Immunohistochemistry Guide
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Making IHC as easy as ABC

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

A. Reliable and reproducible reagents that instill trust and confidence.

B. Simple and robust product designs that streamline workflows and allow elucidation of complex biological systems.

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

It’s as simple as that.

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

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

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* For more information visit: vectorlabs.com/lectins
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

**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

**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
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.

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However, the ability to identify the subcellular structure of tissues became visible and the science of histochemistry emerged.

The use of IHC as a research tool grew dramatically over the next decade. The technique began to be used in clinical settings at large university hospitals. The HRP assay system was further improved in the early 1980’s when Dr. Su-Ming Hsu showed that the high affinity of avidin for biotin could be used to increase the stability of the enzyme antibody complex and improve the sensitivity of the assay. Vector Laboratories was instrumental in the development of the IHC field by commercializing such key technologies. The use of avidin- and biotin-based detection systems dominated the IHC market for the next two decades.

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

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

**References**


Cholds DJ “History of Immunohistochemistry” Pathobiology of Human Disease. 3775-3796 (2014)
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.

Choosing a Detection System

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<th>Modular Mouse Primary on Mouse Tissue</th>
<th>Ready-to-Use (R.T.U.) Format</th>
<th>Typical number of steps</th>
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HPR - Horseradish peroxidase
AP - Alkaline phosphatase
VR - Veterinary Reagents

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.

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

VECTASTAIN® ABC Systems

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

Recommended applications:
- Tissue and cell staining
- Protein and nucleic acid blotting
- In situ hybridization
- ELISAs
- Neuronal tracing

Using the VECTASTAIN® ABC System

1. Add Primary Antibody
2. Add Biotinylated Secondary Antibody
3. Add Avidin/Biotinylated Enzyme Complex (ABC)
4. Add Enzyme Substrate

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

**Peroxidase-Based Kits:**

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

**VECTASTAIN® Elite® ABC System (Peroxidase)**

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

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

**Original VECTASTAIN® ABC Kit (Peroxidase)**

Our original avidin-biotin ABC complex formulation.

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

**VECTASTAIN® Elite®, ABC Kits (Peroxidase)**

- Standard (Elite®, ABC Reagent only)
- Rabbit IgG
- Mouse IgG
- Human IgG
- Rat IgG
- Goat IgG
- Sheep IgG
- Universal

**VECTASTAIN® Elite® ABC System (Peroxidase)**

- R.T.U. VECTASTAIN® Elite® ABC Reagent
- R.T.U. VECTASTAIN® Elite® ABC

**VECTASTAIN® Universal Quick Kits (Peroxidase)**

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

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

**Alkaline Phosphatase-Based Kits:**

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

**VECTASTAIN® ABC-AP Kits (Alkaline Phosphatase)**

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

Product | Elite® (Peroxidase) | Original (Peroxidase) | Quick (Peroxidase) | Alkaline Phosphatase
--- | --- | --- | --- | ---
VECTASTAIN® ABC Kit, Standard | PK-6100 | PK-4000 | PK-5000 | 4A-5005
VECTASTAIN® ABC Kit, Rabbit IgG | PK-6101 | PK-4001 | PK-5001 | 4A-5005
VECTASTAIN® ABC Kit, Mouse IgG | PK-6102 | PK-4002 | PK-5002 | 4A-5005
VECTASTAIN® ABC Kit, Mouse IgM | PK-6103 | PK-4010 | PK-5003 | 4A-5005
VECTASTAIN® ABC Kit, Human IgG | PK-6104 | PK-4004 | PK-5004 | 4A-5005
VECTASTAIN® ABC Kit, Rat IgG | PK-6105 | PK-4005 | PK-5005 | 4A-5005
VECTASTAIN® ABC Kit, Goat IgG | PK-6106 | PK-4006 | PK-5006 | 4A-5005
VECTASTAIN® ABC Kit, Sheep IgG | PK-6107 | PK-4007 | PK-5007 | 4A-5005
VECTASTAIN® ABC Kit, Guinea Pig IgG | PK-6108 | PK-4008 | PK-5008 | 4A-5005
VECTASTAIN® ABC Kit, Universal | PK-6109 | PK-4009 | PK-5009 | 4A-5009
R.T.U. VECTASTAIN® ABC Reagent | PK-7100 | PK-7100 | PK-7100 | 4A-7200
R.T.U. VECTASTAIN® ABC Kit, Universal | PK-7200 | PK-7200 | PK-7200 | 4A-7200
R.T.U. VECTASTAIN® Universal Quick Kit | PK-8100 | PK-8100 | PK-8100 | 4A-8100
VECTASTAIN® Universal Quick Kit (concentrate) | PK-8200 | PK-8200 | PK-8200 | 4A-8200

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

*Stains: • Anti-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).
Choosing a VECTASTAIN® ABC Kit

Choose the detection enzyme

- Peroxidase
- Alkaline phosphatase

Choose the appropriate ABC kit

To detect a biotinylated target, you will need:
- VECTASTAIN® ABC Reagent contained in the standard VECTASTAIN® ABC Kit
- An appropriate substrate

To detect an unlabeled primary antibody or lectin, you will need:
- A biotinylated secondary antibody that binds to the primary antibody species or lectin you have chosen
- VECTASTAIN® ABC Reagent
- An appropriate substrate

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

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

Consider Species Cross-Reactivity

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

The following options may minimize background staining in these instances:
- Directly label the primary antibody with biotin (ProtOn™ Biotin Labeling Kit, PK-1202) and detect it using the VECTASTAIN® Elite® ABC Kit (Standard – PK-5100, includes VECTASTAIN® Elite® ABC Reagent only) followed by an HRP substrate. Alternatively, label the primary with HRP (ProtOn™ HRP Labeling Kit, PK-1203) and develop with an HRP substrate.
- Use a biotinylated secondary antibody specifically adsorbed to remove cross-reacting antibodies of closely-related species (e.g. biotinylated anti-mouse IgG, rat adsorbed).
- Use the Mouse on Mouse (M.O.M.®) Immunodetection System for applications of mouse primary antibodies on mouse tissue (p. 24-25).

Substrates

After choosing the VECTASTAIN® ABC Kit for your application, select a substrate that matches the enzyme system of the kit (p. 26-29).
If a VECTASTAIN® ABC system is not available with a biotinylated secondary antibody of your required specificity, you can custom-build the exact kit that you require. All of our biotinylated, affinity-purified secondary antibodies (p. 38-39) are designed for use with VECTASTAIN® ABC Standard Kits and the appropriate blocking serum. Our mix-and-match kit components allow you to both design a custom kit to suit your needs and to use kit components interchangeably. The reagents can be purchased individually, allowing you to combine them to suit your specific needs.

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

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

**Product Concentrate R.T.U.**
- Anti-Goat IgG (H+L) made in rabbit, biotinylated
- Anti-Goat IgG (H+L) made in horse, biotinylated
- Anti-Human IgG (H+L) made in goat, biotinylated
- Anti-Mouse IgG (H+L) made in horse, biotinylated
- Anti-Mouse IgG (H+L) made in goat, rat adsorbed, biotinylated
- Anti-Mouse IgG (H+L) made in goat, biotinylated
- Anti-Rabbit IgG (H+L) made in rabbit, biotinylated
- Anti-Rabbit IgG (H+L) made in horse, biotinylated
- Anti-Rat IgG (H+L) made in rabbit, biotinylated
- Anti-Rat IgG (H+L) made in goat, biotinylated
- Universal Anti-Mouse/Rabbit IgG (H+L) made in horse, biotinylated
- Universal Pan-Specific Anti-Mouse/Rabbit IgG (H+L) made in horse, biotinylated

**Concentration**
- R.T.U.

**Catalog Number**
- PK-6100
- PK-7100
- PK-4000
- AK-5000
- S-1012
- S-5000
- S-2012
- SP-5035
- SP-5030

*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).

Choosing a Detection System

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 (pp. 23, 30-33)

ImmPRESS® VR Polymer Kits

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

Using the ImmPRESS® Polymer Kits

1. Add Primary Antibody
2. Add ImmPRESS® Reagent
3. Add Enzyme Substrate
ImmPRESS® One-Step Double Staining Polymer Systems
(Double Antigen Detection)

ImmPRESS® one-step double staining polymer systems combine the advantages of the ImmPRESS® Amplified Peroxidase (HRP) Polymer System technology with the simplicity of a single-step staining procedure. This method is ideal for applications requiring detection of two different antigens in a single tissue section.

**ImmPRESS® Duet Double Staining Polymer Kits**
- **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)**

**Using the ImmPRESS® One-Step Double Staining Polymer Systems**

1. Add Primary Antibodies
2. Add ImmPRESS® Duet Reagent
3. Add Enzyme Substrates

*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® two-step amplified polymer systems are designed for applications requiring high sensitivity and low background. This approach utilizes an amplification step to increase the signal-to-noise ratio, making it ideal for detecting weakly-expressed antigens.

**ImmPRESS® Excel Amplified Peroxidase (HRP) Polymer Staining Systems**
- **Anti-Rabbit IgG**
- **Anti-Mouse IgG**

**ImmPRESS® Excel Amplified Polymer Staining Systems**
- **Anti-Rabbit IgG**
- **Anti-Mouse IgG**

**ImmPRESS® Duet Double Staining Polymer Systems**
- **Anti-Rabbit IgG (HRP - brown)**
- **Anti-Mouse IgG (AP - magenta)**

**ImmPRESS® Duet Double Staining Polymer Kits**
- **Anti-Rabbit IgG (HRP - brown)**
- **Anti-Mouse IgG (AP - magenta)**

**ImmPRESS® Duet Polymer Kits**
- **Add Primary Antibody**
- **Add Amplifier Antibody**
- **Add ImmPRESS® Duet Reagent**
- **Add Enzyme Substrate**
- **Add Enzyme Substrate**

**ImmPRESS® One-Step Double Staining Polymer Systems**
- **Add Primary Antibodies**
- **Add ImmPRESS® Duet Reagent**
- **Add Enzyme Substrates**
- **Add Enzyme Substrate**

**ImmPRESS® Two-Step Amplified Polymer Systems**
- **Add Primary Antibody**
- **Add Amplifier Antibody**
- **Add ImmPRESS® Excel HRP Polymer Reagent**
- **Add Enzyme Substrate**
- **Add Enzyme Substrate**

**Using the ImmPRESS® Two-Step Amplified Polymer Systems**

1. Add Primary Antibody
2. Add Amplifier Antibody
3. Add ImmPRESS® Excel HRP Polymer Reagent
4. Add Enzyme Substrate

*Primary antibodies may be applied separately or mixed together.
† Enzyme substrates are applied sequentially. Refer to instructions for recommended procedure.

**Column**: Anti-CD34 (m), ImmPRESS® Excel Amplified Anti-Mouse IgG Staining Kit, ImmPACT® DAB EqV (brown), Hematoxylin QS counterstain (blue).
Choosing an ImmPRESS® Polymer Kit

Choose the detection enzyme  Choose an appropriate ImmPRESS® kit  Choose an appropriate enzyme substrate

- Peroxidase
- Alkaline phosphatase

For optimal results with little to no background staining interference, the following options should be considered when selecting an ImmPRESS® Polymer Kit:
- Species in which the primary antibody was raised
- Species of the tissue under examination – choose a specifically adsorbed detection kit where potential species cross-reactivity may occur.
- Single or double labeling requirements
- Sensitivity (ImmPRESS® Excel two-step kit formats are the most sensitive though not recommended for use on goat, bovine or sheep tissue)
- Applying a mouse primary antibody on mouse tissue (see Species on Species section, p. 24-25)

For appropriate selection consider:
- Enzyme used for detection system (Peroxidase and/or Alkaline Phosphatase)
- Desired color or color combinations for single or double labeling
- Sensitivity
- Counterstain compatibility
- Aqueous or non-aqueous mounting requirements

*ImmPRESS® Duet and ImmPRESS® Excel Kits contain substrate.

Choosing an ImmPRESS® Polymer Kit

Choose the appropriate ImmPRESS® kit

Choose the detection enzyme

For optimal results with little to no background staining interference, the following options should be considered when selecting an ImmPRESS® Polymer Kit:
- Species in which the primary antibody was raised
- Species of the tissue under examination – choose a specifically adsorbed detection kit where potential species cross-reactivity may occur.
- Single or double labeling requirements
- Sensitivity (ImmPRESS® Excel two-step kit formats are the most sensitive though not recommended for use on goat, bovine or sheep tissue)
- Applying a mouse primary antibody on mouse tissue (see Species on Species section, p. 24-25)

For appropriate selection consider:
- Enzyme used for detection system (Peroxidase and/or Alkaline Phosphatase)
- Desired color or color combinations for single or double labeling
- Sensitivity
- Counterstain compatibility
- Aqueous or non-aqueous mounting requirements

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: • M2A antigen (m), ImmPRESS® Reagent (HRP; 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® Anti-Mouse IgG, Rat Adsorbed, Kit (made in horse).

Using the ImmPRESS® Polymer Kits for multiple antigen labeling

1 Add First Primary Antibody
2 Add ImmPRESS® Reagent
3 Add Enzyme Substrate I
4 Add Second Primary Antibody
5 Add ImmPRESS® Reagent
6 Add Enzyme Substrate II

For the detection of two antigens on the same section (i.e. human/primates 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/primates 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.

* Substrates included.
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.

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

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

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

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

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

Species on Species Detection (Mouse)

Product | Catalog Number
--- | ---
M.O.M.® Peroxidase Kit | PK-2200
M.O.M.® Fluorescein Kit | FL-2201
M.O.M.® Basic Kit | BMK-2202
M.O.M.® ImmPRESS® HRP Polymer Kit | MP-2400
M.O.M.® Mouse Ig Blocking Reagent | MB-2213
M.O.M.® Biotinylated Anti-Mouse Ig Reagent* | MB-2215
M.O.M.® ImmPRESS® HRP Polymer Anti-Mouse Reagent | MP-2402

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

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).
Choosing an Enzyme Substrate

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

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

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

**Enzyme Substrates**

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

**Relative sensitivity of substrates in IHC**

<table>
<thead>
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<th>Lower Sensitivity</th>
<th>High Sensitivity</th>
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<td>Polymer Detection</td>
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<tr>
<td>Reagent.</td>
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</table>

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

---

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

Enzyme Substrate Properties

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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Color</th>
<th>Catalog Number</th>
<th>Brightfield</th>
<th>Darkfield</th>
<th>Electron</th>
<th>Fluorescence</th>
<th>Spectral Imaging</th>
<th>Mounting</th>
<th>Contrast in Pigmented Tissue</th>
<th>Multiple Labeling</th>
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Alkaline Phosphatase

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* Substrates that are designated "heat resistant" were developed on tissue then subjected to heat induced epitope retrieval (HER) using a pressure cooker technique (stained tissue was pressure cooked for 3 minutes in Antigen Unmasking Solution, returned to room temperature, and rinsed in buffer). Resulting sensitivity after this treatment was found to be equivalent to non-HER treated tissue.

Peroxidase Substrates

- **Tumor**: Cytokeratin (s), VECTASTAIN® Elite ABC Kit, TMB (blue).
- **Tonsil**: Cytokeratin AE1/AE3 (m), ImmPRESS® Reagent (HRP), ImmPRESS® DAB (brown).
- **Tonsil**: Cytokeratin AE1/AE3 (m), ImmPRESS® Reagent (HRP), ImmPRESS® DAB-Ni (gray-black).
- **Prostate**: Prostate Specific Antigen (m), ImmPRESS® Reagent (HRP), ImmPRESS® DAB (brown).
- **Tonsil**: CD20 (m), ImmPRESS® Reagent (HRP), ImmPRESS® VIP (purple).
- **Tonsil**: CD20 (m), ImmPRESS® Reagent (HRP), ImmPRESS® AMEC Red (red).
- **Prostate**: Prostate Specific Antigen (m), VECTASTAIN® ABC-AP Kit, Vector® Black (brown-black).

Alkaline Phosphatase Substrates

- **Tumor**: Cytokeratin AE1/AE3 (m), ImmPRESS®-AP Reagent, Vector® Blue (blue).
- **Prostate**: Prostate Specific Antigen (m), ImmPRESS®-AP Reagent, Vector® Black (brown-black).
- **Colon Carcinoma**: 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.

Enzyme Substrate Combinations

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

<table>
<thead>
<tr>
<th>Second Substrate</th>
<th>ImmPACT® VIP &amp; Vector® VIP (purple)</th>
<th>ImmPACT® DAB, ImmPACT® DAB EqV &amp; Vector® DAB HRP Substrate (brown)</th>
<th>ImmPACT® AEC, ImmPACT® AEC Red HRP Substrate (red)</th>
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</tbody>
</table>

Key:  
+ Indicates good contrast  
– Indicates incompatibility of substrates for various reasons

Colon:  
- Cytokeratin (AE1/AE3, m), ImmPRESS®-AP Anti-Mouse IgG Reagent, Vector® Blue AP Substrate (blue)  
- CD3 (m), ImmPRESS® Anti-Rabbit IgG HRP Reagent

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)

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)  
- Cytokeratin 8/18 (m), VECTASTAIN® Elite, ABC Kit, Vector® NovaRED™ HRP Substrate (purple)  
- Ki67 (m), ImmPRESS® Anti-Mouse IgG Reagent, Vector® SG HRP Substrate (blue/gray)
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® Blue AP Substrate (blue).

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

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).

Melanoma:  • Cyclin A (m), ImmPRESS®-AP Anti-Mouse IgG, ImmPACT® Vector® Blue AP Substrate (blue)  • 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).

Colon Carcinoma: • 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).

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

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

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

**Vector® Hematoxylin QS (blue)**
- Modification of Mayer’s hematoxylin developed especially for immunocytochemistry
- Ready-to-use without filtration or ‘blueing’ step
- Stains in less than 45 seconds
- Excellent color contrast with most commonly used peroxidase and alkaline phosphate substrates
- Suitable for use with non-aqueous and aqueous mounting media
- Mercury-free

**Vector® Methyl Green (light green)**
- Superior formulation of methyl green suitable for use with a wide range of enzyme substrates
- Simple, two-step procedure
- Excellent alternative in multiple antigen labeling when hematoxylin obscures the substrate colors
- Suitable for use with non-aqueous mounting media
- Alcohol- and mercury-free

**Vector® Nuclear Fast Red (pink)**
- Fast one-step protocol
- Excellent alternative in multiple antigen labeling when hematoxylin obscures the substrate colors
- Good contrast with a variety of substrates
- Mercury-free

**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.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Catalog Number</th>
<th>Vector® Hematoxylin and Hematoxylin QS</th>
<th>Vector® Methyl Green</th>
<th>Vector® Nuclear Fast Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImmPACT® DAB (brown)</td>
<td>H-3405</td>
<td>Excellent Contrast</td>
<td>Excellent Contrast</td>
<td>Fair Contrast</td>
</tr>
<tr>
<td>ImmPACT® DAB EqV</td>
<td>H-3403</td>
<td>Excellent Contrast</td>
<td>Excellent Contrast</td>
<td>Fair Contrast</td>
</tr>
<tr>
<td>Vector® DAB (brown)</td>
<td>H-3400</td>
<td>Excellent Contrast</td>
<td>Excellent Contrast</td>
<td>Fair Contrast</td>
</tr>
<tr>
<td>Vector® DAB-Ni (gray-black)</td>
<td>H-3402</td>
<td>Excellent Contrast</td>
<td>Fair Contrast *</td>
<td>Good Contrast</td>
</tr>
<tr>
<td>ImmPACT® AEC (red)</td>
<td>H-3406</td>
<td>Excellent Contrast</td>
<td>Counterstain Incompatibility **</td>
<td>Color Incompatibility</td>
</tr>
<tr>
<td>ImmPACT® AEC Red (red)</td>
<td>H-3405</td>
<td>Excellent Contrast</td>
<td>Counterstain Incompatibility **</td>
<td>Color Incompatibility</td>
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<tr>
<td>Vector® AEC (red)</td>
<td>H-3400</td>
<td>Excellent Contrast</td>
<td>Counterstain Incompatibility **</td>
<td>Color Incompatibility</td>
</tr>
<tr>
<td>TNB (blue)</td>
<td>H-4400</td>
<td>Color Incompatibility</td>
<td>Counterstain Incompatibility</td>
<td>Excellent Contrast</td>
</tr>
<tr>
<td>ImmPACT® VIP (purple)</td>
<td>H-4405</td>
<td>Fair Contrast</td>
<td>Excellent Contrast</td>
<td>Poor Contrast</td>
</tr>
<tr>
<td>Vector® VIP (purple)</td>
<td>H-4400</td>
<td>Fair Contrast</td>
<td>Excellent Contrast</td>
<td>Poor Contrast</td>
</tr>
<tr>
<td>ImmPACT® SG (blue-gray)</td>
<td>H-4700</td>
<td>Poor Contrast</td>
<td>Good Contrast</td>
<td>Excellent Contrast</td>
</tr>
<tr>
<td>Vector® SG (blue-gray)</td>
<td>H-4704</td>
<td>Poor Contrast</td>
<td>Good Contrast</td>
<td>Excellent Contrast</td>
</tr>
<tr>
<td>ImmPACT® NovaRED® (red)</td>
<td>H-4800</td>
<td>Excellent Contrast</td>
<td>Excellent Contrast ***</td>
<td>Color Incompatibility</td>
</tr>
<tr>
<td>Vector® NovaRED® (red)</td>
<td>H-4800</td>
<td>Excellent Contrast</td>
<td>Excellent Contrast ***</td>
<td>Color Incompatibility</td>
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<tr>
<td>ImmPACT® Vector® Red (magenta)</td>
<td>H-5105</td>
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<td>Excellent Contrast</td>
<td>Color Incompatibility</td>
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<tr>
<td>Vector® Red (magenta)</td>
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<td>Excellent Contrast</td>
<td>Excellent Contrast</td>
<td>Color Incompatibility</td>
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<tr>
<td>Vector® Black (black)</td>
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<td>Vector® Blue (blue)</td>
<td>H-5300</td>
<td>Color Incompatibility</td>
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<td>Excellent Contrast</td>
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<tr>
<td>BCP/NBT (indigo)</td>
<td>H-5400</td>
<td>Color Incompatibility</td>
<td>Excellent Contrast</td>
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</table>

* This substrate shows a slight decrease in sensitivity following the methyl green protocol. This decrease can be reversed 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.

Mountants

- Non-aqueous and Aqueous

<table>
<thead>
<tr>
<th>Product</th>
<th>Mmountants</th>
<th>Catalog Number</th>
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<tbody>
<tr>
<td>Vector® Hematoxylin</td>
<td>Non-aqueous and Aqueous</td>
<td>H-3401</td>
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<td>Vector® Hematoxylin QS</td>
<td>Non-aqueous and Aqueous</td>
<td>H-3404</td>
</tr>
<tr>
<td>Vector® Methyl Green</td>
<td>Non-aqueous</td>
<td>H-3402</td>
</tr>
<tr>
<td>Vector® Nuclear Fast Red</td>
<td>Non-aqueous and Aqueous</td>
<td>H-3403</td>
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</table>
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.

**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.

**Bovine Serum Albumin (BSA)**

Immunohistochemical Grade.

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

**10x Casein Solution**

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

**Carbo-Free™ Blocking Solution**

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

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

**R.T.U. Animal-Free Block and Diluent**

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.
### 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) |<|---|--|<|---|--|<|---|--|<|---|--|
| Anti-Cat IgG (H+L) | BA-9000 | Goat | Rabbit | Horse |<|---|--|<|---|--|<|---|--|<|---|--|
| Anti-Chicken IgG (H+L) | BA-9100 |<|---|--|<|---|--|<|---|--|<|---|--|
| Anti-Goat IgG (H+L) | BA-1000 |<|---|--|<|---|--|<|---|--|<|---|--|
| Anti-Goat IgG (H+L) | BA-5000 | BP-9500 | A-1000 |<|---|--|<|---|--|<|---|--|
| Anti-Horse IgG (H+L) | BA-7000 |<|---|--|<|---|--|<|---|--|<|---|--|
| Anti-Hamster IgG (H+L) | BA-9300 | A-1100 |<|---|--|<|---|--|<|---|--|
| Anti-Horse IgG (H+L) | BA-9000 |<|---|--|<|---|--|<|---|--|<|---|--|
| Anti-Mouse IgG (H+L) | BA-2000 | BP-2000 | A-3000 |<|---|--|<|---|--|<|---|--|
| Anti-Mouse IgG (H+L), rat absorbed | BA-2001 |<|---|--|<|---|--|<|---|--|<|---|--|
| Anti-Mouse IgM (H+L) | BA-2010 | BP-2000 | A-3000 |<|---|--|<|---|--|<|---|--|
| Anti-Mouse IgM (H+L), mu chain specific | BA-2020 |<|---|--|<|---|--|<|---|--|<|---|--|
| Anti-Rabbit IgG (H+L) | BA-1000 | BP-9100 | A-1000 |<|---|--|<|---|--|<|---|--|
| Anti-Rabbit IgG (H+L) | BA-1100 | BP-1100 |<|---|--|<|---|--|<|---|--|
| Anti-Rat IgG (H+L) | BA-4000 | A-4000 |<|---|--|<|---|--|<|---|--|
| Anti-Rat IgG (H+L), mouse absorbed | BA-4001 | A-4001 |<|---|--|<|---|--|<|---|--|
| Anti-Rat IgG (H+L) | BA-9400 | BP-9400 |<|---|--|<|---|--|<|---|--|
| Anti-Rat IgG (H+L), mouse absorbed | BA-9401 |<|---|--|<|---|--|<|---|--|<|---|--|
| Anti-Sheep IgG (H+L) | BA-9500 |<|---|--|<|---|--|<|---|--|<|---|--|
| 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

### 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.

<table>
<thead>
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<th>Product</th>
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<tr>
<td>Alkaline Phosphatase</td>
<td>AP-2000</td>
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<tr>
<td>Alkaline Phosphatase made in goat</td>
<td>AP-1000</td>
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<td>Alkaline Phosphatase made in horse</td>
<td>AP-1100</td>
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<tr>
<td>Alkaline Phosphatase Strepavidin</td>
<td>SA-5100</td>
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<td>Alkaline Phosphatase Avidin D</td>
<td>A-7100</td>
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<tr>
<td>Avidin and Streptavidin Enzyme Conjugates</td>
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<tr>
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</tr>
<tr>
<td>Avidin and Streptavidin Enzyme Conjugates</td>
<td>&lt;</td>
</tr>
</tbody>
</table>

### 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.
Mounting Media

**VectaMount® Permanent Mounting Medium**

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

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

**VectaMount® AQ Aqueous Mounting Medium**

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

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

### Mounting Media / Substrate Compatibility

<table>
<thead>
<tr>
<th>Substrate</th>
<th>VectaMount® Permanent Mounting Medium</th>
<th>VectaMount® AQ Aqueous Mounting Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRP</td>
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<tr>
<td>Vector® DAB</td>
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<tr>
<td>Vector® DAB-HRP</td>
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<tr>
<td>ImmPACT® DAB</td>
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<td>ImmPACT® DAB EqV</td>
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<td>Vector® NovaRED®</td>
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<td>Vector® Blue</td>
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<tr>
<td>Vector® Black</td>
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</tr>
<tr>
<td>Vector® BCIP/NBT</td>
<td>•</td>
<td></td>
</tr>
</tbody>
</table>

**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 (magenta).

**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 sections 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

**Imprint™ Histology Pen**

The Imprint™ 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 Imprint™ 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.

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Contact Details

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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).