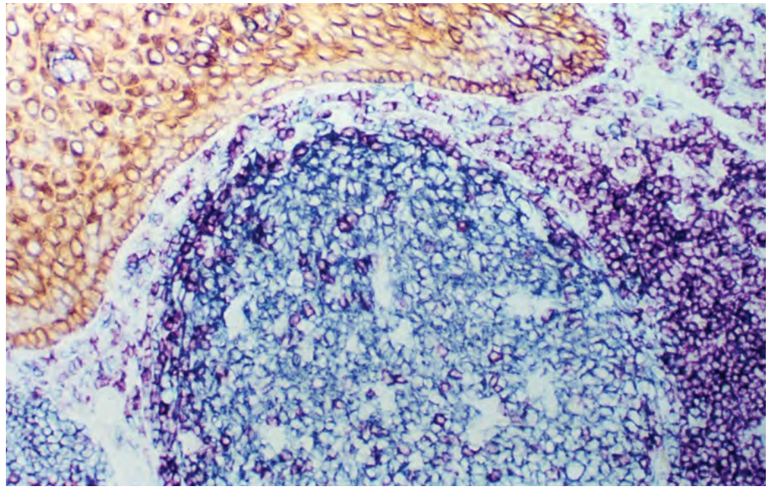
The VECTASTAIN® Elite® ABC complex is smaller and more uniform than conventional avidin-biotin complexes. They have greater accessibility to biotinylated targets within tissue samples. VECTASTAIN® Elite® ABC Kits are our most sensitive avidin-biotin based peroxidase systems.

- 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 Kits (Peroxidase)

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

VECTASTAIN® ABC Kits (Peroxidase)

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

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

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:

The sensitivity of the VECTASTAIN® ABC-AP system is comparable to that of the peroxidase VECTASTAIN® Elite® ABC system. You may prefer to use VECTASTAIN® ABC-AP Kits for tissues that have high endogenous peroxidase activity. The system also offers additional substrate color choices. (For alkaline phosphatase substrates see p. 26-29.)

VECTASTAIN® Quick Kits (Peroxidase)

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

VECTASTAIN® ABC-AP Kits (Alkaline Phosphatase)

- Standard (ABC Reagent only)
- Rabbit IgG
- Mouse IgG
- Mouse IgM
- Human IgG
- Rat IgG
- Goat IgG
- Sheep IgG
- Universal

Product	Elite® (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		AK-5010
VECTASTAIN® ABC Kit, Human IgG	PK-6103	PK-4003		AK-5003
VECTASTAIN® ABC Kit, Rat IgG	PK-6104	PK-4004		AK-5004
VECTASTAIN® ABC Kit, Goat IgG	PK-6105	PK-4005		AK-5005
VECTASTAIN® ABC Kit, Sheep IgG	PK-6106	PK-4006		AK-5006
VECTASTAIN® ABC Kit, Guinea Pig IgG		PK-4007		
VECTASTAIN® ABC Kit, Universal	PK-6200			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	

Choosing a VECTASTAIN® ABC Kit

Choose the detection enzyme → **Choose the appropriate ABC kit** → **Choose a convenient format**

- Peroxidase
- Alkaline phosphatase

To detect a biotinylated target, you will need:

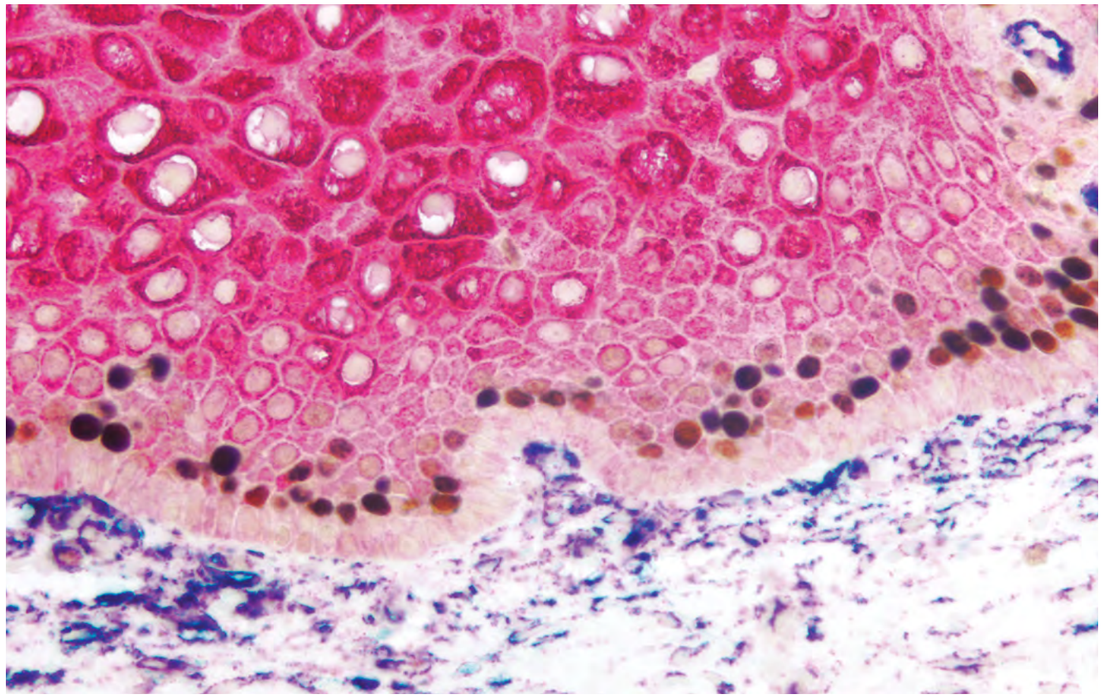
- VECTASTAIN® ABC Reagent contained in the standard VECTASTAIN® ABC Kit
- An appropriate substrate

To detect an unlabeled primary antibody or lectin, you will need:

- A biotinylated secondary antibody that binds to the primary antibody species or lectin you have chosen
- VECTASTAIN® ABC Reagent
- An appropriate substrate

For example, to detect a primary antibody made in rabbit, the appropriate choice is a VECTASTAIN® ABC Kit designated Rabbit IgG.

VECTASTAIN® ABC Kits are available in economical concentrated formats. For additional convenience and ease of use some peroxidase-based kits are offered in ready-to-use prediluted stabilized formats.



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

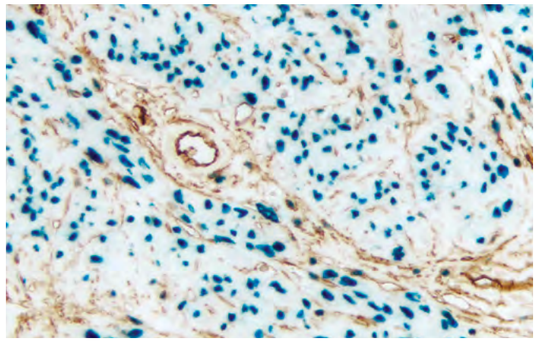
When choosing the optimal detection system for your application, it is important to consider not only the species of the primary antibody but also the species of the tissue under examination. If the species of the primary antibody and the species of the tissue are closely related (for example, rat and 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:

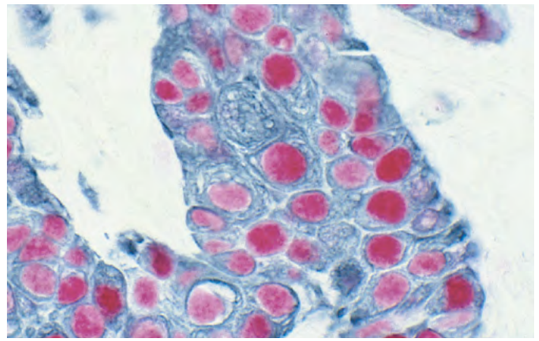
- Directly label the primary antibody with biotin (ProtOn™ Biotin Labeling Kit, PLK-1202) and detect it using the VECTASTAIN® Elite® ABC Kit (Standard – PK-6100, includes VECTASTAIN® Elite® ABC Reagent only) followed by an HRP substrate. Alternatively, label the primary with HRP (ProtOn™ HRP Labeling Kit, PLK-1203) and develop with 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 Mouse on Mouse (M.O.M.™) Immunodetection System for applications of mouse primary antibodies on mouse tissue (p. 24-25).

Substrates

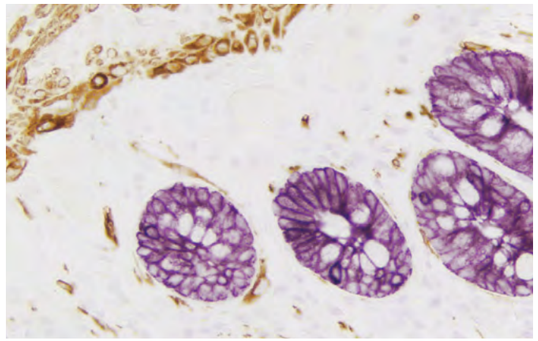
After choosing the VECTASTAIN® ABC Kit for your application, select a substrate that matches the enzyme system of the kit (p. 26-29).



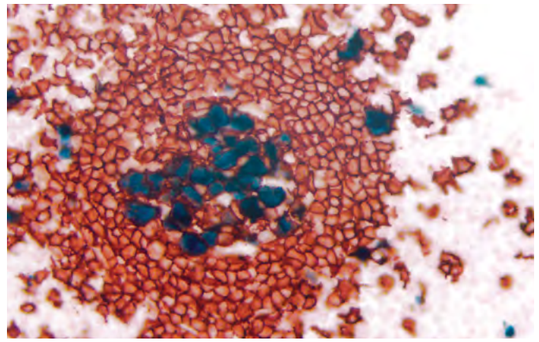
Endometrium: • Progesterone Receptor (rm), VECTASTAIN® Universal ABC- AP Kit, Vector® Blue AP Substrate (blue) • CD34 (m), VECTASTAIN® Universal Elite® *ABC Kit, Vector® DAB HRP Substrate (brown).*



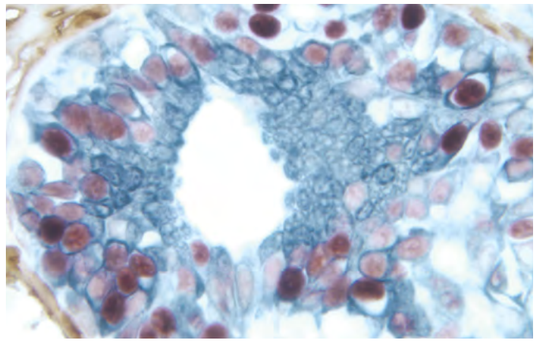
Tumor: • p53 protein (m), VECTASTAIN® ABC-AP Kit, Vector® Red AP Substrate (red) • Pan-Cytokeratin (sheep), VECTASTAIN® Elite® *ABC Kit, Vector® SG HRP Substrate (blue/gray).*



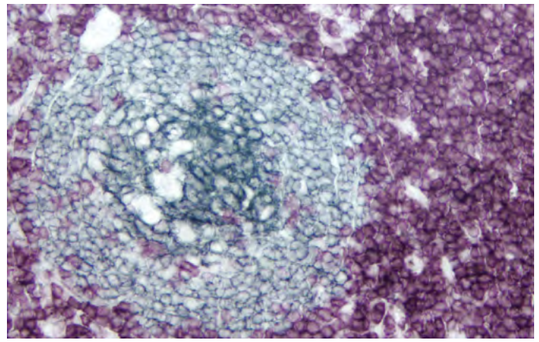
Intestine: • Desmin (m), VECTASTAIN® Elite® *ABC Kit, ImmPACT™ DAB (brown) substrate. • Cytokeratin (m), VECTASTAIN®* Elite® *ABC Kit, Vector® VIP (purple) substrate.*



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) μ 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 Block and Diluent	SP-5030	SP-5035

* For a complete list of all biotinylated secondary antibodies please visit: https://vectorlabs.com/b-2nd-abs/
† Ready-to-use, prediluted stabilized solutions.

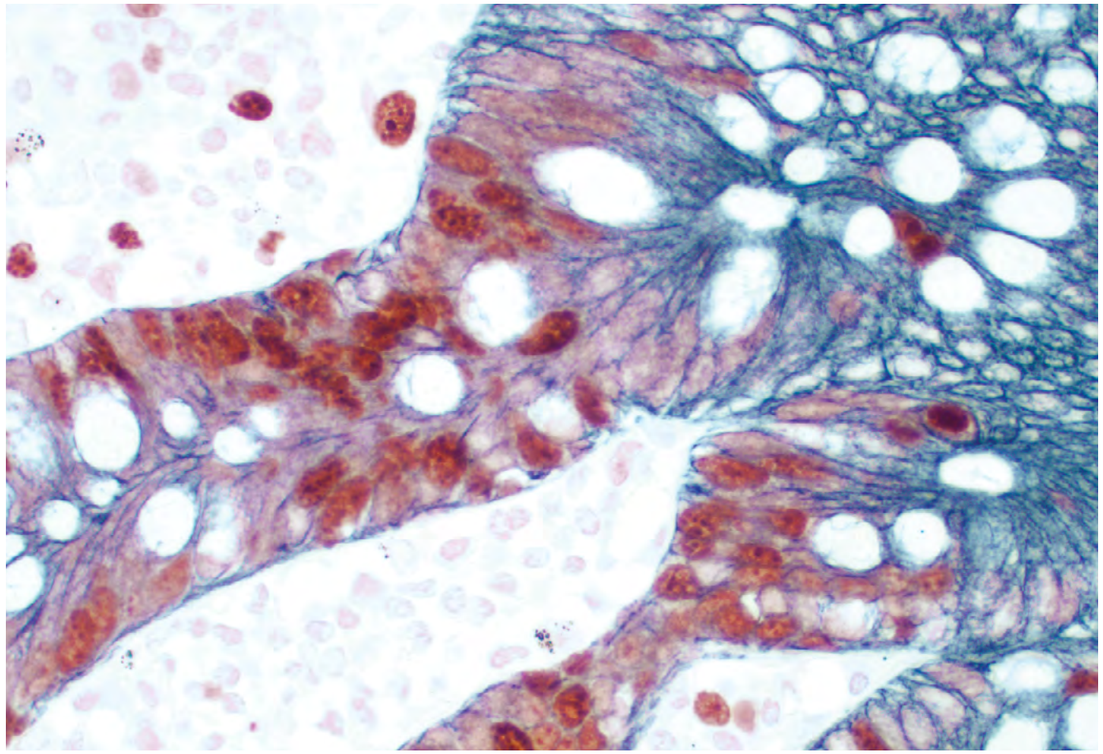
Polymer-Based Detection

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

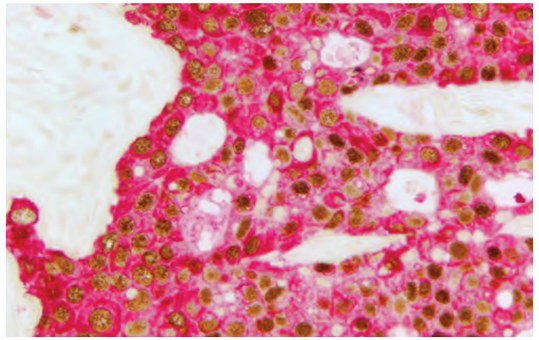
Polymer-based reagents are a more recent introduction into IHC detection methodology than traditional avidin and biotin conjugates, such as ABC kit formats. Polymers offer distinct advantages over these traditional methods particularly for applications such as multiple antigen labeling (multiplexing) 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.

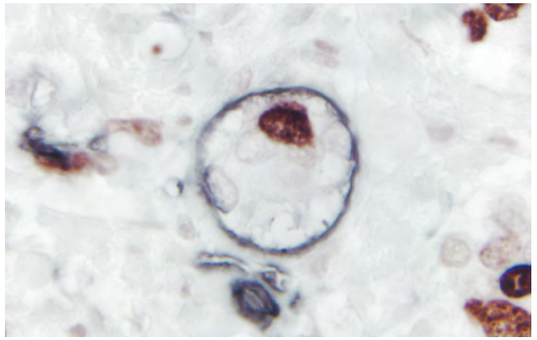
Polymer-based systems were initially introduced consisting of dextran or similar molecules that had inherent issues in some tissues due to their large size. Our ImmPRESS™ polymer systems have been highly refined and consist of micropolymers that penetrate more easily into thicker sections, avoid steric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



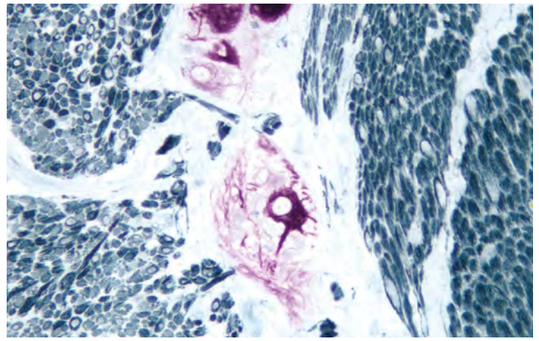
Small Bowel: • Ki67 (rp), ImmPRESS™ Reagent (HRP; Universal), Vector® NovaRED™ (red) • Cytokeratin 8/18 (m), ImmPRESS™ Reagent (HRP; Universal), Vector® SG (blue-gray).



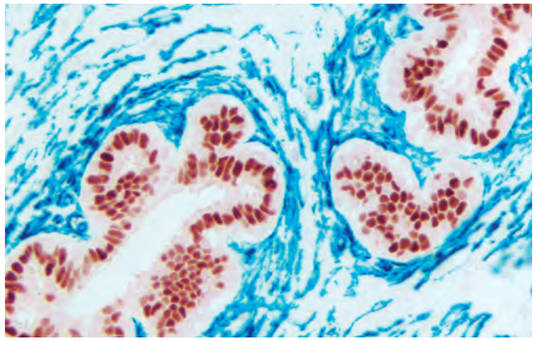
Breast Carcinoma: • Estrogen Receptor (m), ImmPRESS™ Reagent (HRP; Universal), Vector® DAB (brown) • Cytokeratin AE1/AE3 (m), VECTASTAIN® ABC-AP Kit (Universal), Vector® Red (magenta).



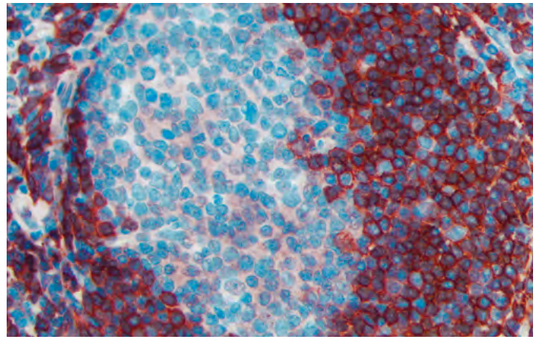
Breast Carcinoma: • Estrogen Receptor (m), ImmPRESS™ Reagent (HRP; Universal), Vector® NovaRED™ HRP Substrate (red) • CD34 (m), ImmPRESS™ Reagent (HRP; Universal), Vector® DAB+Ni HRP Substrate (gray-black).



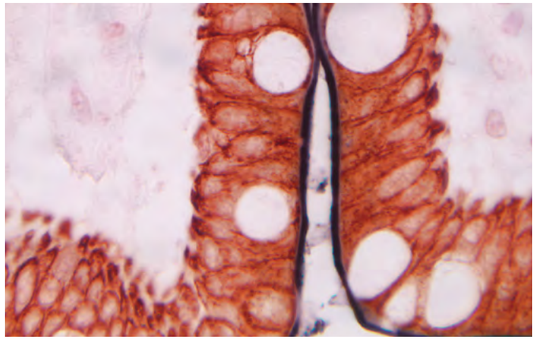
Small Bowel: • Neurofilament 200 kDa (m), ImmPRESS™ Reagent (HRP) Anti-Mouse IgG, Vector® VIP (purple) • Desmin (m), ImmPRESS™ Reagent (HRP) Anti-Mouse IgG, Vector® SG (blue-gray).



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



Mouse Tonsil: CD45 (rat), ImmPRESS™ Reagent (HRP) Anti-Rat IgG, Mouse Adsorbed, ImmPACT™ AEC (red), Hematoxylin QS (blue).



Small Bowel: • CD10 (m), ImmPRESS™ Reagent (HRP) Anti-Mouse IgG, Vector® DAB-Ni (black) • Cytokeratin 20 (m), ImmPRESS™ Reagent (HRP) Anti-Mouse IgG, Vector® NovaRED™ (red).

ImmPRESS™ One-Step Polymer Systems

ImmPRESS™ Polymer Detection Systems

ImmPRESS™ Polymer Reagents 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 Reagents 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. 30-33)

ImmPRESS™ VR Polymer Kits

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).

ImmPRESS™ Polymer Kits (Peroxidase)

- 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

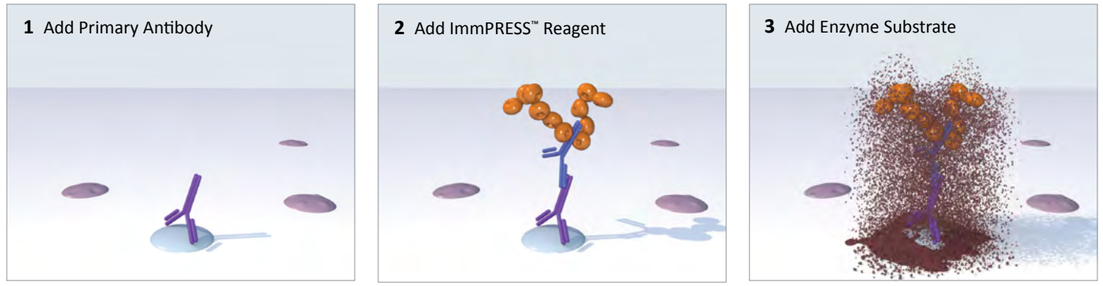
ImmPRESS™ Polymer Kits (Alkaline Phosphatase)

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

ImmPRESS™ VR Polymer Kits (Peroxidase)

- Anti-Rabbit IgG
- Anti-Mouse IgG

Using the ImmPRESS™ Polymer Kits



ImmPRESS™ Two-Step Amplified Polymer Systems

ImmPRESS™ Excel Amplified Polymer Staining Systems

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.

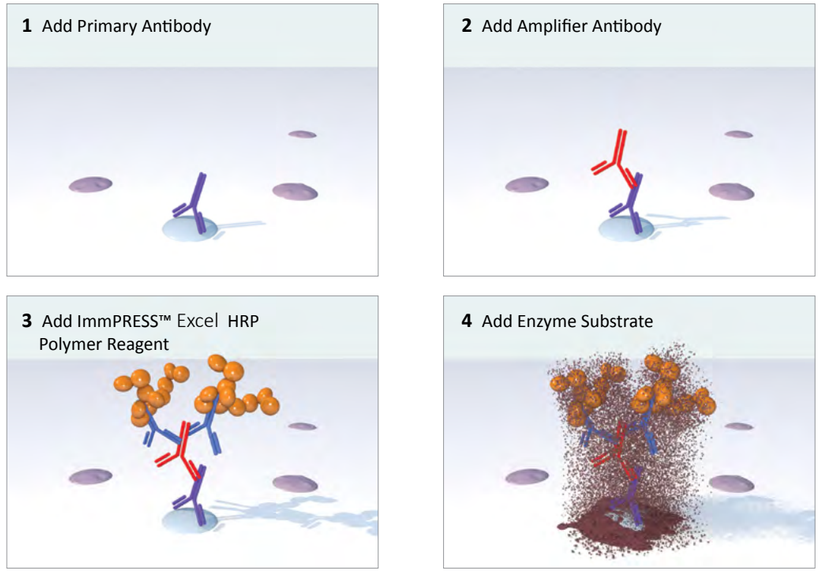
Each ImmPRESS™ Excel Amplified Staining Kit includes the following:

- 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™ Excel Amplified Polymer Kits (Peroxidase)

- Anti-Rabbit IgG
- Anti-Mouse IgG

Using the ImmPRESS™ Two-Step Amplified Polymer Systems

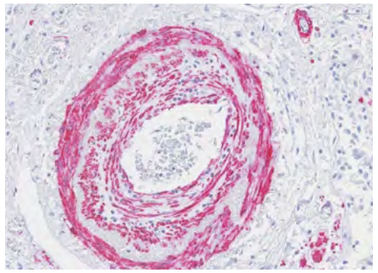


Choosing an ImmPRESS™ Polymer Kit

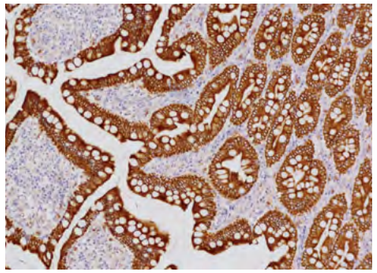
When choosing the optimal detection system for your application, consider not only the species of the primary antibody but also the species of the tissue under examination. If the species of the primary antibody and the species of the tissue are closely related (for example, rat and mouse), the secondary antibody may cross-react with endogenous IgG in the tissue section. This can lead to background staining. For the two-step ImmPRESS™ Excel Amplified Polymer Staining Kit, the species of the Amplifier Antibody (made in goat) should be considered in addition to the primary and tissue species.

The following options can minimize background staining in these instances:

- Use a secondary antibody specifically adsorbed to remove cross-reacting antibodies of closely-related species (for example, ImmPRESS™ Anti-Mouse IgG, Rat Adsorbed).
- Use the ImmPRESS™ M.O.M.™ Kit for applications of mouse primary antibodies on mouse tissue. (p. 25)
- Use ImmPRESS™ VR Polymer Detection Reagents, which eliminate off-target binding and thus increase signal specificity in these animal tissues: bovine, goat, sheep, swine, horse, cat, dog, rabbit, rat, mouse.
- Since the Amplifier Antibody in both the ImmPRESS™ Excel Anti-Mouse IgG Kit and the ImmPRESS™ Excel Anti-Rabbit Kit is made in goat, these kits are not recommended for goat, bovine and sheep tissue - the ImmPRESS™ Excel Polymer Detection Reagent (an anti-goat IgG) may cross-react with endogenous immunoglobulins.



Lung: aSMA (m), ImmPRESS™-AP Anti-Mouse IgG, ImmPACT™ Vector® Red AP Substrate. Counterstained with Hematoxylin (blue). Image kindly provided by the Centre for Inflammation and Tissue Repair, University College London.



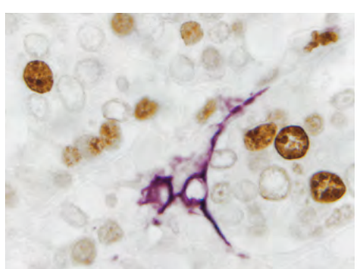
Canine small intestine: Cytokeratin (MNF 116), ImmPRESS™ VR Anti-Mouse IgG and Vector® DAB Substrate (brown). Counterstained with hematoxylin (blue).

Product	Peroxidase	Veterinary Reagents (VR)	Excel Amplified (Peroxidase)	Alkaline Phosphatase
ImmPRESS™ Anti-Rabbit IgG Kit (made in horse)	MP-7401	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-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 horse)	MP-7404			MP-5404
ImmPRESS™ Anti-Rat IgG, Mouse Adsorbed, Kit (made in horse)	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			

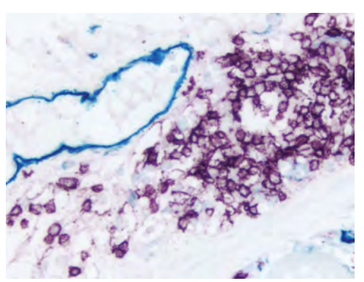
Multiple Antigen Labeling Simplified

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

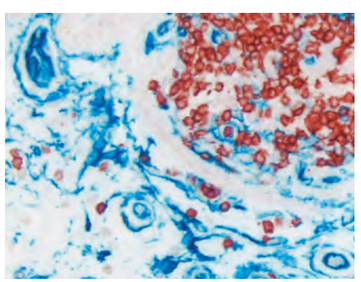
- Fewer steps than conventional protocols decreases slide handling.
- Eliminates the need for avidin/biotin blocking steps in samples with endogenous biotin.



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

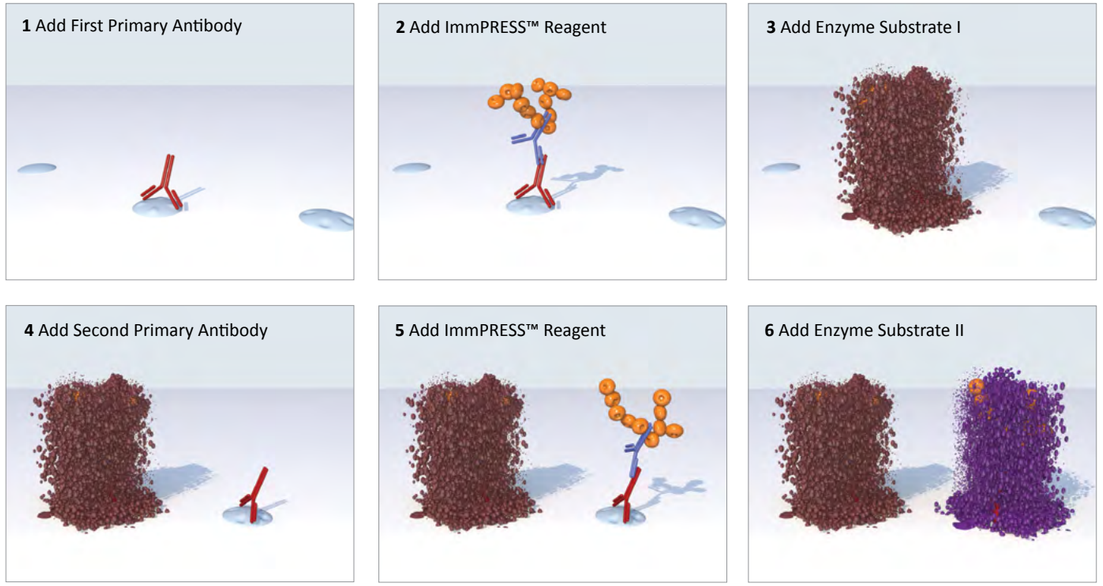


Colon: • M2A antigen (m), VECTASTAIN® ABC-AP Kit (Universal), Vector® Blue (blue) • CD20 (m), ImmPRESS™ Reagent (HRP; Universal), Vector® VIP (purple).



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

Using the ImmPRESS™ Polymer Kits for multiple antigen labeling



Read more: *Discovery Through Color: A Guide to Multiple Antigen Labeling* (vectorlabs.com/brochures)

Species on Species Detection (Mouse)

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.

Newborn Mouse Tongue: • Synapsin (m), M.O.M.™ Peroxidase Kit, Vector® NovaRED™ (red) • Desmin (m), M.O.M.™ Peroxidase Kit, Vector® DAB-Ni (black).

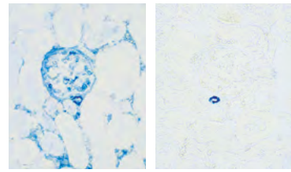
Mouse on Mouse (M.O.M.™) Immunodetection Kits

Vector Laboratories M.O.M.™ Immunodetection systems 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 Ig Blocking Reagent. M.O.M.™ Kits are available based on either avidin-biotin technology (M.O.M.™ Elite® ABC 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 M.O.M.™ System.

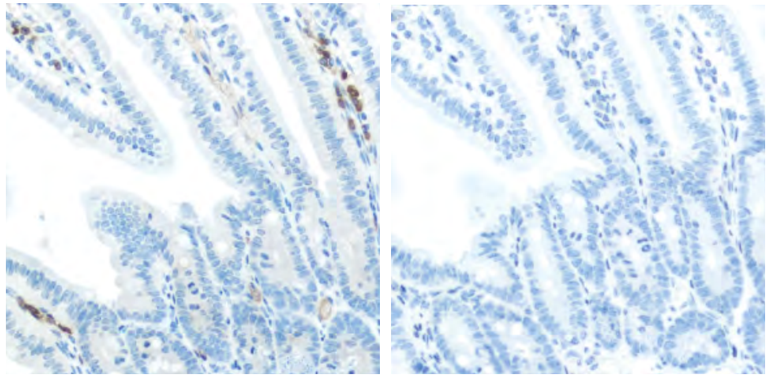
- Significantly reduces endogenous mouse Ig staining when using mouse primary antibodies on mouse tissue
- Simple protocols
- Eliminates tedious calculations
- Eliminates primary antibody prebinding steps
- Clear, crisp, specific staining of antigens of interest
- Compatible with fluorescent or enzyme-based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

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).



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.)

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

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

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.

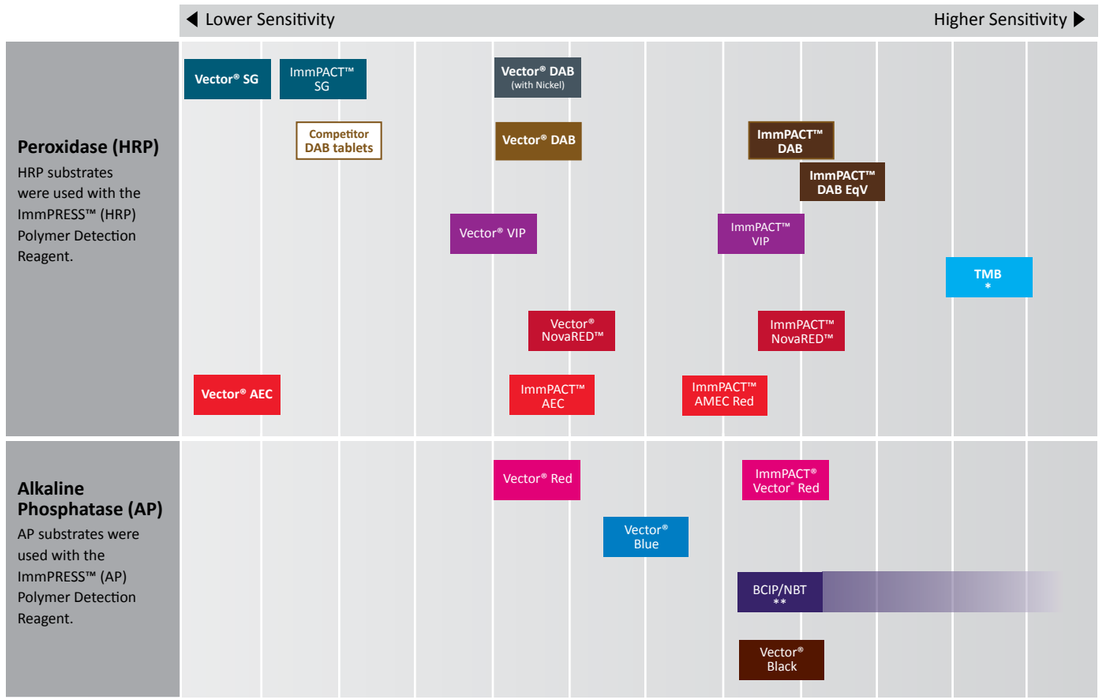
- **Sensitivity.** Substrates differ in sensitivity. Some may increase in sensitivity with longer incubation times.
- **Color.** Color contrast is essential in multiple antigen labeling applications, in pigmented tissues such as melanomas, and in counterstained tissues. Where performance is equal, color 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.

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

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



* Reaction product deposition not discreet and can be variable
** Longer incubation times increase sensitivity

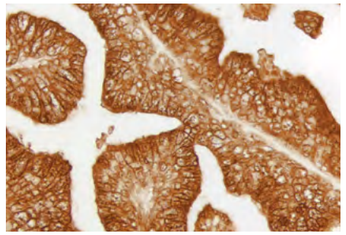
Enzyme Substrate Properties

See also Enzyme Substrate Combinations for multiple antigen labeling (p. 30-33) and Counterstain/Substrate Compatibility (p. 35).

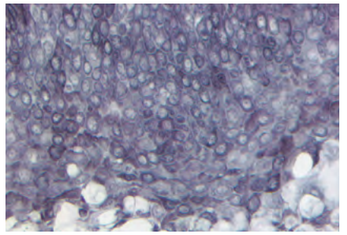
Substrate	Color	Catalog Number	Microscopy					Mounting	Contrast in Pigmented Tissue	Multiple Labeling	Heat Resistant*
			Bright-field	Darkfield	Electron	Fluore-scence	Spectral Imaging				
Peroxidase											
Vector® DAB	Brown	SK-4100	•	•	•		•	Non-aqueous or Aqueous		•	•
Vector® DAB +Ni	Gray-Black	SK-4100	•	•	•		•	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			
Alkaline Phosphatase											
Vector® Red	Magenta	SK-5100	•			•	•	Non-aqueous or Aqueous	•	•	•
ImmPACT™ Vector® Red	Magenta	SK-5105	•			•	•	Non-aqueous or Aqueous	•	•	•
Vector® Blue	Blue	SK-5300	•			•	•	Non-aqueous or Aqueous	•	•	•
Vector® Black	Brown-Black	SK-5200	•					Non-aqueous			
BCIP/NBT	Indigo	SK-5400	•			•	•	Non-aqueous or Aqueous		•	•

* Substrates that are designated “heat resistant” were developed on tissue then subjected to heat induced epitope retrival (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.

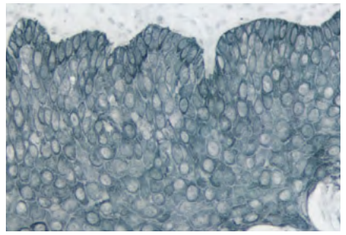
Peroxidase Substrates



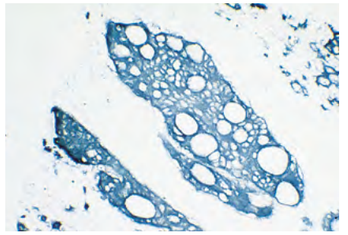
Prostate: Prostate Specific Antigen (m), ImmPRESS™ Reagent (HRP), ImmPACT™ DAB (brown).



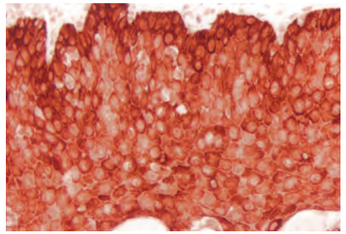
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), Vector® DAB-Ni (gray-black).



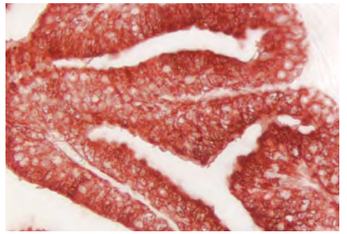
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ SG (blue-gray).



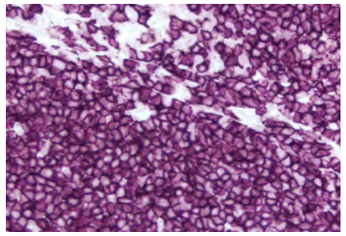
Tumor: Cytokeratin (s), VECTASTAIN® Elite® ABC Kit, TMB (blue).



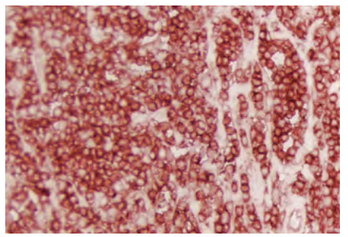
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ NovaRED™ (red).



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

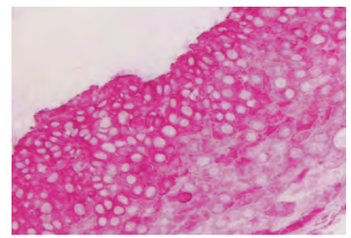


Tonsil: CD20 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ VIP (purple).

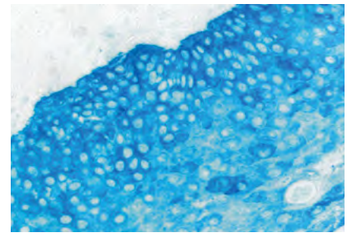


Tonsil: LCA (m), ImmPRESS™ Reagent (HRP), ImmPACT™ AMEC Red (red).

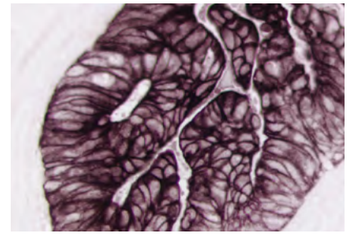
Alkaline Phosphatase Substrates



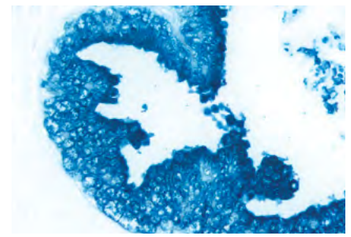
Tonsil: Cytokeratin AE1/AE3 (m), Vector® ImmPRESS™-AP Reagent, ImmPACT™ Vector® Red (magenta).



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).

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, *Discovery Through Color: A Guide to Multiple Antigen Labeling*.

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).

Enzyme Substrate Combinations

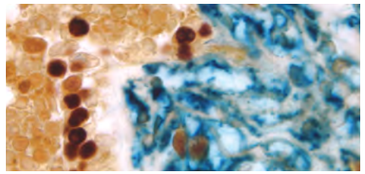
Recommended combinations of substrates and the recommended order in which they should be used.

First Substrate \ Second Substrate	ImmPACT™ Vector® Red & Vector® Red (magenta) SK-5105 & SK-5100	Vector® Blue (blue) SK-5300	BCIP/NBT (indigo) SK-5400	ImmPACT™ VIP & Vector® VIP (purple) SK-4605 & SK-4600	ImmPACT™ DAB, mmPACT™ DAB EqV & Vector® DAB (brown) SK-4105, SK-4103, SK-4100	DAB-Ni (gray-black) SK-4100	ImmPACT™ NovaRED™ & Vector® NovaRED™ (red) SK-4805 & SK-4800	ImmPACT™ SG & Vector® SG (blue-gray) SK-4705 & SK-4700	ImmPACT™ AEC & Vector® AEC (red) SK-4205 & SK-4200	ImmPACT™ AMEC Red (red) SK-4285
ImmPACT™ Vector® Red & Vector® Red (magenta) Cat. No. SK-5105 & SK-5100		–	–	–	+	+	–	+	–	–
Vector® Blue (blue) Cat. No. SK-5300	+		–	+	+	+	+	+	+	+
BCIP/NBT (indigo) Cat. No. SK-5400	+	–		+	+	+	+	+	+	+
ImmPACT™ VIP & Vector® VIP (purple) Cat. No. SK-4605, SK-4600	–	+	–		+	+	–	+	–	–
ImmPACT™ DAB, ImmPACT™ DAB EqV & Vector® DAB (brown) Cat. No. SK-4105, SK-4103, SK-4100	+	+	+	+		–	–	+	+	+
Vector® DAB-Ni (gray-black) Cat. No. SK-4100	+	–	–	+	+		+	–	–	–
ImmPACT™ NovaRED™ & Vector® NovaRED™ (red) Cat. No. SK-4805, SK-4800	–	+	+	–	+	+		+	–	–
ImmPACT™ SG & Vector® SG (blue-gray) Cat. No. SK-4705, SK-4700	+	–	–	+	+	–	–		+	+
ImmPACT™ AEC & Vector® AEC (red) Cat. No. SK-4205, SK-4200	–	–	–	–	+	–	–	+		–
ImmPACT™ AMEC Red (red) SK-4285	–	–	–	–	+	–	–	+	–	

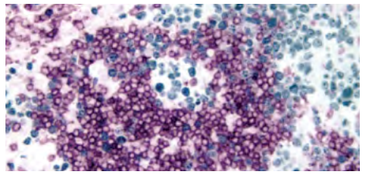
Key: Alkaline Phosphatase | Peroxidase

\+ Indicates good contrast

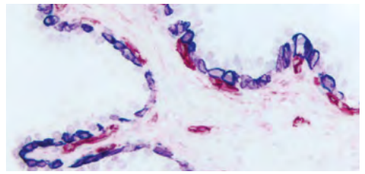
– Indicates incompatiblity of substrates for various reasons



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).

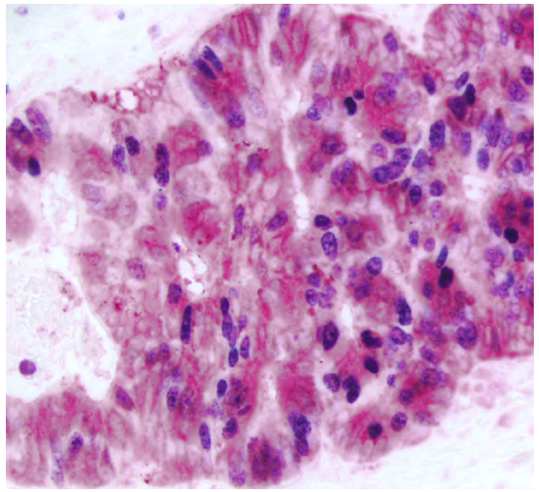


Tonsil: • CD3 (m), ImmPRESS™ Anti-Mouse IgG Reagent, Vector® VIP HRP Substrate (purple) • Ki67 (m), ImmPRESS™ Anti-Mouse IgG Reagent, Vector® SG HRP Substrate (blue/gray).

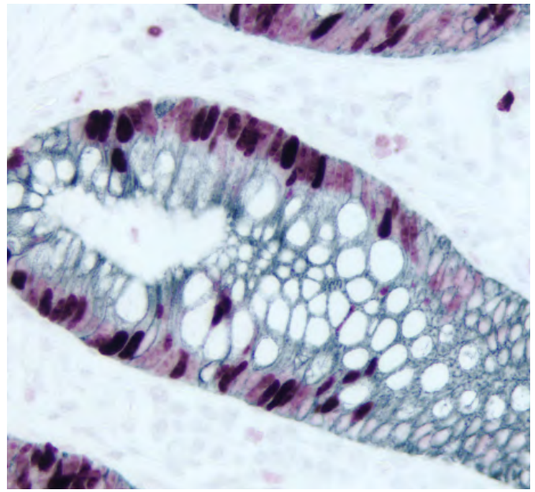


Prostate: • Cytokeratin 5 (m), VECTASTAIN® Universal ABC-AP Kit, Vector® Blue AP Substrate (blue) • CD34 (m), VECTASTAIN® Universal ABC-AP Kit, Vector® Red AP Substrate (red).

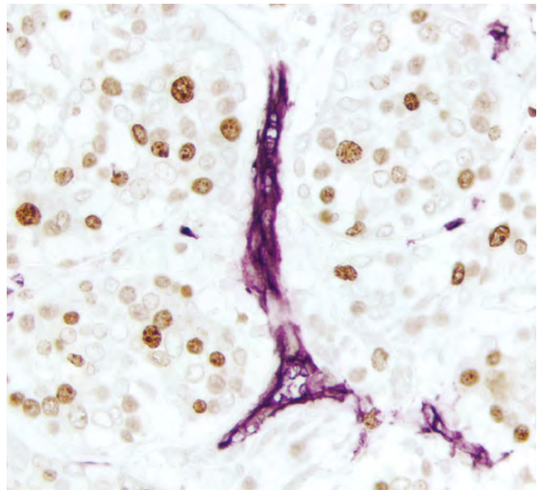
Multiple Labeling Examples



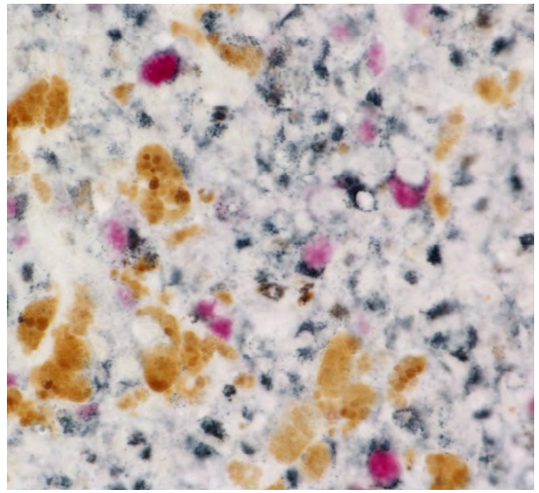
Colon Cancer: • Ki67 (rm), ImmPRESS™-AP Anti-Rabbit IgG Reagent, Vector® Blue AP Substrate (blue) • Cox2 (rm), ImmPRESS™-AP Anti-Rabbit IgG Reagent, ImmPACT™ Vector® Red AP Substrate (red).



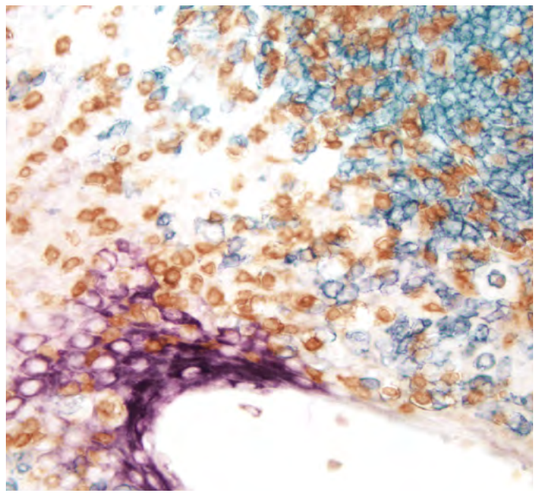
Colon: • Ki67 (rm), ImmPRESS™ Universal (Anti-Mouse/Anti-Rabbit IgG) HRP Reagent, Vector® VIP (purple) • Cytokeratin (m), ImmPRESS™ Universal (Anti-Mouse/Anti-Rabbit IgG) HRP Reagent, Vector® SG HRP Substrate (gray).



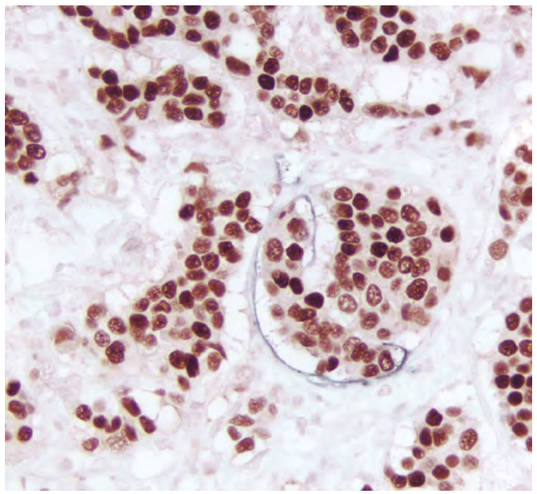
Breast Carcinoma: • Ki67 (rm), ImmPRESS™ Reagent (HRP; Universal), Vector® DAB (brown) • CD34 (m), ImmPRESS™ Reagent (HRP; Universal), Vector® VIP (purple).



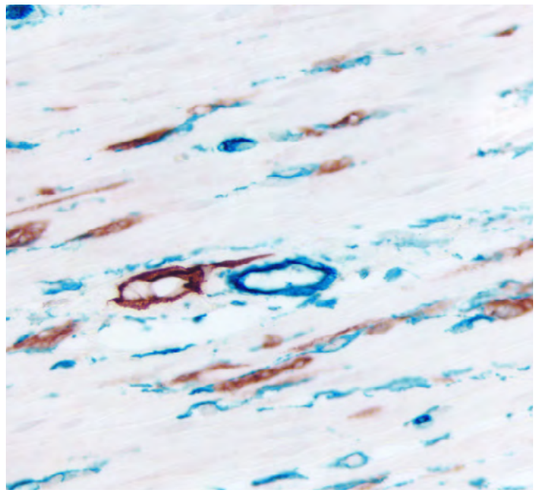
Melanoma: • Cyclin A (m), ImmPRESS™-AP Anti-Mouse IgG, ImmPACT™ Vector® Red AP Substrate (magenta) • Melanoma Marker (m) ImmPRESS™ HRP Anti-Mouse IgG, Vector® SG HRP Substrate (gray). Note contrast of double stain with the brown pigments in the tissue.



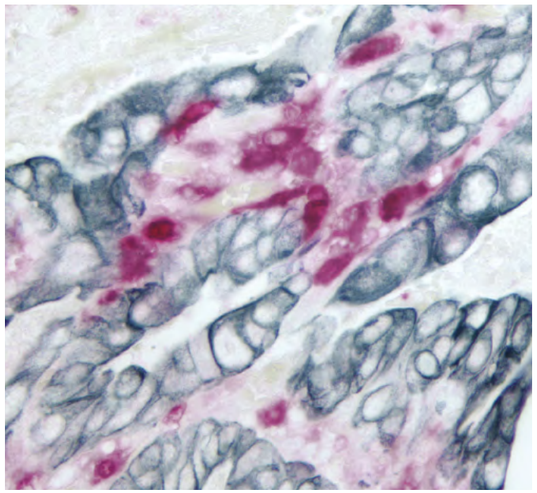
Tonsil: • CD3 (m), VECTASTAIN® Elite® ABC Kit (Universal), Vector® DAB (brown) • CD20 (m), VECTASTAIN® Elite® ABC Kit (Universal), Vector® SG (blue-gray) • Multi-Cytokeratin (m), VECTASTAIN® Elite® ABC Kit (Universal), Vector® VIP (purple).



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).



Colon: • M2A Antigen (m), ImmPRESS™ Universal (Anti-Mouse/Rabbit IgG) HRP Reagent, Vector® NovaRED HRP Substrate (red) • CD34 (m), VECTASTAIN® Universal ABC-AP kit, Vector® Blue AP Substrate (blue).



Colon Carcinoma: • S100 (rp), VECTASTAIN® Universal ABC-AP Kit, Vector® Red AP Substrate (red) • Cytokeratin 8/18 (m), VECTASTAIN® Universal Elite® ABC Kit, Vector® SG HRP Substrate (blue/gray).

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.

Vector® 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

Vector® Hematoxylin QS (blue)

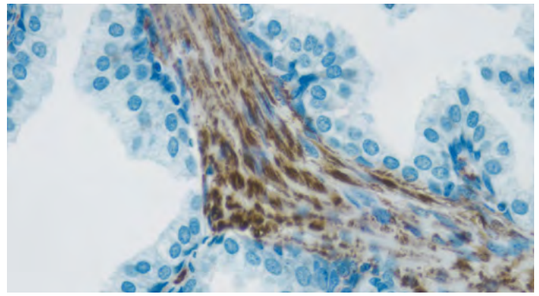
- Modification of Mayer's hematoxylin developed especially for immunocytochemistry
- Ready-to-use without filtration or 'blueing' step
- Stains in less than 45 seconds
- Excellent color contrast with most commonly used peroxidase and alkaline phosphatase substrates
- Suitable for use with non-aqueous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

Vector® Methyl Green (light green)

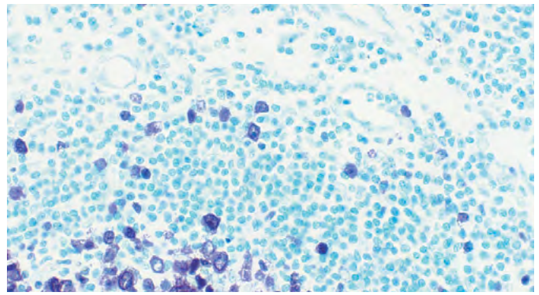
- Superior formulation of methyl green suitable for use with a wide range of enzyme substrates
- Simple, two-step procedure
- Excellent alternative in multiple antigen labeling when hematoxylin obscures the substrate colors
- Suitable for use with non-aqueous mounting media

Vector® Nuclear Fast Red (pink)

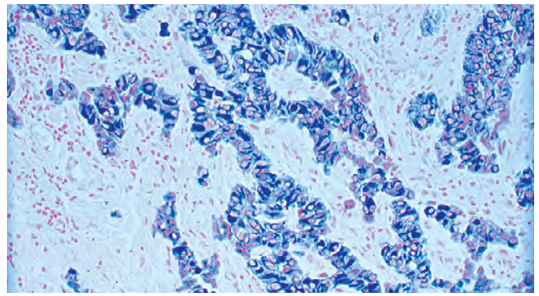
- Fast one-step protocol
- Excellent alternative in multiple antigen labeling when hematoxylin obscures the substrate colors
- Good contrast with a variety of substrates



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



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



Tumor tissue section showing specific cytoplasmic cell staining (Vector® SG, blue/gray) with Vector® 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
Vector® DAB (brown)	SK-4100	Excellent Contrast	Excellent Contrast	Fair Contrast
Vector® 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
Vector® 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
Vector® 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.

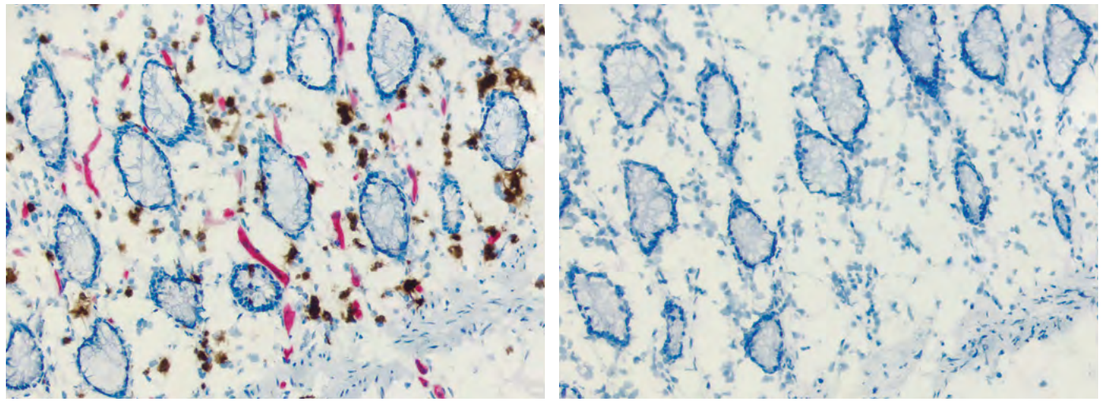
** Substrate dissolves in acetone wash.

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

Product	Mountants	Catalog Number
Vector® Hematoxylin	Non-aqueous and Aqueous	H-3401
Vector® Hematoxylin QS	Non-aqueous and Aqueous	H-3404
Vector® Methyl Green	Non-aqueous	H-3402
Vector® Nuclear Fast Red	Non-aqueous and 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 Peroxidase/Alkaline Phosphatase Blocking Solution

Tissues may contain endogenous peroxidase, pseudoperoxidase, and/or alkaline phosphatase activity that will produce background staining. BLOXALL™ Endogenous Peroxidase/Alkaline Phosphatase 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 Block 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™ 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 Block 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.

	Biotinylated					Unconjugated		
	Host Species (Concentrate)			Host Species (R.T.U.)†		Host Species (Concentrate)		
Secondary Antibodies	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					AI-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						
Anti-Swine IgG (H+L)	BA-9020							
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 Host Species (Concentrate) Goat	Unconjugated Host Species (Concentrate) Goat
Anti-Human IgG (H+L)	BA-3000	AI-3000
Anti-Human IgE, ε (Epsilon) chain specific	BA-3040	AI-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
Alkaline Phosphatase	
Anti-Mouse IgG (H+L) made in horse Alkaline Phosphatase labeled	AP-2000
Anti-Rabbit IgG (H+L) made in goat Alkaline Phosphatase labeled	AP-1000
Peroxidase	
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
Alkaline Phosphatase	
Alkaline Phosphatase Streptavidin	SA-5100
Alkaline Phosphatase Avidin D	A-2100
Peroxidase	
Horseradish Peroxidase Streptavidin, concentrate	SA-5004
Horseradish Peroxidase Streptavidin, R.T.U.	SA-5704
Horseradish Peroxidase Avidin D, concentrate	A-2004
Horseradish Peroxidase Avidin D, R.T.U.	A-2704

Mounting Media

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.

- Non-aqueous
- Toluene- and xylene-free (low toxicity)
- Resin-based media
- Odorless
- Viscosity formulated for easy application and uniform spreading
- Compatible with most horseradish peroxidase and alkaline phosphatase substrates
- Dries clear with an ideal refractive index suitable for high-resolution oil-immersion microscopy

VectaMount™ AQ Aqueous Mounting Medium

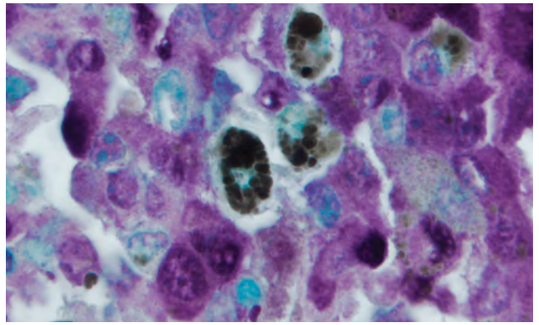
VectaMount™ AQ Aqueous Mounting Medium preserves the color and clarity of enzyme substrates whose reaction products are soluble in alcohol or other organic solvents. Stained and mounted sections can be stored in a slide box at room temperature for at least two years without fading.

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

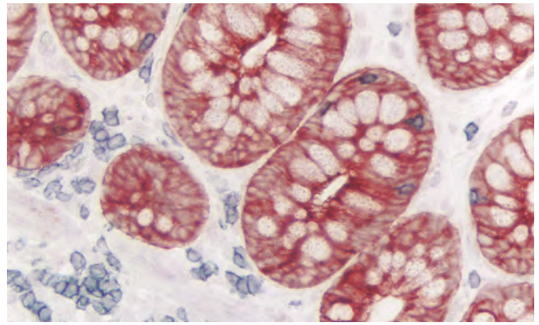
Product	Catalog Number
VectaMount™ Permanent Mounting Medium	H-5000
VectaMount™ AQ Aqueous Mounting Medium	H-5501

Mounting Media / Substrate Compatibility

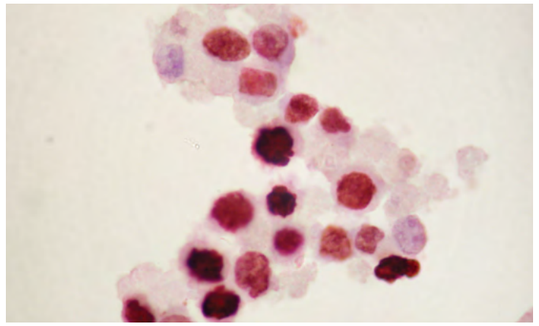
Substrate	VectaMount™ Permanent Mounting Medium	VectaMount™ AQ Aqueous Mounting Medium
HRP		
Vector® DAB	•	•
Vector® DAB-Ni	•	
ImmPACT™ DAB	•	•
ImmPACT™ DAB EqV	•	•
Vector® VIP	•	
ImmPACT™ VIP	•	
Vector® NovaRED™	•	
ImmPACT™ NovaRED™	•	
Vector® SG	•	•
ImmPACT™ SG	•	•
Vector® AEC		•
ImmPACT™ AEC		•
ImmPACT™ AMEC Red		•
Vector® TMB	•	
AP		
Vector® Red	•	•
ImmPACT™ Vector® Red	•	•
Vector® Blue	•	•
Vector® Black	•	
Vector® BCIP/NBT	•	•



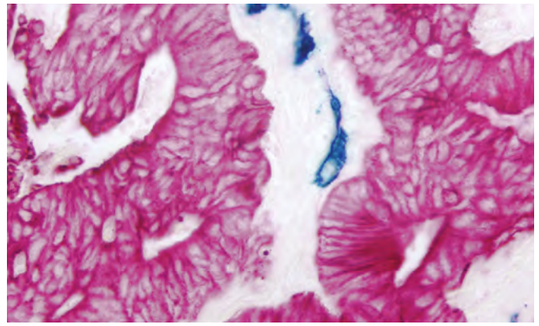
Melanoma: S100 (rp), VECTASTAIN® Elite® ABC Kit, Vector® VIP (purple), Vector® Methyl Green counterstain (green). Note color contrast with brown pigments in tissue.



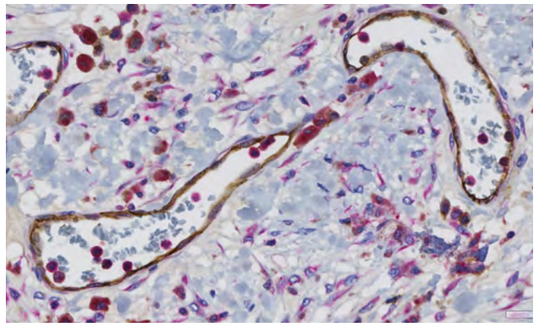
Colon: • CD3 (rm), ImmPRESS™ Reagent (HRP) Anti-Rabbit IgG, ImmPACT™ SG (blue-gray) • Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP) Anti-Mouse IgG, ImmPACT™ AMEC Red (red).



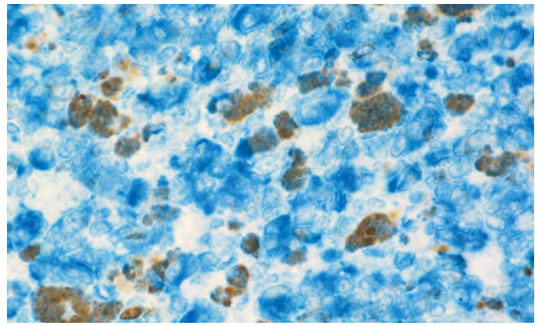
Cytospin of EBV+ cell line: Epstein-Barr virus nuclear antigen 1 (EBNA-1; rat), ImmPRESS™ Reagent (HRP) Anti-Rat IgG, ImmPACT™ NovaRED™ (red). (Image courtesy of Dr. GM Reynolds, Centre for Liver Research, University of Birmingham, U.K.)



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

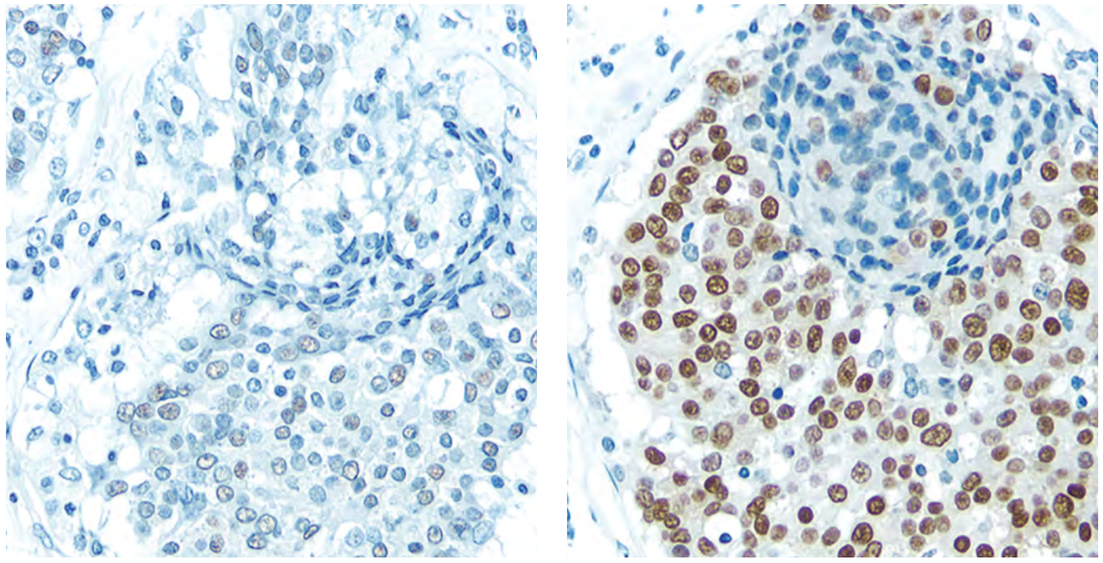
Control Antibodies

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.

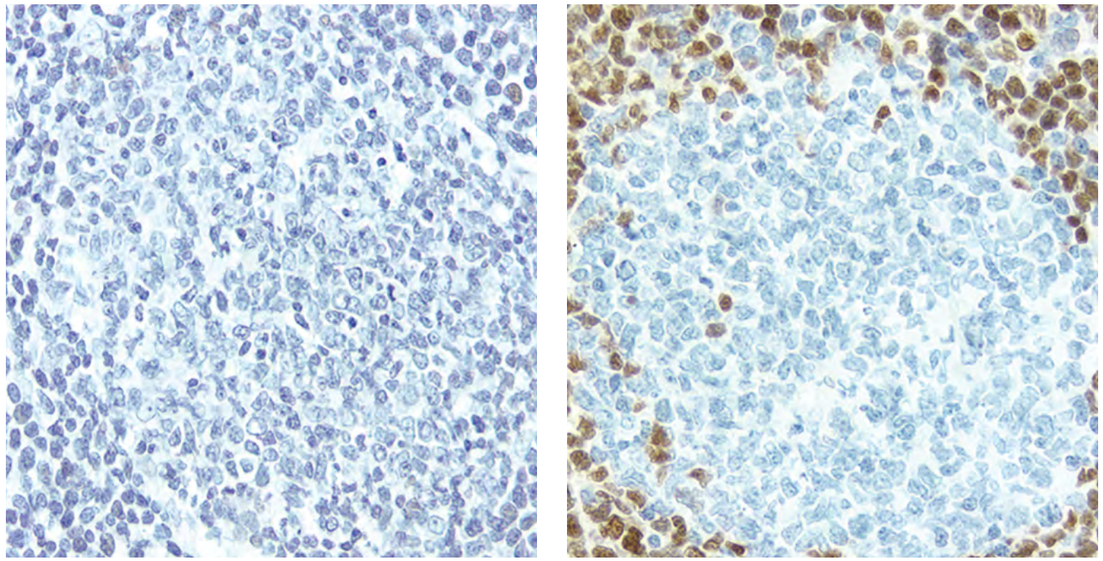
Antigen Unmasking Solutions

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 Pen	H-4000
ImmPrint™ Histology Pen	H-6100
Control Antibodies	
Rabbit IgG	I-1000
Mouse IgG	I-2000
Rat IgG	I-4000
Goat IgG	I-5000
Antigen Unmasking Solutions	
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.

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Ordering Information

Order online at: www.vectorlabs.com

Orders may also be placed by mail, telephone, fax, email or secure online ordering. 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 $2000 may require written faxed 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.

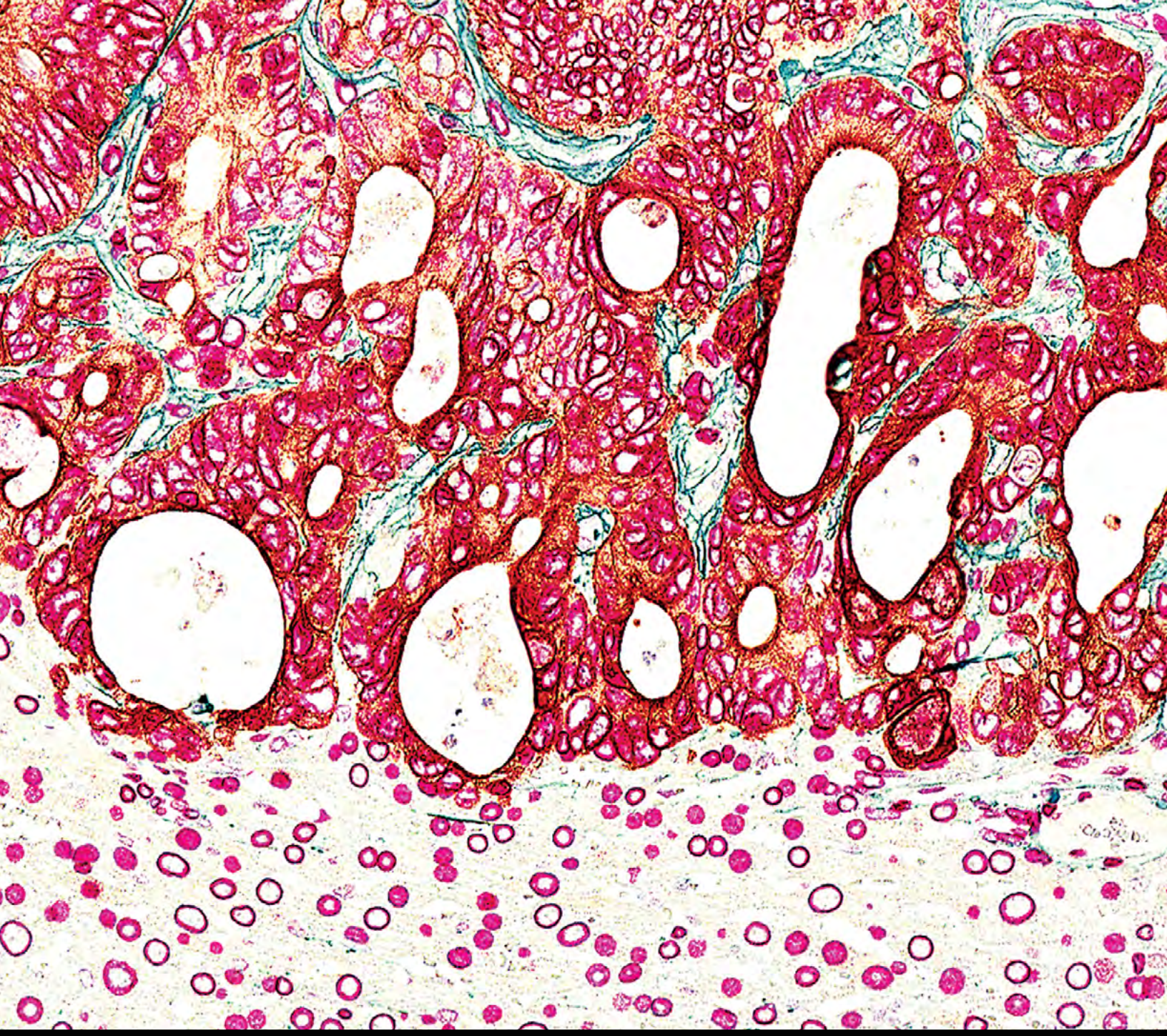
Payment / shipping terms:

Payment terms: net 30 days. Prices are FCA Burlingame, California. Shipping charges will be prepaid and added to the invoice. Orders are usually shipped the same day they are received. Unless requested otherwise, all products are shipped 2nd-day air. RCA_{60} products are shipped in the USA only according to federal transportation regulations requiring additional shipping charges. We require a written confirmation for all RCA_{60} products.

International Orders

Vector products are also available from over 50 distributors worldwide. Please contact us or visit our website for a complete listing of these representatives.

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).



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