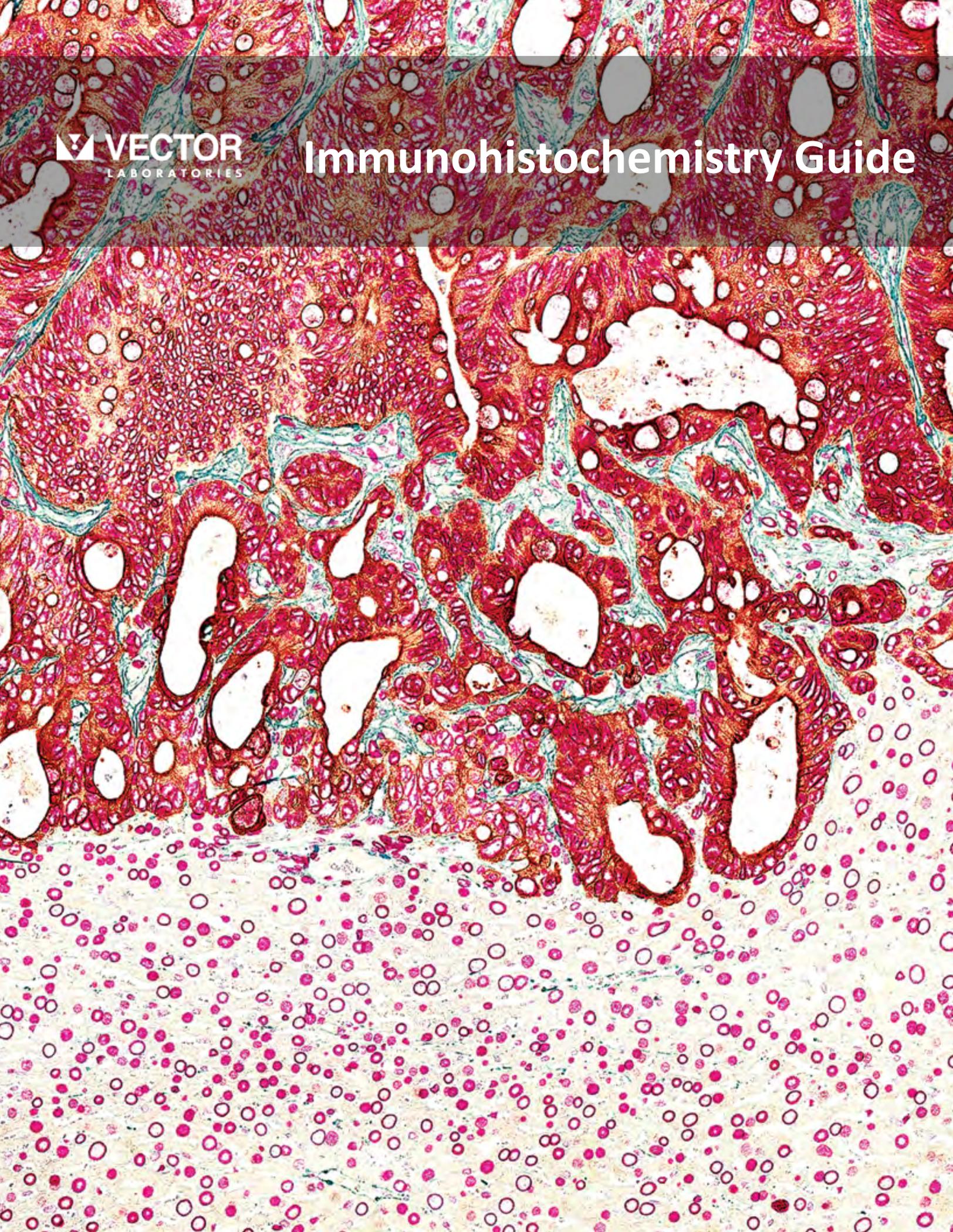




Immunohistochemistry Guide



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).

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- BLOXALL® Endogenous HRP/AP Blocking Solution
- Avidin/Biotin and Streptavidin/Biotin Blocking Reagents
- Normal Sera
- Animal-Free Blocking Solutions
- General Protein Blocking Reagents

39 Secondary and Tertiary Detection Reagents

- Biotinylated and Unconjugated Secondary Antibodies
- Enzyme Conjugated Secondary Antibodies
- Avidin and Streptavidin Enzyme Conjugates

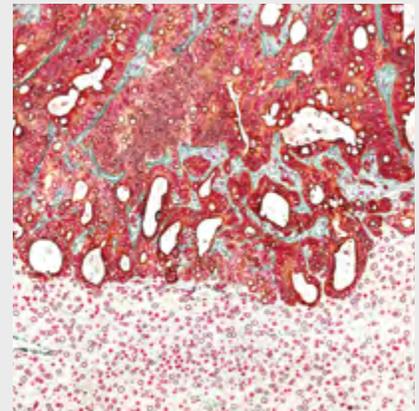
41 Mounting Media

- VectaMount® Mounting Media

43 Accessory Reagents

- VECTABOND® Reagent Tissue Section Adhesive
- ImmEdge™ Hydrophobic Barrier Pen
- Histology Pens, Control Antibodies, and Antigen Unmasking Solutions

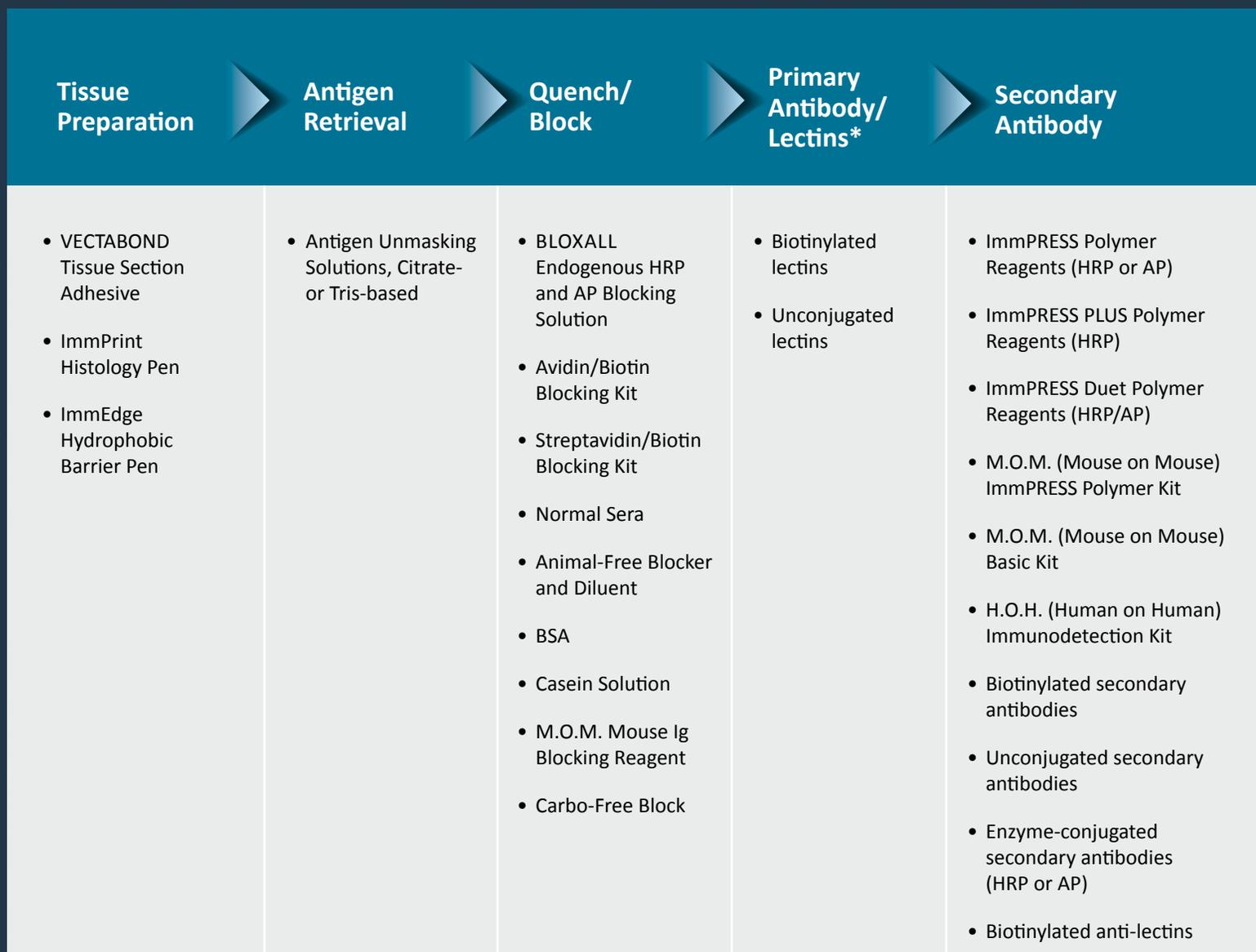
Vector Laboratories was founded on a growing portfolio of purified lectins and lectin conjugates that helped to pioneer lectin histochemistry. These products remain a key component of our business today. In the early 1980s, we leveraged our expertise in histochemistry to revolutionize the field of IHC 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, M.O.M. (Mouse on Mouse) 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.



* For more information visit: vectorlabs.com/lectins



- 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

Visualize

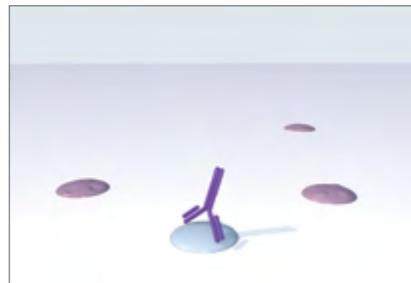


Immunohistochemistry Selection Guide

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)

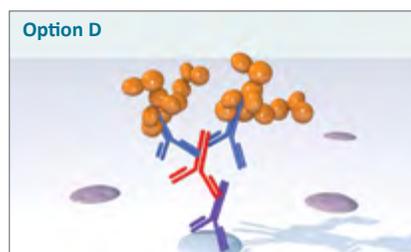
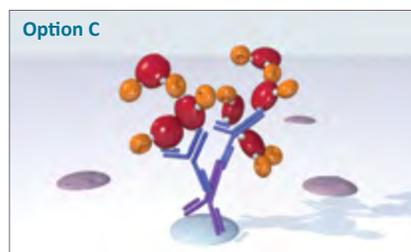
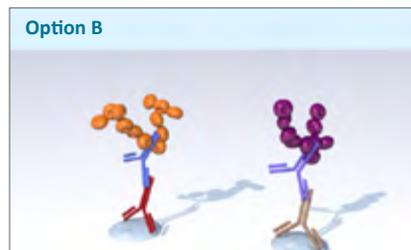
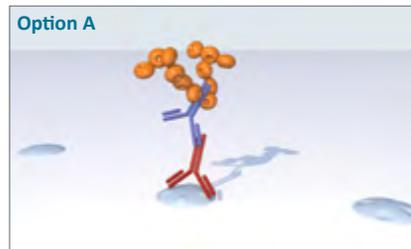
Blocking Reagents

- 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 Blocker 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



One Step

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

- ImmPRESS Polymer Reagents
- ImmPRESS PLUS Polymer Kits



One Step

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

- ImmPRESS Duet Double Staining HRP/AP Polymer Detection Kit



Two Step

Economical. Biotin-based.

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



Two Step

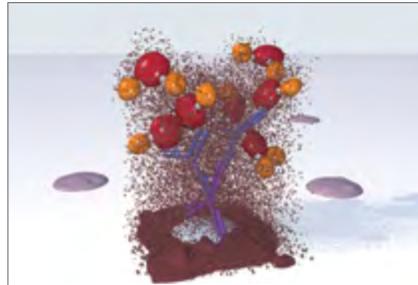
Highest sensitivity. Non-biotin based.

- ImmPRESS Excel Amplified Polymer Staining Systems

3

Choose Enzyme Substrate

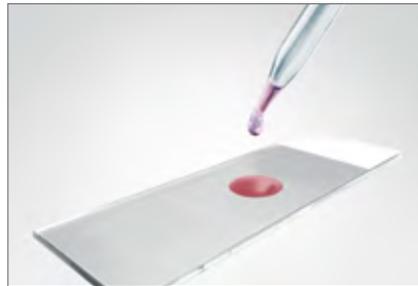
- Color
- Compatibility with other system reagents (counterstains, mounting media and other substrates for multiplexing)



4

Choose Nuclear Counterstain

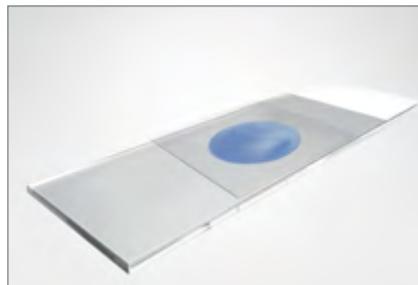
- Blue, green, or red
- Compatibility with substrate, mounting media



5

Choose Mounting Media

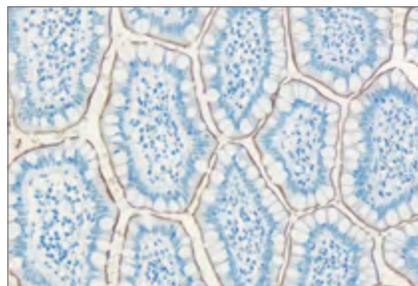
- Aqueous vs. non-aqueous
- Compatibility with substrate(s) and counterstain



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

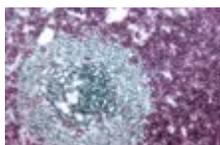
1976

Vector Laboratories, Inc. is founded.



1981

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



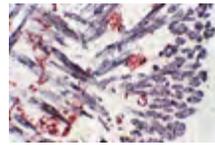
1993

Vector Labs introduces first antifade mounting media for fluorescence (VECTASHIELD® Mounting Media).



1999

Vector Labs introduces M.O.M.® detection kits for use of mouse primary antibodies on mouse tissue sections.



2005

Vector Labs develops ImmPRESS® enzyme polymer detection reagents enabling greater access to antigens within tissues.



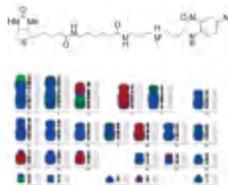
1980

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



1986

Vector Labs launches PHOTOPROBE® Biotin



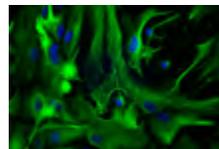
1999

Vector Labs introduces a next-generation PAP pen conforming with environmental regulations (ImmEdge® Pen).



2004

Vector Labs expands antifade mounting media with hardening formulation (VECTASHIELD® HardSet Mounting Medium).



2007

Vector Labs introduces proprietary substrates enabling multiplexing IHC (ImmPACT® HRP substrates).



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".

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2011
 Vector Labs introduces BLOXALL® Endogenous HRP/AP Blocking Solution

2014
 Vector Labs introduces additional proprietary AP substrate (ImmPACT® Vector Red substrate).

2016
 Strategic investment by Maravai LifeSciences

2017
 Vector Labs introduces TrueVIEW® Autofluorescent Quenching Kit.

2018
 Vector Labs further expands its antifade mounting media with VECTASHIELD® Vibrance™ and now offers a 2 ml size.

2018
 Vector Labs achieves ISO 9001:2015 certification.

2019
 Vector TrueVlew® Autofluorescent Quenching with DAPI is released.

2020
 Next generation non-setting VECTASHIELD® PLUS added to our antifade mounting media family.

2020
 Vector adds Bioconjugation product line featuring the SoluLINK® bioconjugation technology.

2021
 VectaMount® Express Mounting Medium is released.

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 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 |
| VECTASTAIN Kits | | | | | | | | | |
| 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 |
| M.O.M. (Mouse on Mouse) Kits | | | | | | | | | |
| M.O.M. (Mouse on Mouse) ImmPRESS Polymer Kit | HRP | ••• | ••• | • | • | | • | | 1 |
| M.O.M. (Mouse on Mouse) 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

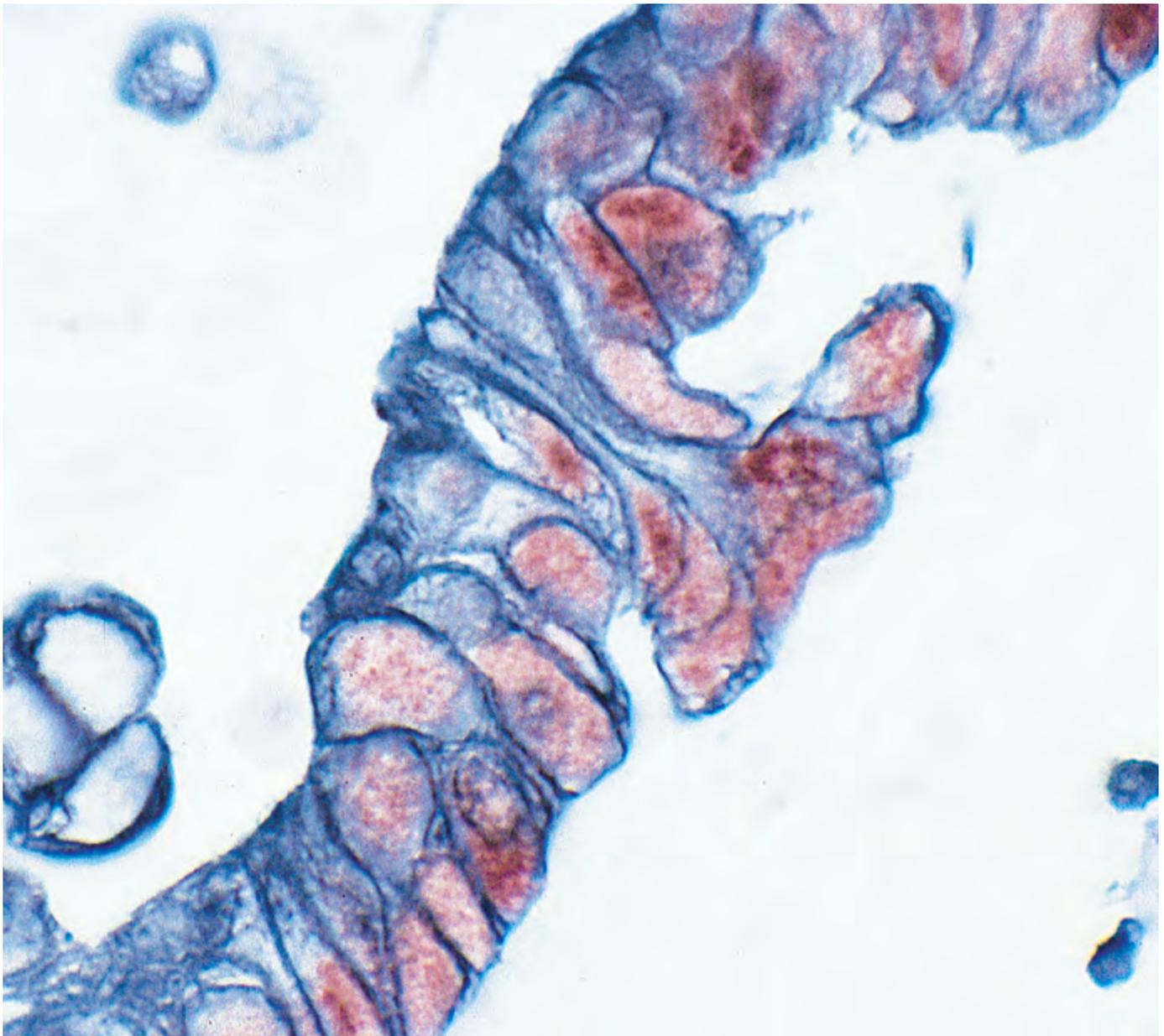
AP - Alkaline phosphatase

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).

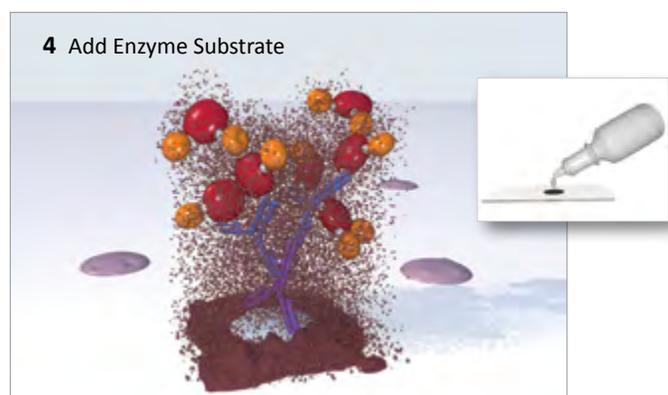
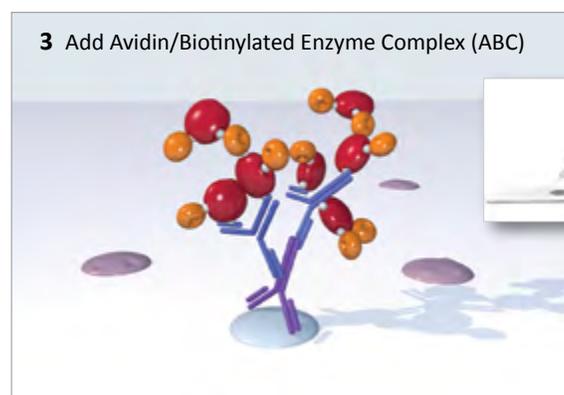
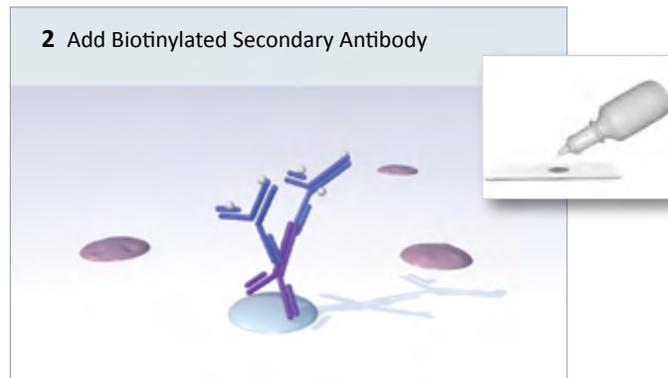
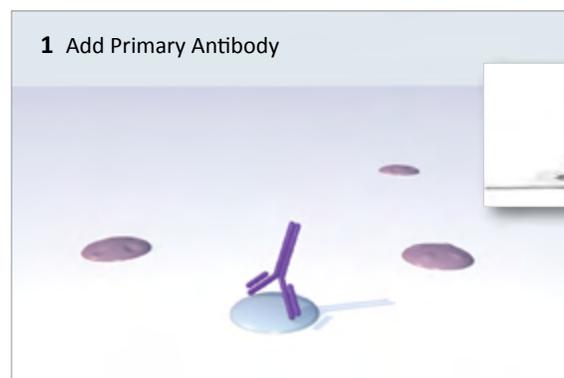
VECTASTAIN[®] ABC Systems

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-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. 28 - 30).

VECTASTAIN Elite ABC System (Peroxidase)

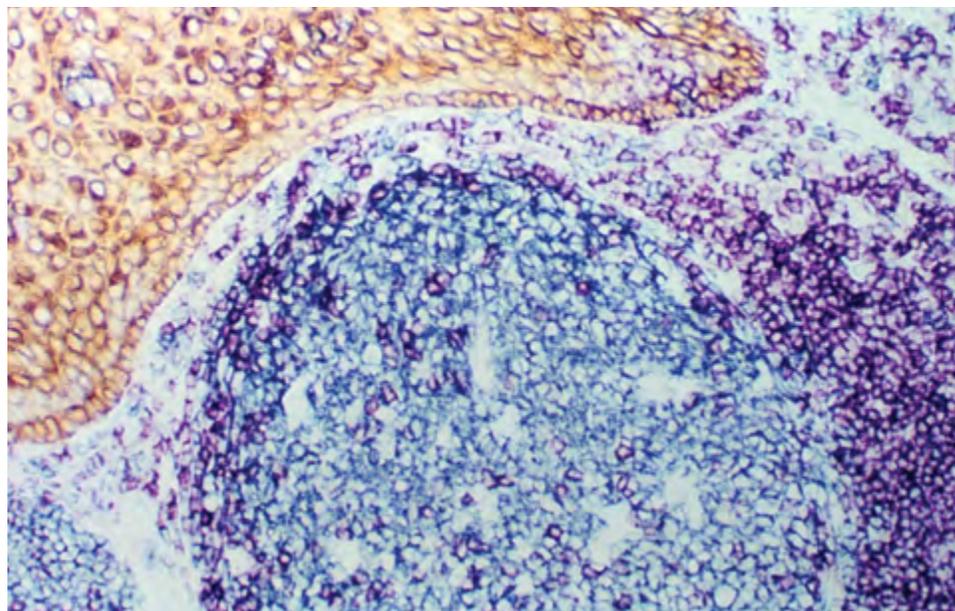
The VECTASTAIN Elite ABC complex is smaller and more uniform than conventional avidin-biotin complexes. They have greater accessibility to biotinylated targets within tissue samples. VECTASTAIN Elite ABC Kits are our most sensitive avidin-biotin based peroxidase systems.

- 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
- > 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 Kits (Peroxidase)

- > 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 recent 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.

| 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 | |

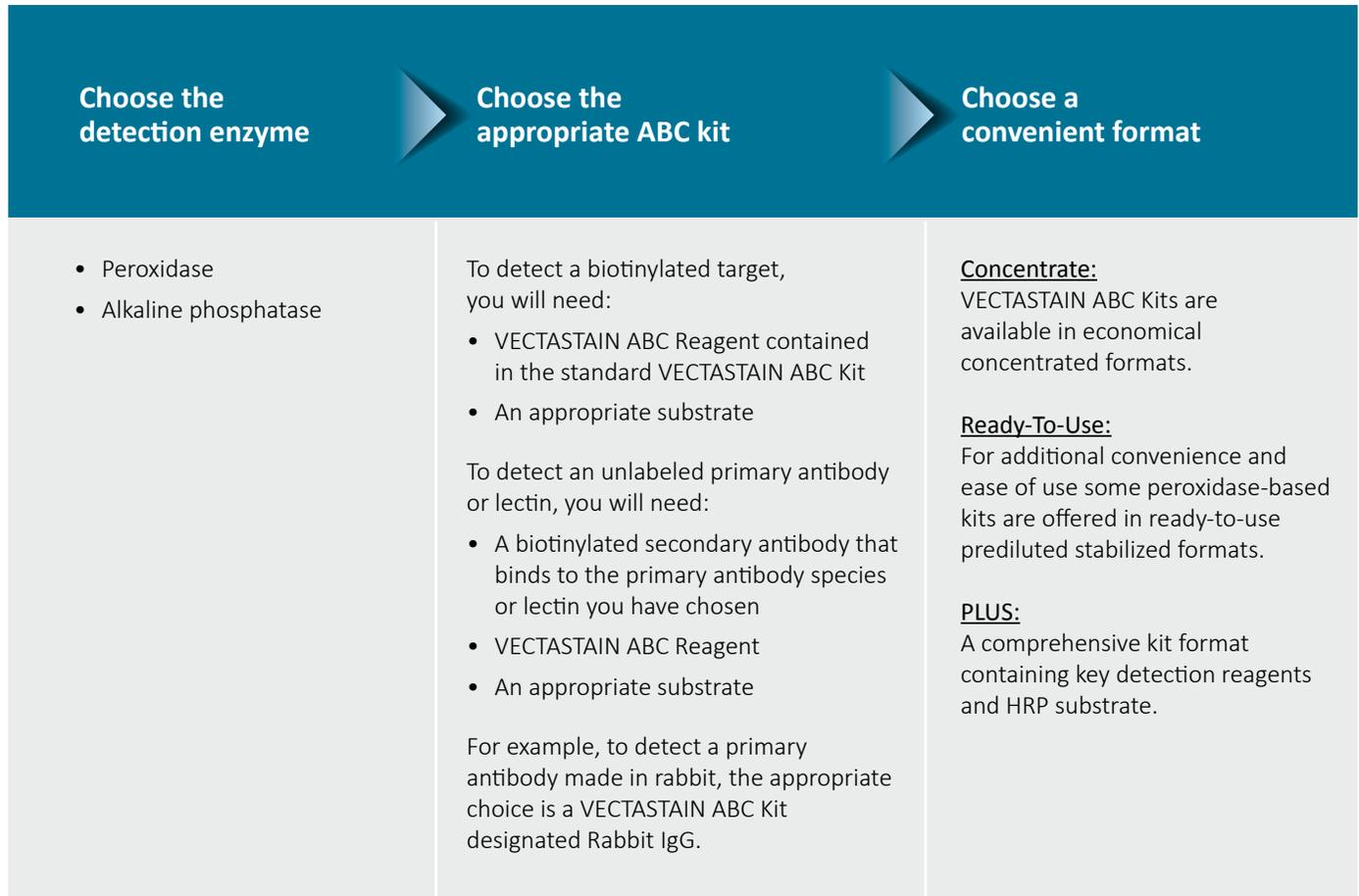
VECTASTAIN Quick Kits (Peroxidase)

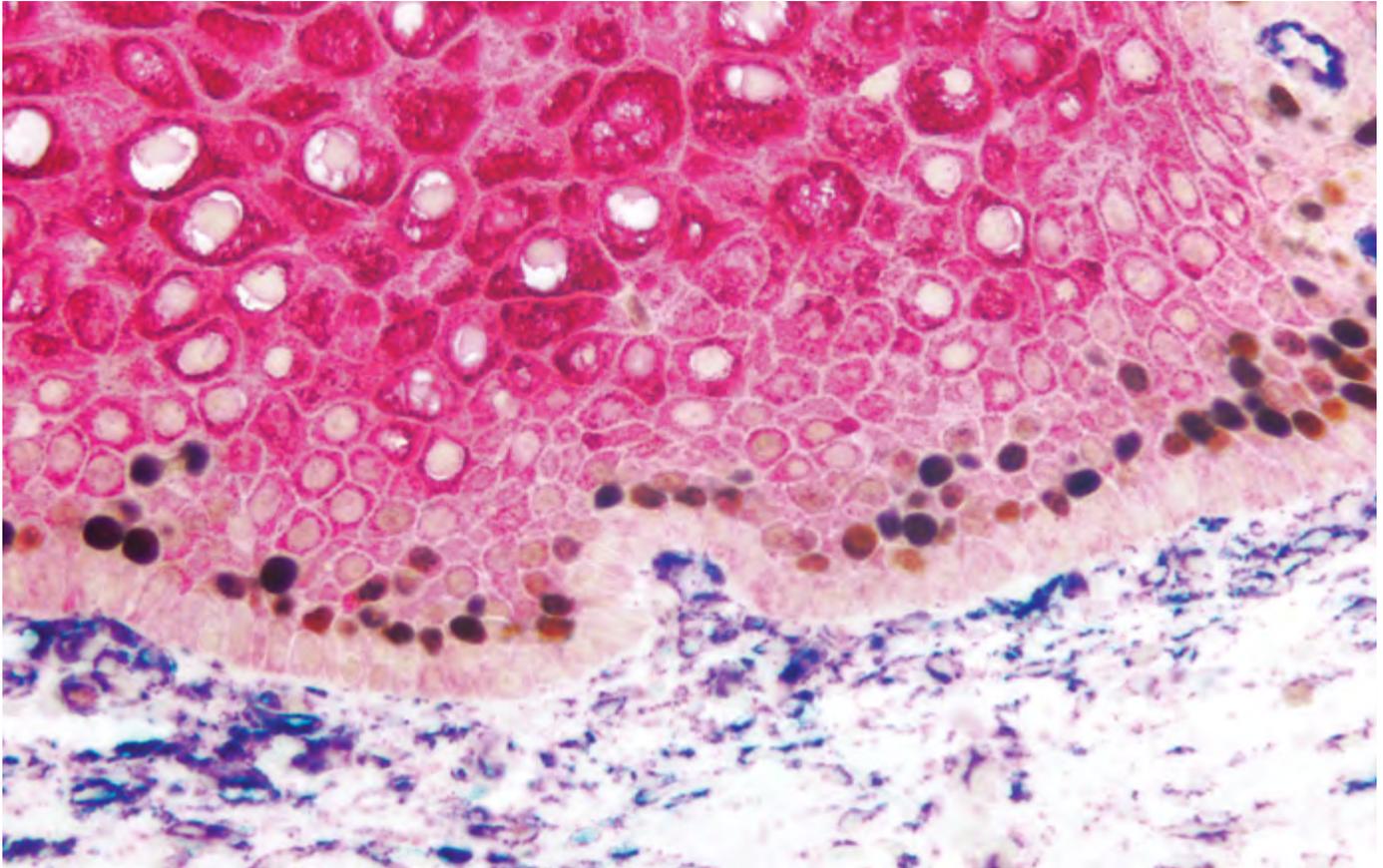
- > R.T.U. VECTASTAIN Quick Kit, Universal
- > VECTASTAIN Quick Kit, Universal

VECTASTAIN ABC-AP Kits (Alkaline Phosphatase)

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

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:

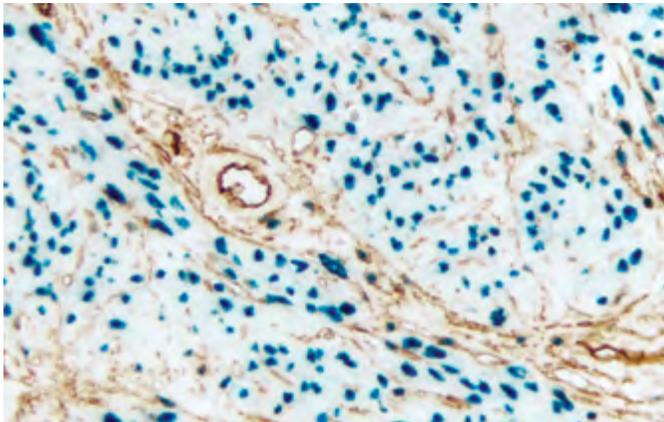
- Directly label the primary antibody with biotin (SP-1200 or SP-1210) and detect it using the VECTASTAIN Elite ABC Kit (Standard, PK-6100), followed by an HRP Substrate.

- 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).

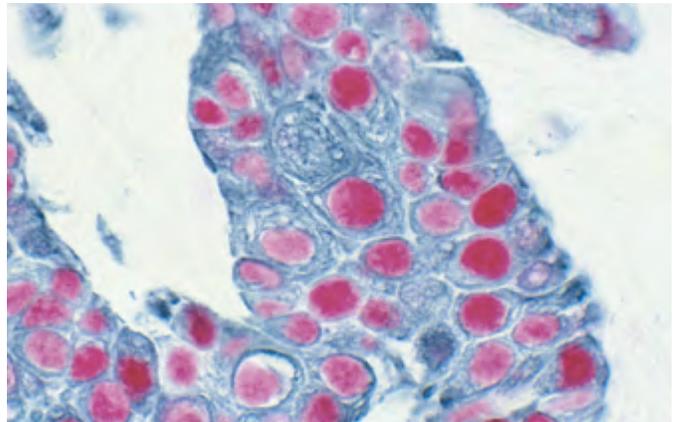
Substrates

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

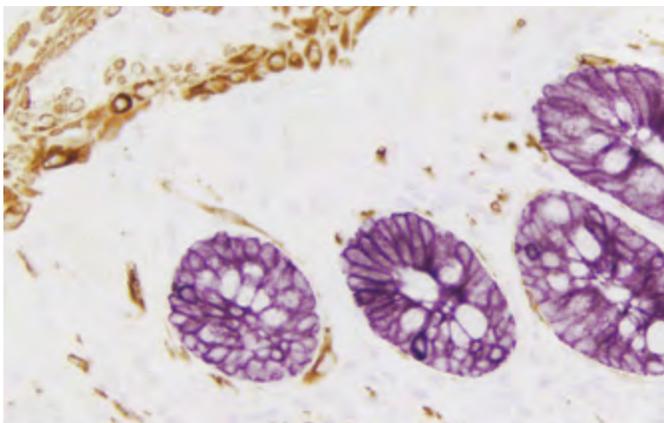
Avidin-Biotin Complex (ABC)-Based Detection



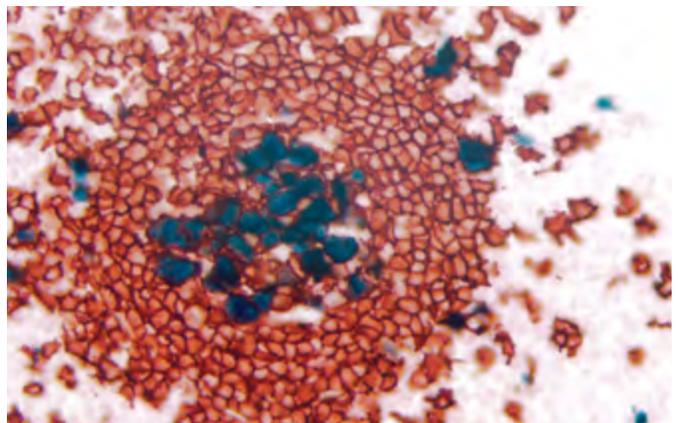
Endometrium: • Progesterone Receptor (m), VECTASTAIN Universal ABC- AP Kit, Vector Blue AP Substrate (blue) • CD34 (m), VECTASTAIN Universal Elite ABC Kit, Vector DAB HRP Substrate (brown).



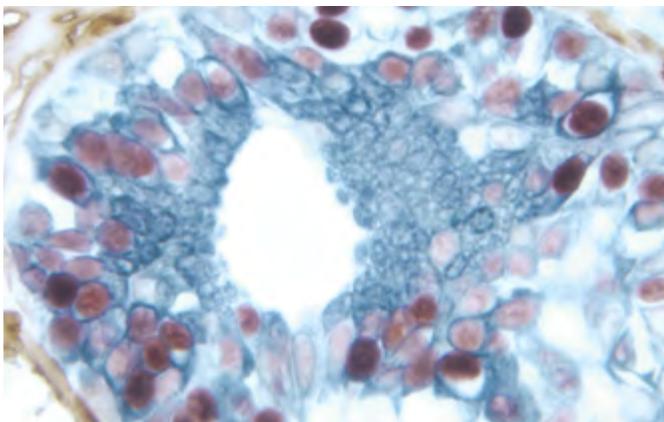
Tumor: • p53 protein (m), VECTASTAIN ABC-AP Kit, Vector Red AP Substrate (red) • Pan-Cytokeratin (sheep), VECTASTAIN Elite ABC Kit, Vector SG HRP Substrate (blue/gray).



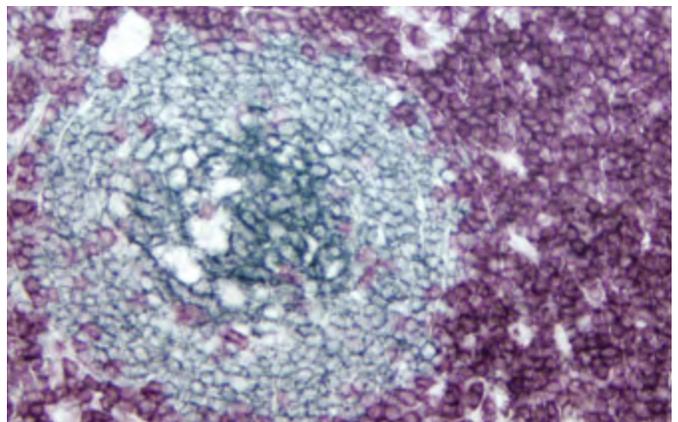
Intestine: • Desmin (m), VECTASTAIN Elite ABC Kit, ImmPACT DAB (brown) substrate. • Cytokeratin (m), VECTASTAIN Elite ABC Kit, Vector VIP (purple) substrate.



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. 39-40) 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.

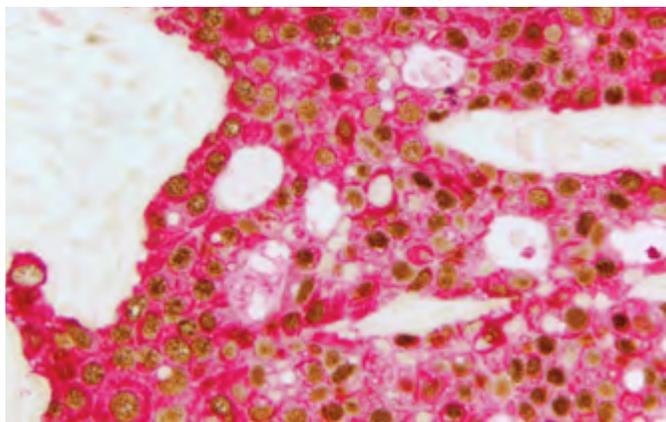
Polymer-Based Detection

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

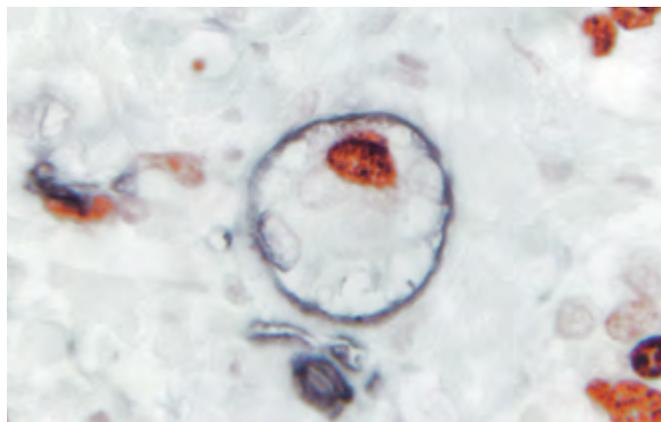
Polymer-based reagents are a more recent introduction into IHC detection methodology than traditional avidin and biotin conjugates, such as ABC kit formats. Polymers offer distinct advantages over these traditional methods particularly for applications such as multiple antigen labeling (multiplexing)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

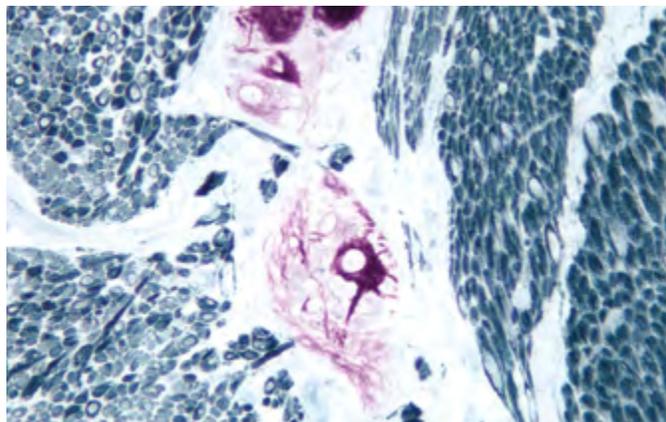
Polymer-based systems were initially introduced consisting of dextran or similar molecules that had inherent issues in some tissues due to their large size. Our ImmPRESS polymer systems have been highly refined and consist of micropolymers that penetrate more easily into thicker sections, avoid steric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



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 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. 23, 31-33)

ImmPRESS HRP PLUS Polymer Kits

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.

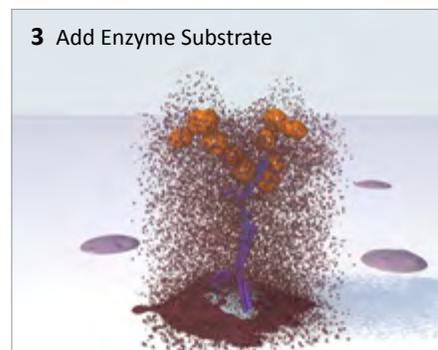
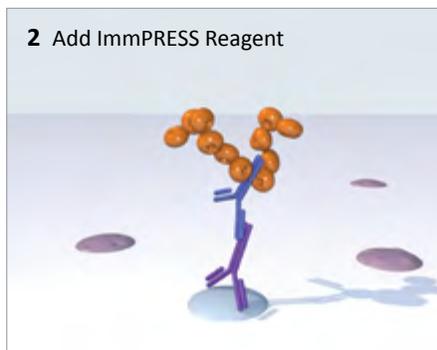
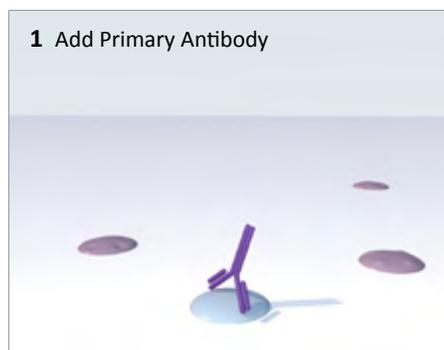
Each ImmPRESS PLUS polymer Kit includes the following:

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

ImmPRESS VR Polymer Kits

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 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 PLUS Polymer Kits (Peroxidase)

- > Anti-Rabbit IgG
- > Anti-Mouse 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

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

ImmPRESS Duet Double Staining Polymer Systems

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.

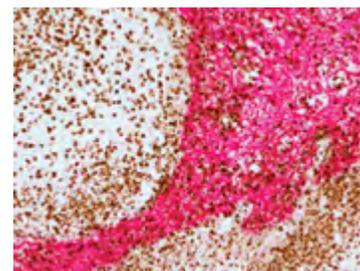
The ImmPRESS Duet Double Staining Polymer Kit is intended for use on non-rodent tissue specimens. Reliable and reproducible results are obtained on tissue sections where the two target antigens do not over-lap (co-localize) in the same structure of the same cell, but rather are expressed in different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

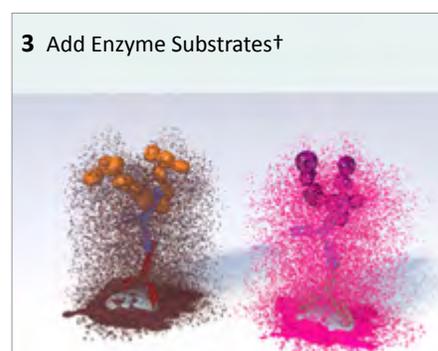
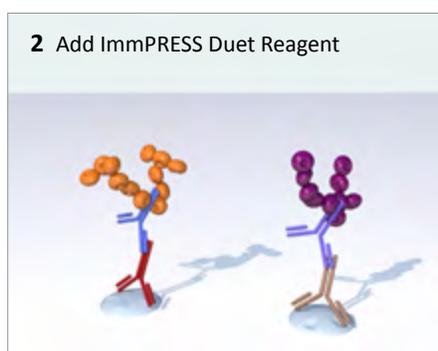
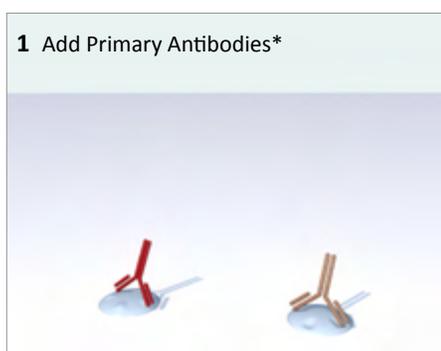
ImmPRESS Duet Double Staining Polymer Kits

- > 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 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.

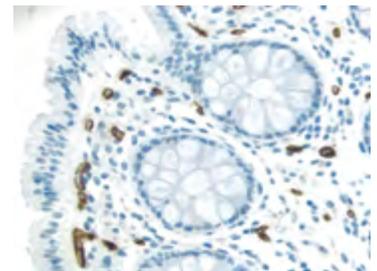
The ImmPRESS Excel Amplified HRP Kits are recommended for applications that require detection of weakly-expressed antigens, in cases of unknown expression levels such as gene knock-in studies, or in determining up-regulation of a given target. This straightforward kit format yields reliable, consistent 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)

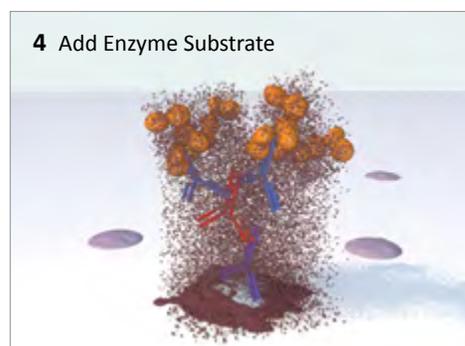
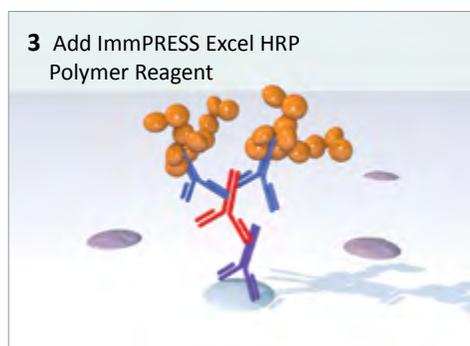
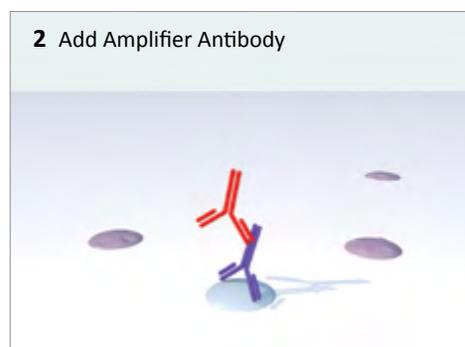
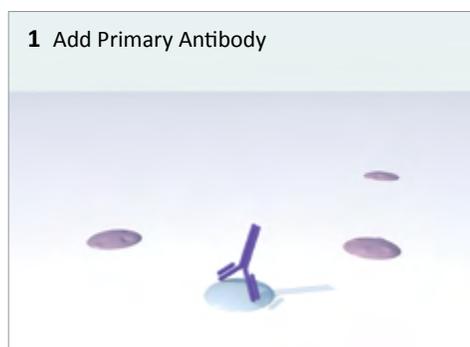
ImmPRESS Excel Amplified Polymer Kits (Peroxidase)

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

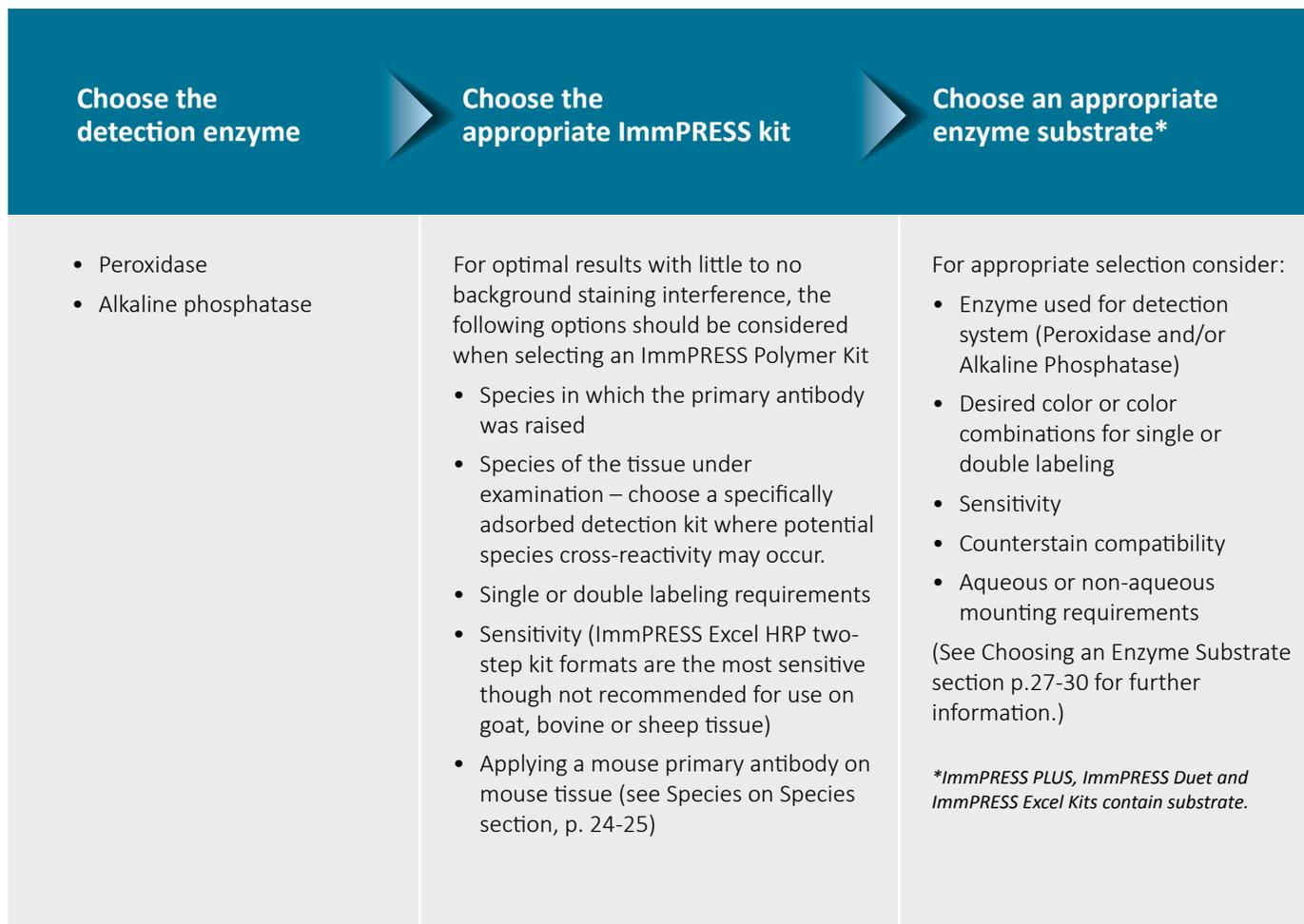


Colon: Anti-CD34 (m), ImmPRESS Excel Amplified Anti-Mouse IgG Staining Kit, ImmPACT DAB EqV (brown), Hematoxylin QS counterstain (blue).

Using the ImmPRESS Two-Step Amplified Polymer Systems



Choosing an ImmPRESS® Polymer Kit



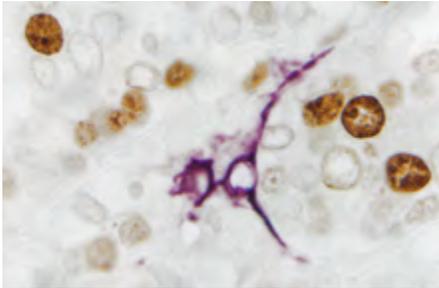
| Product | Peroxidase (HRP) | Peroxidase PLUS* (HRP) | Veterinary Reagents (HRP) | Excel Amplified* (HRP) | Alkaline Phosphatase (AP) | Duet Double Staining* (HRP & AP) |
|---|------------------|------------------------|---------------------------|------------------------|---------------------------|----------------------------------|
| 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.

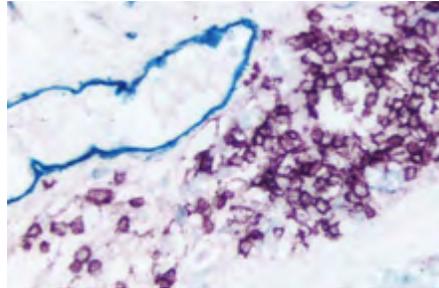
Multiple Antigen Labeling Simplified

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

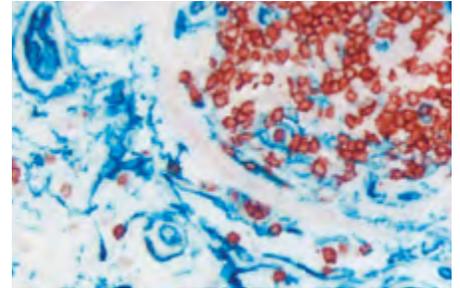
- Fewer steps than conventional protocols decrease slide handling.
- Eliminates the need for avidin/biotin blocking steps in samples with endogenous biotin.



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).

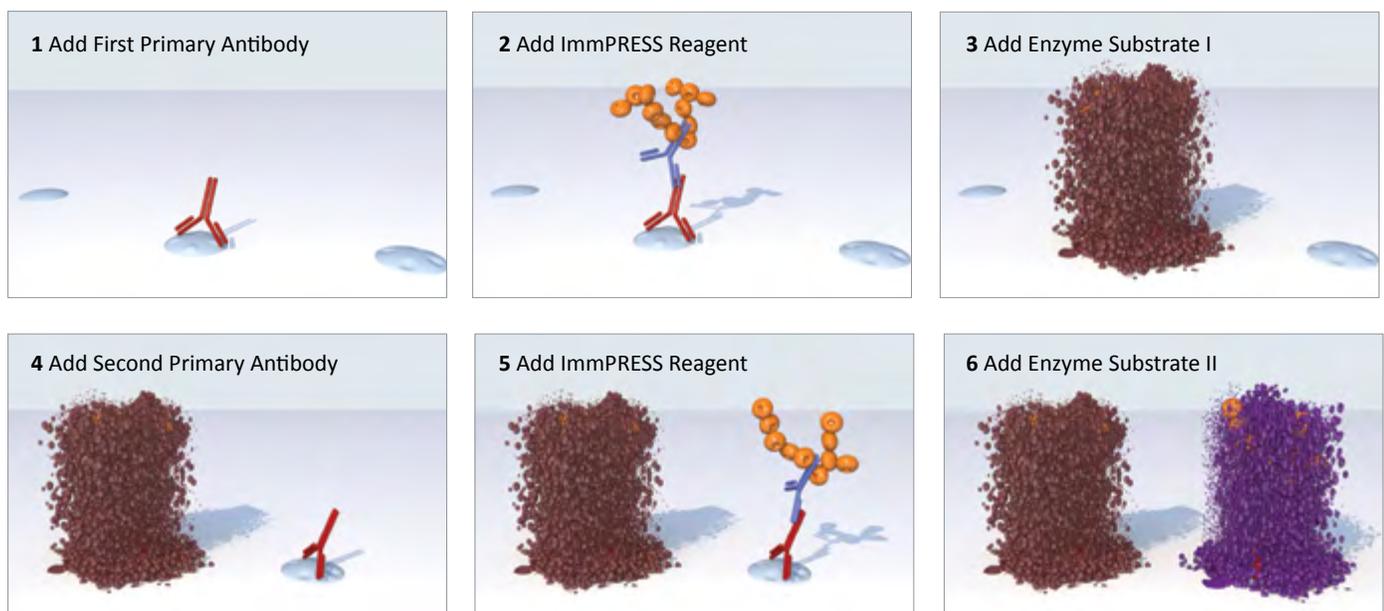


Colon: • CD3 (rm), ImmPRESS Reagent (HRP) Anti-Rabbit IgG, ImmPACT AMEC Red • CD34 (m), ImmPRESS-AP Anti-Mouse IgG Reagent, Vector Blue (blue).

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



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.

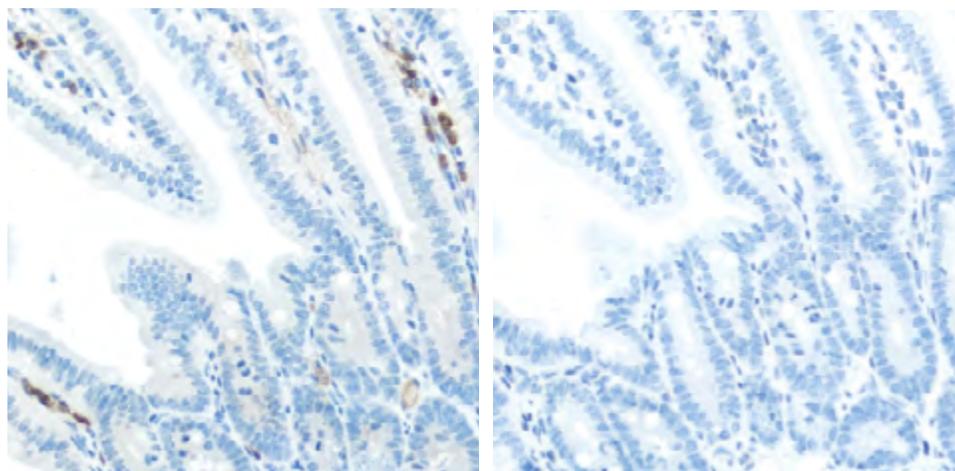
When a primary antibody is the same species as the specimen, the secondary antibody cannot distinguish between the endogenous immunoglobulins and the primary antibody. This can result in high background staining that obscures antigen-specific staining. Mouse on Mouse detection is especially important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

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

M.O.M.[®] (Mouse on Mouse) Immunodetection Kits

The 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 Labs 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



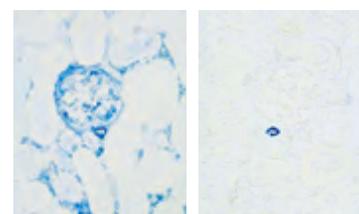
Mouse intestine stained with standard anti-mouse IgG polymer system (left) and Vector M.O.M. ImmPRESS HRP Polymer Kit (right). Brown signal indicates IgG background staining. (Both: No primary antibody, Vector DAB stain, hematoxylin counterstain.)

| 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.

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).

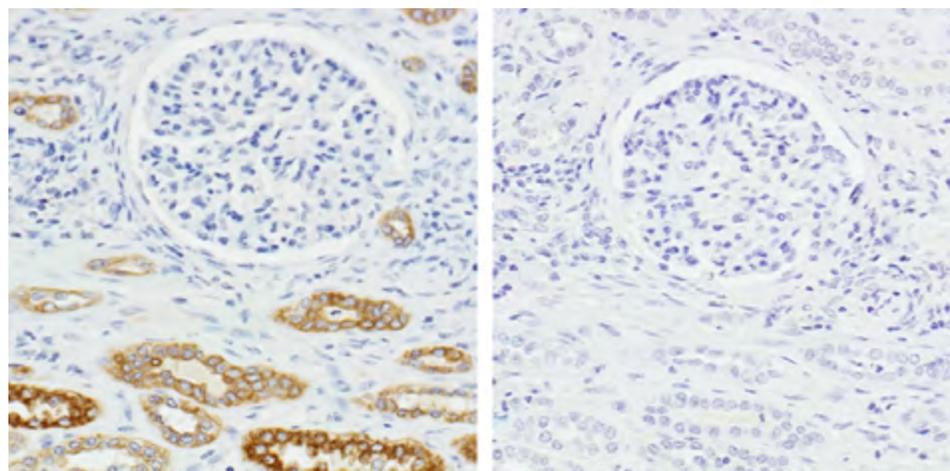
H.O.H. (Human on Human) Immunodetection Kit

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.

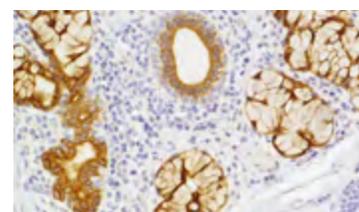
- Essentially background-free results even on frozen lymphoid tissue sections
- A simplified IHC procedure for H.O.H. detection
- Minimal optimization requirements
- A comprehensive kit format with matched volume reagents
- Kit includes the DAB substrate



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).

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).

| 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.

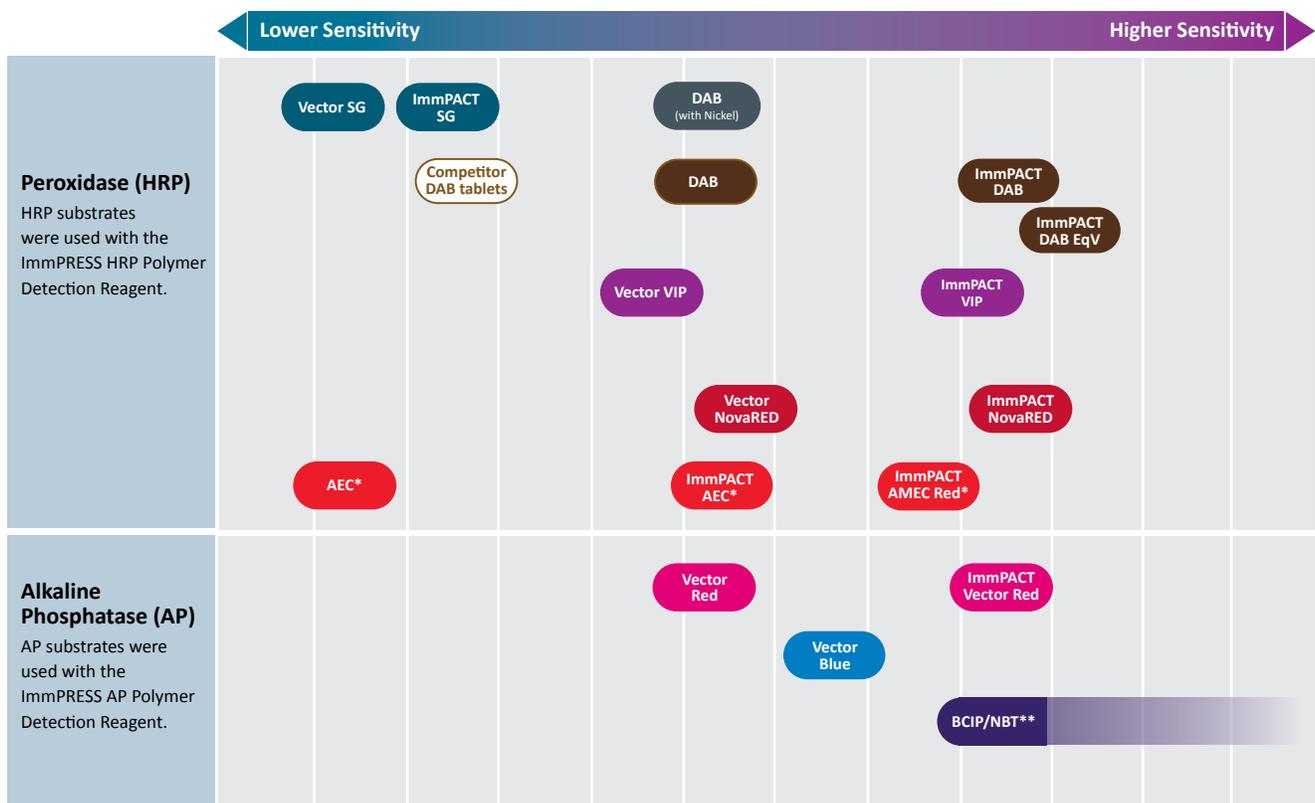
- **Sensitivity.** Substrates differ in sensitivity. Some may increase in sensitivity with longer incubation times.
- **Color.** Color contrast is essential in multiple antigen labeling applications, in pigmented tissues such as melanomas, and in counterstained tissues. Where performance is equal, color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

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



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

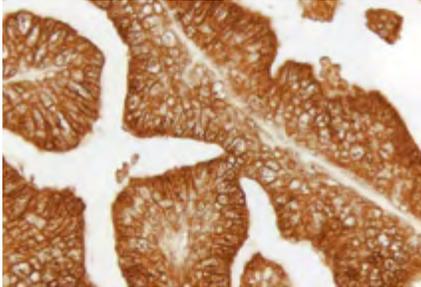
Enzyme Substrate Properties

See also Enzyme Substrate Combinations for multiple antigen labeling (p. 31-34) and Counterstain/Substrate Compatibility (p. 36).

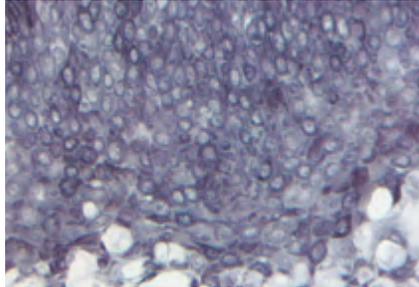
| Substrate | Color | Catalog Number | Microscopy | | | | | Mounting | Contrast in Pigmented Tissue | Multiple Labeling | Heat Resistant* |
|-----------------------------|---|----------------|--------------|-----------|----------|--------------|------------------|------------------------|------------------------------|-------------------|-----------------|
| | | | Bright-field | Darkfield | Electron | Fluorescence | 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 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.

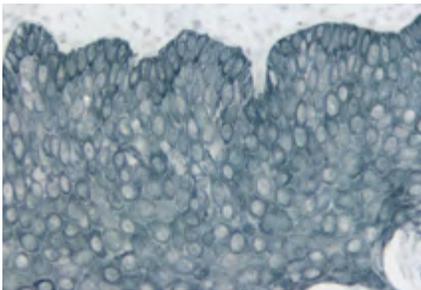
Peroxidase Substrates



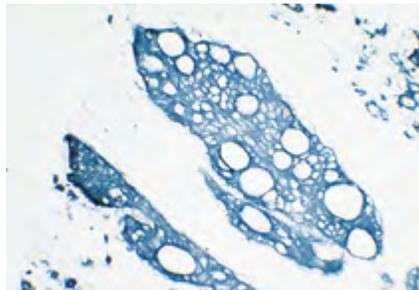
Prostate: Prostate Specific Antigen (m), ImmPRESS Reagent (HRP), ImmPACT DAB (brown).



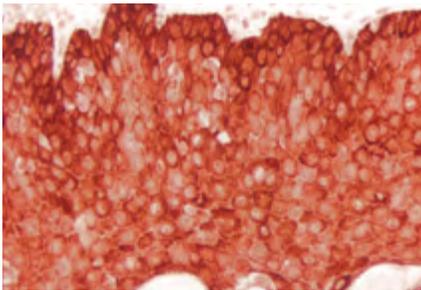
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS Reagent (HRP), Vector DAB-Ni (gray-black).



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



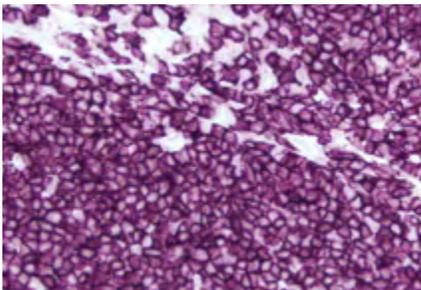
Tumor: Cytokeratin (s), VECTASTAIN Elite ABC Kit, TMB (blue).



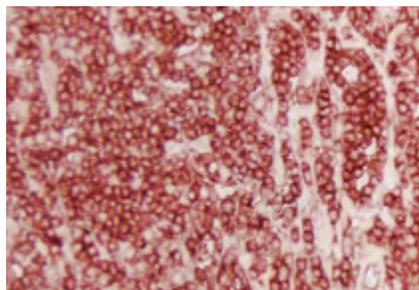
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS Reagent (HRP), ImmPACT NovaRED (red).



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

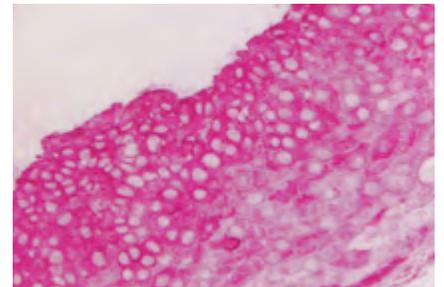


Tonsil: CD20 (m), ImmPRESS Reagent (HRP), ImmPACT VIP (purple).

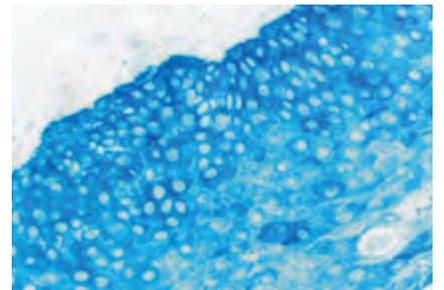


Tonsil: LCA (m), ImmPRESS Reagent (HRP), ImmPACT AMEC Red (red).

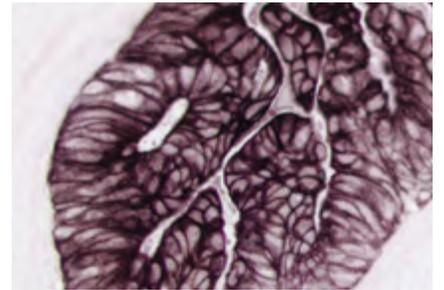
Alkaline Phosphatase Substrates



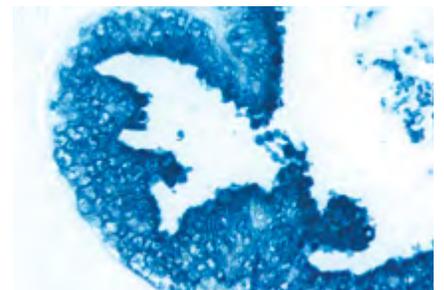
Tonsil: Cytokeratin AE1/AE3 (m), Vector ImmPRESS-AP Reagent, ImmPACT Vector Red (magenta).



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*.

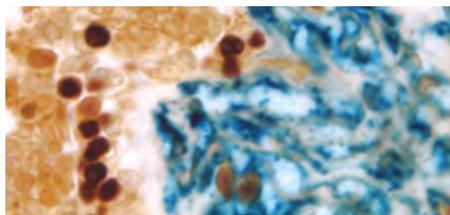
Colon: • Cytokeratin (AE1/AE3, m), ImmPRESS-AP Anti-Mouse IgG Reagent, Vector Blue AP Substrate (blue)
• CD3 (rb), ImmPRESS Anti-Rabbit IgG HRP Reagent, ImmPACT AMEC Red HRP Substrate (red).

Enzyme Substrate Combinations

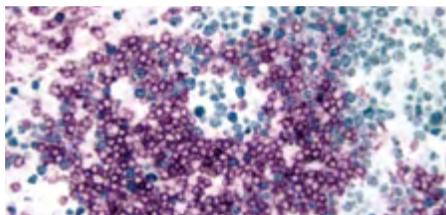
Recommended combinations of substrates and the recommended order in which they should be used.

| 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 | - | - | - | - | + | - | - | + | |

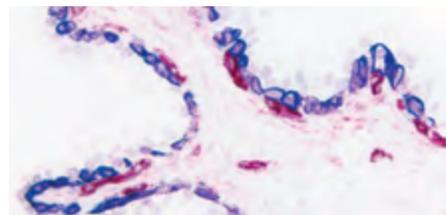
+ Indicates good contrast - Indicates incompatibility of substrates for various reasons



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).

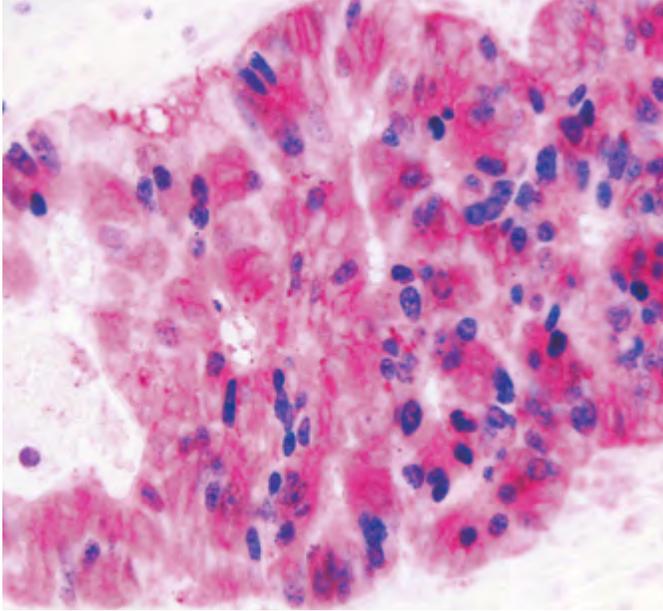


Tonsil: • CD3 (m), ImmPRESS Anti-Mouse IgG Reagent, Vector VIP HRP Substrate (purple) • Ki67 (m), ImmPRESS Anti-Mouse IgG Reagent, Vector SG HRP Substrate (blue/gray).

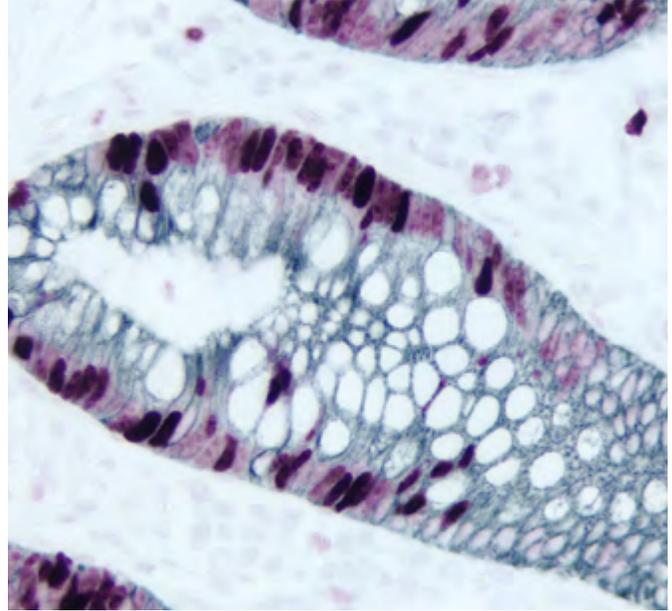


Prostate: • Cytokeratin 5 (m), VECTASTAIN Universal ABC-AP Kit, Vector Blue AP Substrate (blue) • CD34 (m), VECTASTAIN Universal ABC-AP Kit, Vector Red AP Substrate (red).

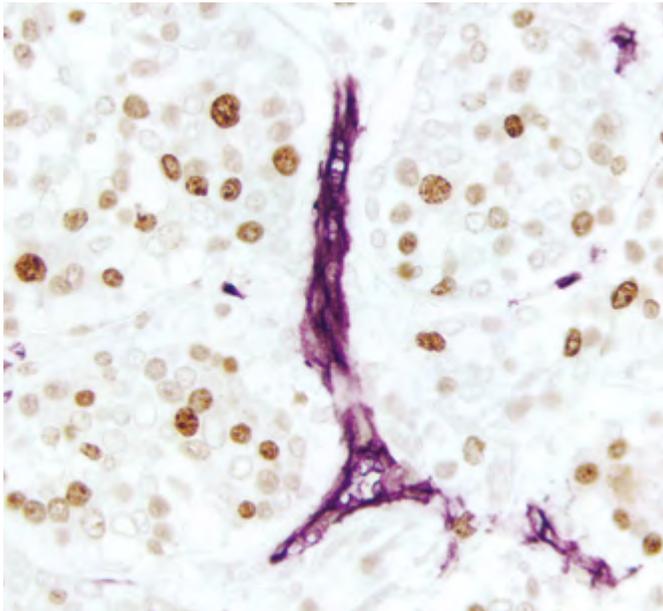
Multiple Labeling Examples



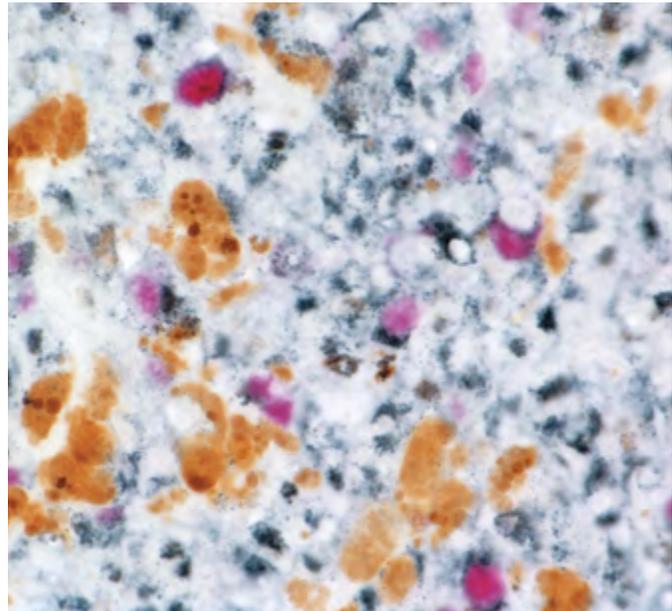
Colon Cancer: • Ki67 (rm), ImmPRESS-AP Anti-Rabbit IgG Reagent, Vector Blue AP Substrate (blue) • Cox2 (rm), ImmPRESS-AP Anti-Rabbit IgG Reagent, ImmPACT Vector Red AP Substrate (red).



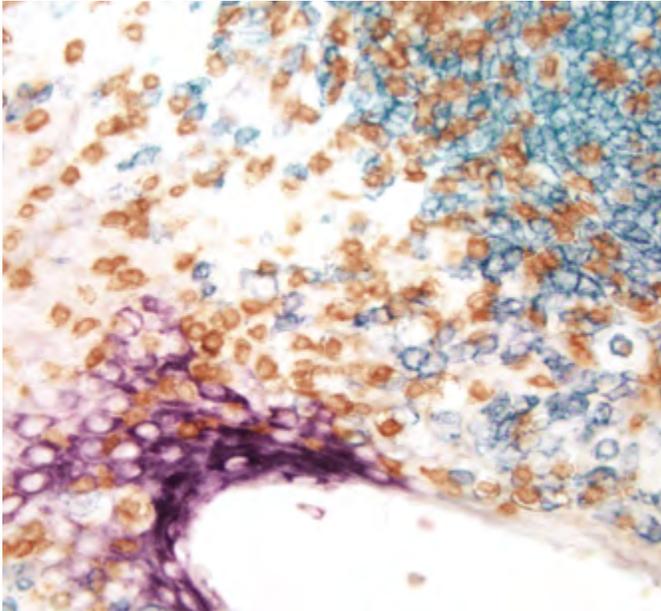
Colon: • Ki67 (rm), ImmPRESS Universal (Anti-Mouse/Anti-Rabbit IgG) HRP Reagent, Vector VIP (purple) • Cytokeratin (m), ImmPRESS Universal (Anti-Mouse/Anti-Rabbit IgG) HRP Reagent, Vector SG HRP Substrate (gray).



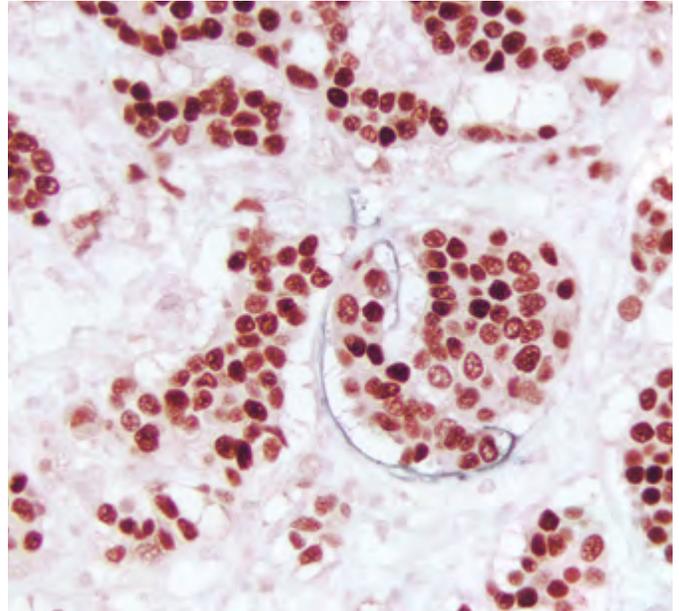
Breast Carcinoma: • Ki67 (rm), ImmPRESS Reagent (HRP; Universal), Vector DAB (brown) • CD34 (m), ImmPRESS Reagent (HRP; Universal), Vector VIP (purple).



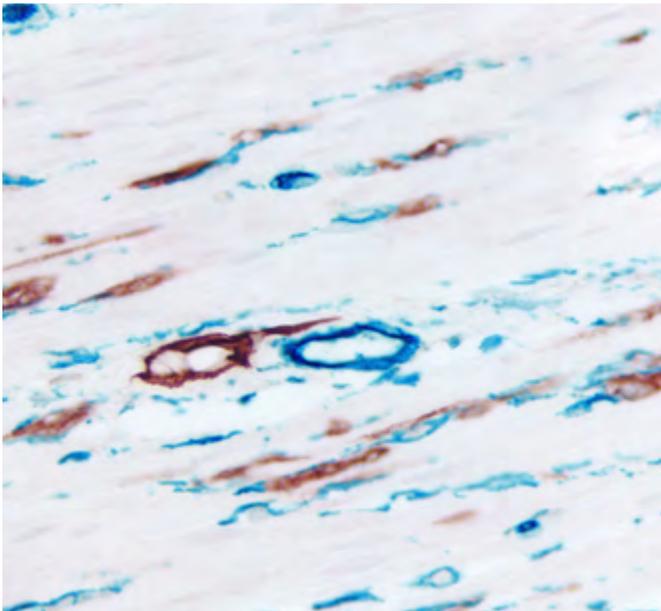
Melanoma: • Cyclin A (m), ImmPRESS-AP Anti-Mouse IgG, ImmPACT Vector Red AP Substrate (magenta) • Melanoma Marker (m) ImmPRESS HRP Anti-Mouse IgG, Vector SG HRP Substrate (gray). Note contrast of double stain with the brown pigments in the tissue.



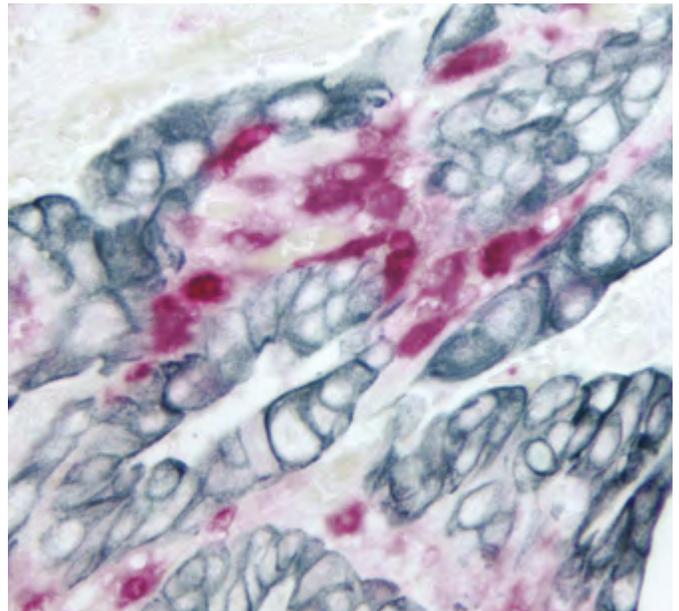
Tonsil: • CD3 (m), VECTASTAIN Elite ABC Kit (Universal), Vector DAB (brown)
 • CD20 (m), VECTASTAIN Elite ABC Kit (Universal), Vector SG (blue-gray) • Multi-Cytokeratin (m), VECTASTAIN Elite ABC Kit (Universal), Vector VIP (purple).



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.

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)

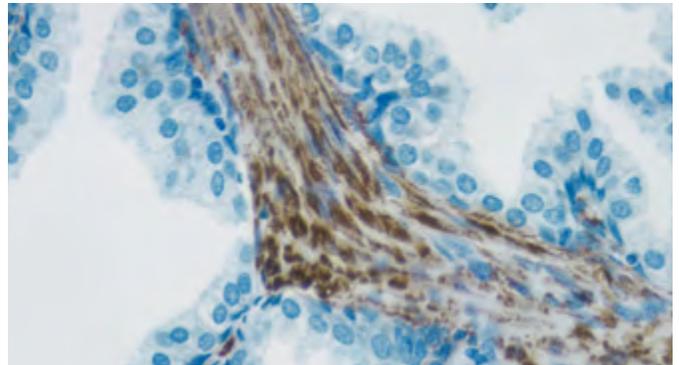
- Modification of Mayer's hematoxylin developed especially for immunocytochemistry
- Ready-to-use without filtration or 'blueing' step
- Stains in less than 45 seconds
- Excellent color contrast with most commonly used peroxidase and alkaline phosphatase substrates
- Suitable for use with non-aqueous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

Methyl Green (light green)

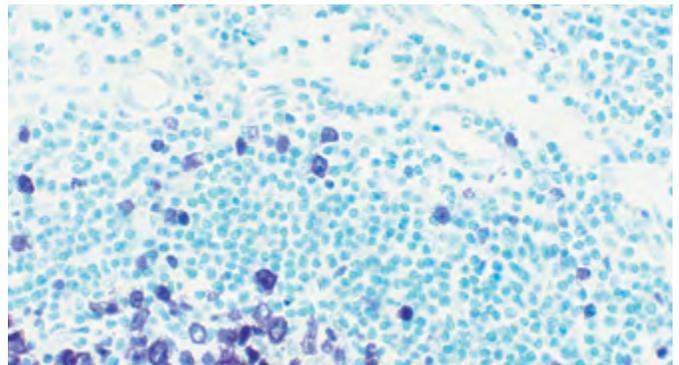
- Superior formulation of methyl green suitable for use with a wide range of enzyme substrates
- Simple, two-step procedure
- Excellent alternative in multiple antigen labeling when hematoxylin obscures the substrate colors
- Suitable for use with non-aqueous mounting media

Nuclear Fast Red (pink)

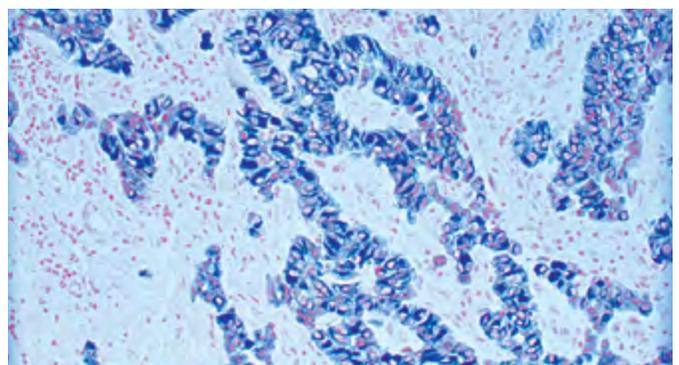
- Fast one-step protocol
- Excellent alternative in multiple antigen labeling when hematoxylin obscures the substrate colors
- Good contrast with a variety of substrates
- Suitable for use with non-aqueous mounting media



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.

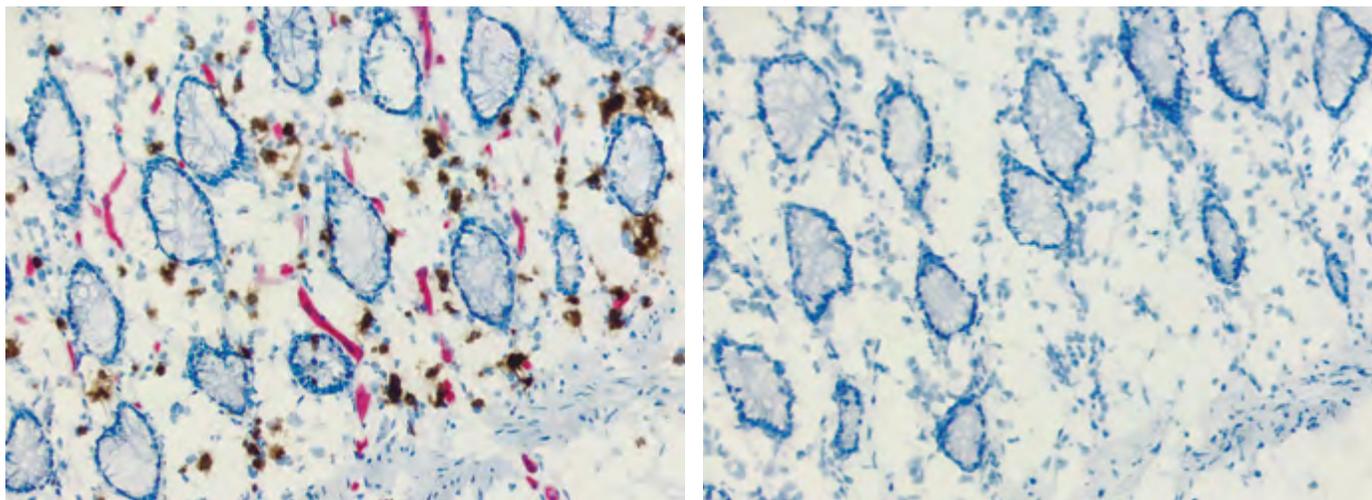
** Substrate dissolves in acetone wash.

*** 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 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 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 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 |
|---|----------------|
| 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 |

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

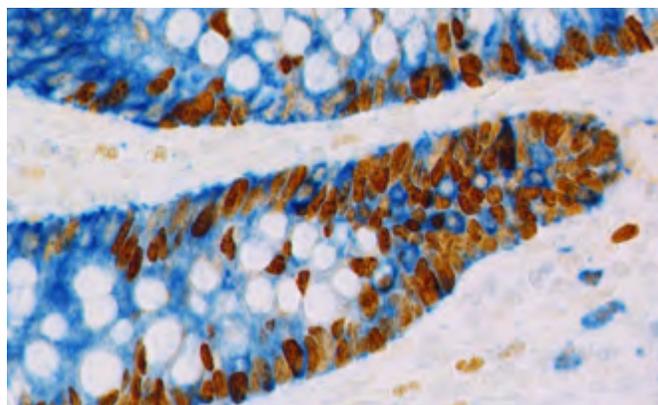
VectaMount AQ Aqueous Mounting Medium preserves the color and clarity of enzyme substrates whose reaction products are soluble in alcohol or other organic solvents. Stained and mounted sections can be stored in a slide box at room temperature for at least two years without fading.

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

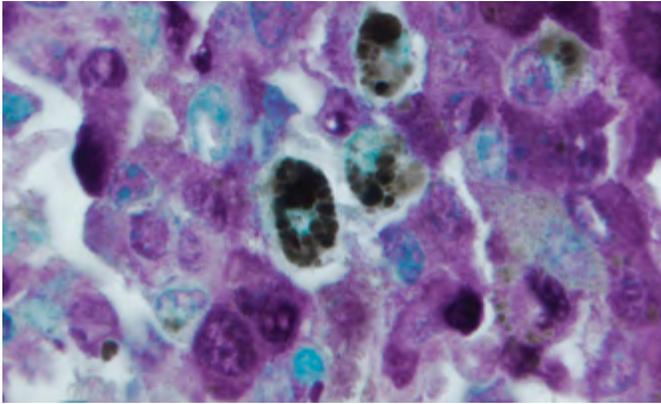
| Product | Catalog Number |
|---------------------------------------|----------------|
| VectaMount Express Mounting Medium | H-5700 |
| VectaMount Permanent Mounting Medium | H-5000 |
| VectaMount AQ Aqueous Mounting Medium | H-5501 |

Mounting Media / Substrate Compatibility

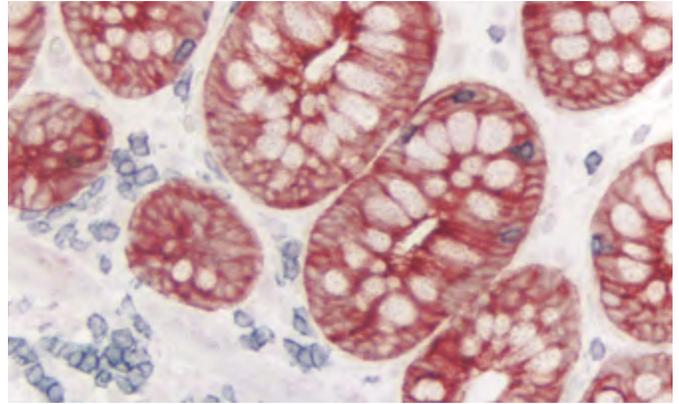
| Substrate | VectaMount Express Mounting Medium | VectaMount Permanent Mounting Medium | VectaMount AQ Aqueous Mounting Medium |
|--------------------|------------------------------------|--------------------------------------|---------------------------------------|
| HRP | | | |
| DAB | • | • | • |
| 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 | | • | |
| AP | | | |
| Vector Red | • | • | • |
| ImmPACT Vector Red | • | • | • |
| Vector Blue | • | • | • |
| Vector Black | | • | |
| BCIP/NBT | • | • | • |



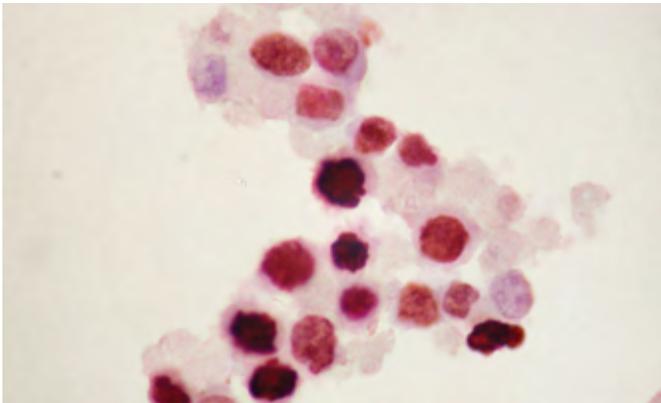
Human Colon – double label: Mouse Anti-Ki67, ImmPRESS-HRP Anti-Mouse IgG, ImmPACT DAB EqV and Rabbit Anti-Cytokeratin ImmPRESS-AP Anti-Rabbit IgG, Vector Blue. Mounted in VectaMount Express Mounting Media.



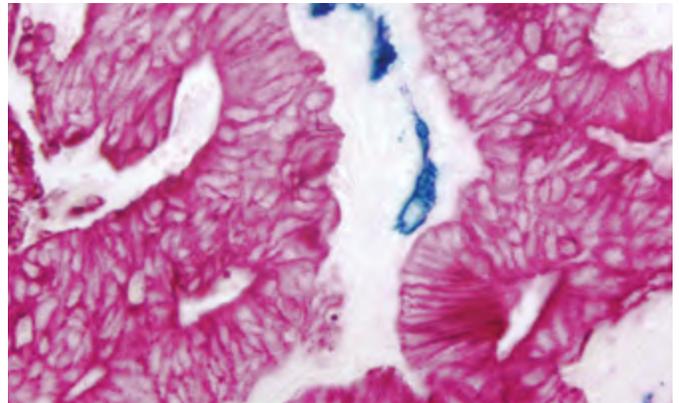
Melanoma: S100 (rp), VECTASTAIN Elite ABC Kit, Vector VIP (purple), Vector Methyl Green counterstain (green). Note color contrast with brown pigments in tissue.



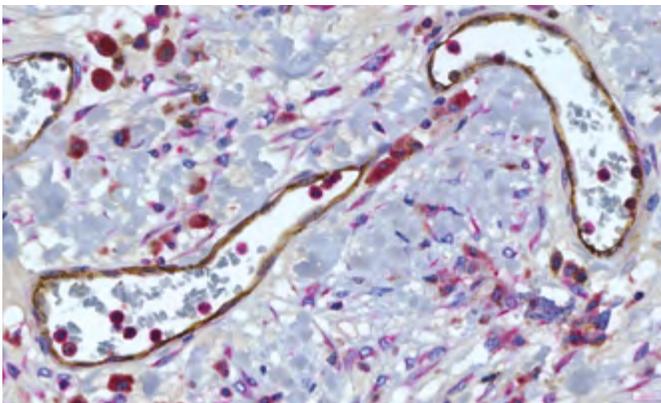
Colon: • CD3 (rm), ImmPRESS Reagent (HRP) Anti-Rabbit IgG, ImmPACT SG (blue-gray) • Cytokeratin AE1/AE3 (m), ImmPRESS Reagent (HRP) Anti-Mouse IgG, ImmPACT AMEC Red (red).



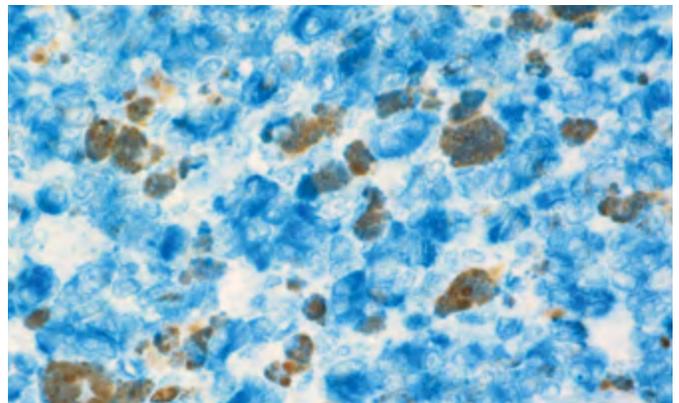
Cytospin of EBV+ cell line: Epstein-Barr virus nuclear antigen 1 (EBNA-1; rat), ImmPRESS Reagent (HRP) Anti-Rat IgG, ImmPACT NovaRED (red). (Image courtesy of Dr. GM Reynolds, Centre for Liver Research, University of Birmingham, U.K.)



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

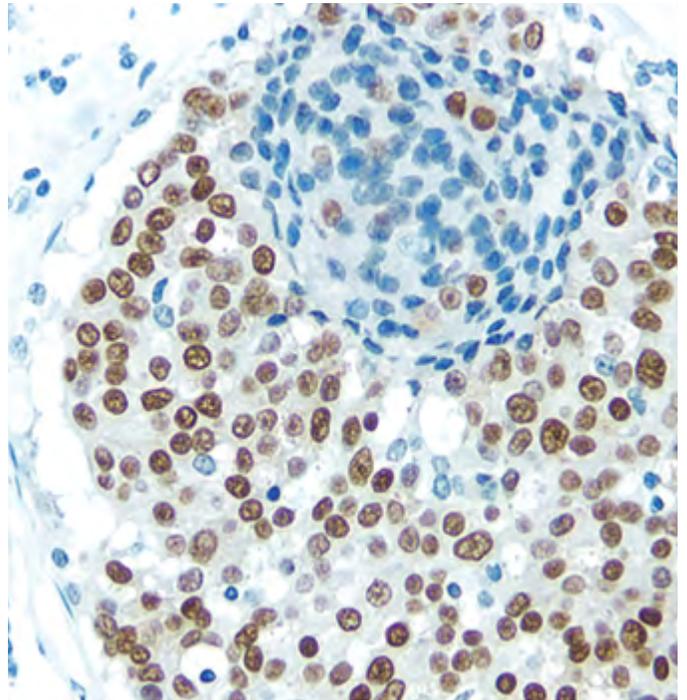
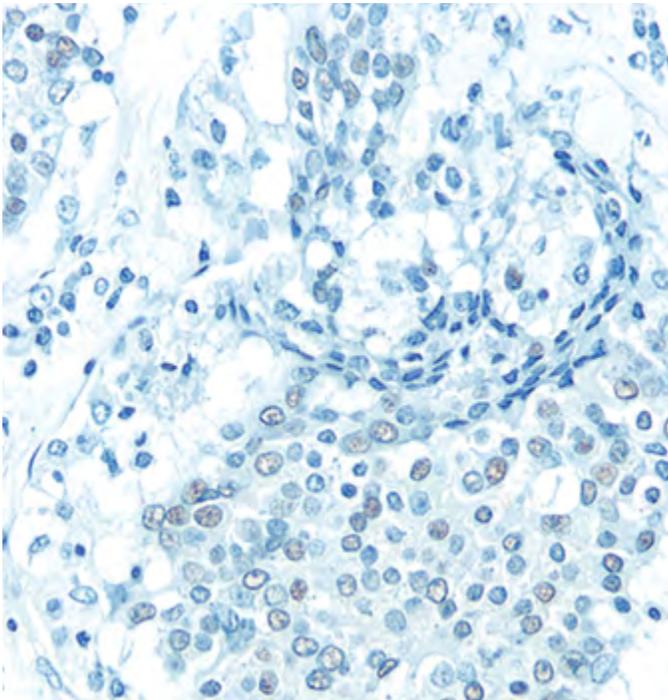
Control Antibodies

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.

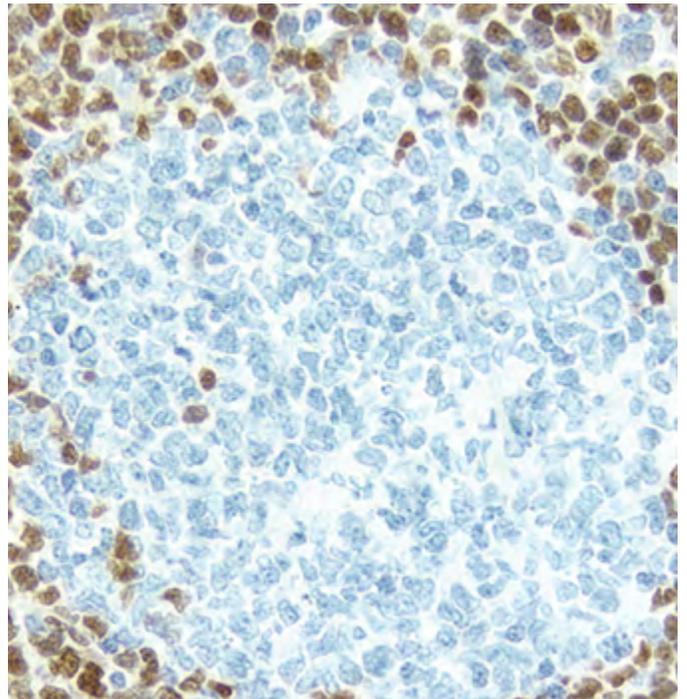
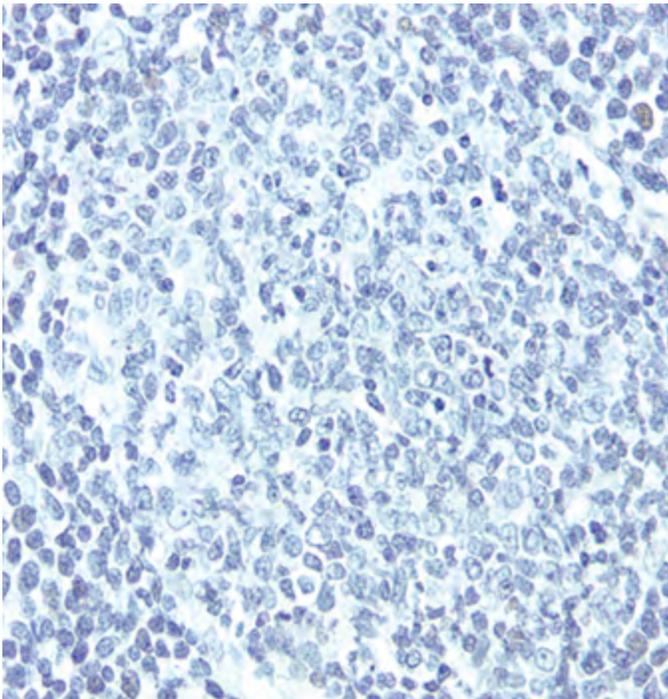
Antigen Unmasking Solutions

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 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.

Contact Details



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Technical Support: technical@vectorlabs.com
International Inquiries: Please contact your local distributor.

Ordering Information

Order online at: www.vectorlabs.com

Orders may also be placed by email, telephone, fax, 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 \$1,000 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.

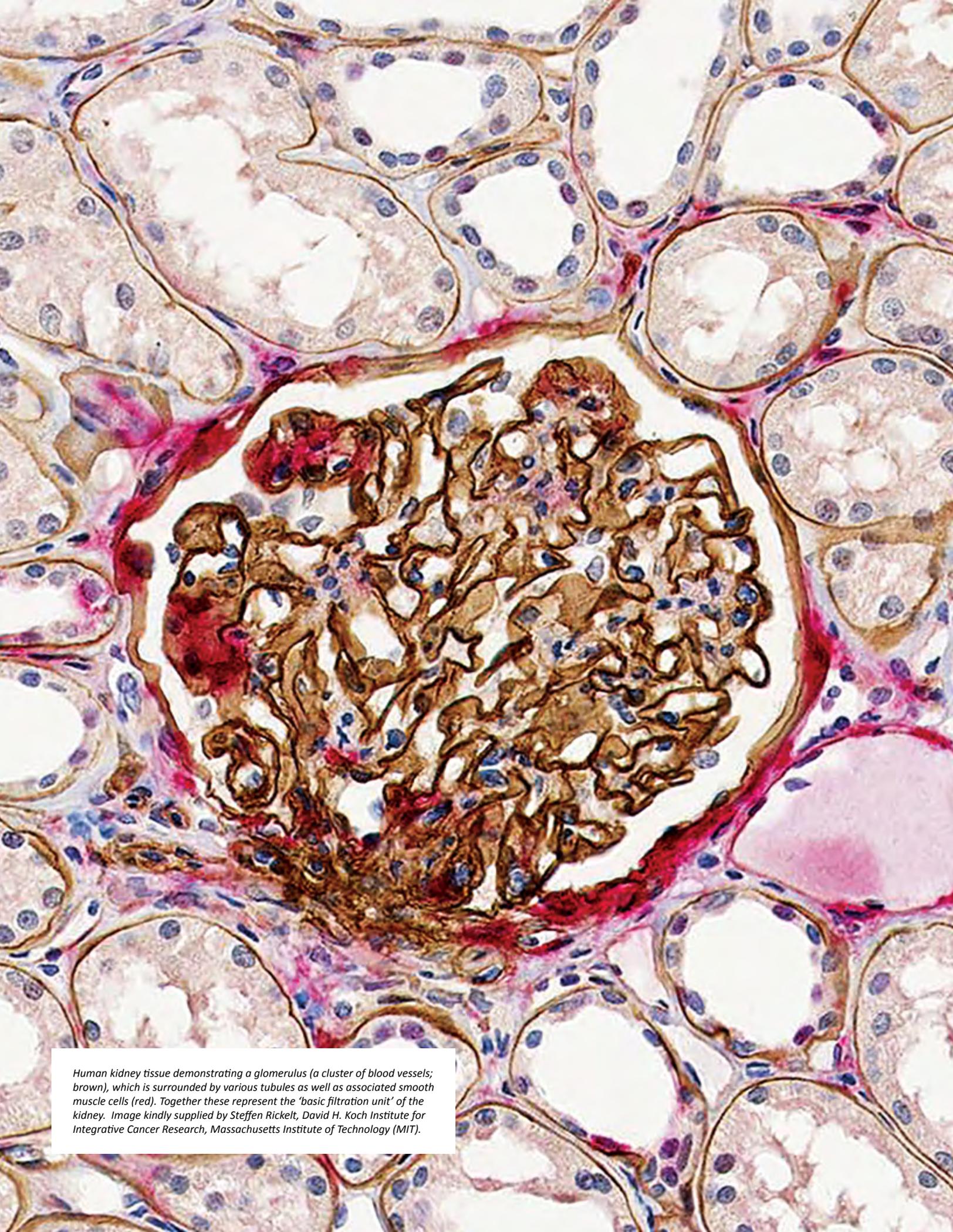
Payment / Shipping Terms:

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, all products are shipped 2nd Day Air.

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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).

