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Immunofluorescence

Immunohistochemistry

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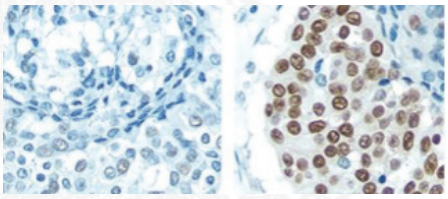
Immunofluorescence Overview

Immunofluorescence (IF) is a powerful method for visualizing proteins expressed directly within tissues. The IF method combines immunology and fluorescent molecules to localize proteins within defined morphological structures, and thus, provides insights into gene expression, protein-protein interactions, and biomarker identification. This method is used in a wide variety of applications, including basic research, assessment of normal and disease states in human and animal health, and in plant pathology studies.

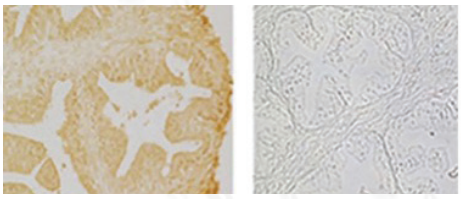
Step 1
Slide / Tissue Preparation

- Maximize tissue section retention and adherence on glass slides.
- Demarcate and isolate individual sections to reduce reagent use or for discrete treatment.



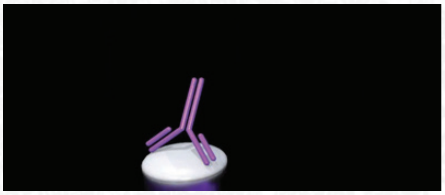
Step 2
Antigen Retrieval

Increase tissue antigenicity ("immunoreactivity") in aldehyde-fixed preparations with defined pH and salt solutions. The combination of aldehyde fixation (e.g., formalin) and heat exposure from the paraffin-embedding process can make epitopes inaccessible to detection. During antigen retrieval, tissues are treated with a combination of buffered solutions and high temperature, which causes conformational changes in the proteins that expose antigens for detection.



Step 3
Quench / Block

Reduce or eliminate unwanted background (non-specific) staining on tissue sections and cell preparations using a blocking solution. Non-specific staining can result from endogenous enzyme or tissue elements, including endogenous enzyme activity, presence of Fc receptors, or interactions of detection reagents with tissue or cell proteins and other macromolecules. Choose a blocking solution based on the results of negative control sections.

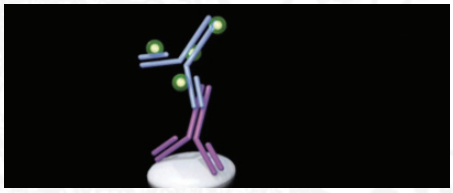


Step 4
Primary Antibody / Lectins

Identify and localize the target protein antigen or glycoprotein moiety in cells or tissue preparations using specific validated markers. When choosing a primary antibody or lectin, consider the tissue species and tissue preparation method (e.g., fixation) to ensure specific binding of your target.

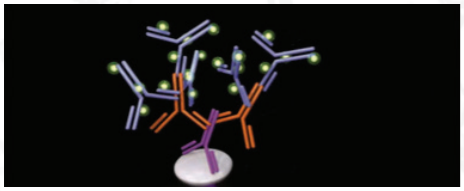
Considerations for Primary Antibody Selection

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



Step 5
Secondary Antibody

Select highly purified and optimally conjugated detection reagents to meet assay conditions, such as primary antibody species, tissue species, target abundance, and ease of use (concentrated or ready-to-use formats).

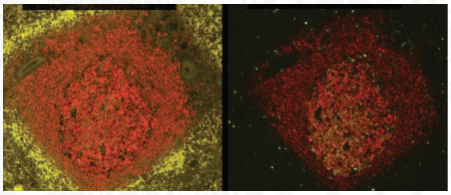


Step 6
Tertiary Reagent

If needed, increase the sensitivity of detection to visualize weakly or transiently expressed, upregulated, or unknown (e.g., gene knock-in studies) antigens.

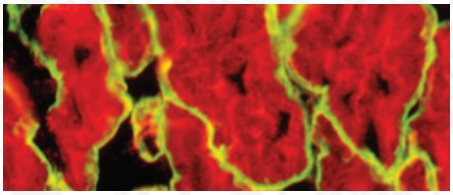
Considerations for Secondary Antibody and Tertiary Reagent Selection

- Choose fluorophore based on wavelengths available in microscope
- Fluorophore-conjugated secondary antibody or biotinylated secondary antibody
- Consider sensitivity requirements
- Consider species of primary antibody
- Consider tissue species



Step 7
Autofluorescence Quencher

Remove unwanted fluorescence in tissue sections due to aldehyde fixation, red blood cells, and structural elements such as collagen and elastin.



Step 8
Counterstain / Mount

Retain and preserve fluorescent signal intensity, with or without optional counterstain, to increase microscopy exposure times and archive for future imaging and reference requirements.

Step 9
Visualize

Fluorescence microscope

View using appropriate excitation/emission filters.

General Immunofluoresence Workflow

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

Tissue Preparation	Antigen Retrieval	Quench/ Block	Primary Antibody/ Lectins*
• VECTABOND Tissue Section Adhesive • ImmPrint Histology Pen • ImmEdge Hydrophobic Barrier Pen	• Antigen Unmasking Solutions, Citrate- or Tris-based	• Vector TrueVIEW Autofluorescence Quenching Kit** • Vector TrueVIEW Autofluorescence Quenching Kit with DAPI** • Avidin/Biotin Blocking Kit • Streptavidin/Biotin Blocking Kit • Normal Sera • Animal-Free Blocker® and Diluent, R.T.U. • BSA • Casein Solution • M.O.M. (Mouse on Mouse) Blocking Reagent • Carbo-Free™ Block • BLOXALL Endogenous HRP/AP Blocking Solution	• Biotinylated lectins • Fluorochrome-conjugated lectins • Unconjugated lectins

* For more information visit: vectorlabs.com/lectins
** TrueVIEW Autofluorescence Quenching is applied just prior to coverslipping

Secondary Antibody

- VectaFluor DyLight™ R.T.U. Secondary Antibodies
- VectaFluor Duet IF Double Labeling Kits
- M.O.M. (Mouse on Mouse) Immunodetection Kits
- Biotinylated secondary antibodies
- Fluorochrome-conjugated secondary antibodies
- Unconjugated secondary antibodies

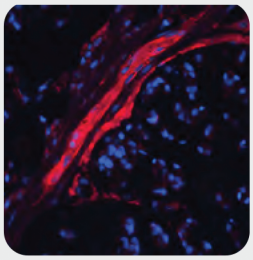
Tertiary Reagent

- VectaFluor Excel DyLight Amplified Fluorescent Staining Systems
- Fluorochrome-conjugated avidin or streptavidin
- Biotinylated anti-avidin amplifying reagent
- Biotinylated anti-streptavidin amplifying reagent

Counterstain/ Mount

- VECTASHIELD Vibrance® Antifade Mounting Medium with or without DAPI counterstain (hard-setting)
- VECTASHIELD PLUS Antifade Mounting Medium with or without DAPI counterstain (non-hardening)
- VECTASHIELD Antifade Mounting Medium with or without DAPI counterstain (non-hardening)
- VECTASHIELD Antifade Mounting Medium with Propidium Iodide (PI) (non-hardening)
- VECTASHIELD HardSet Antifade Mounting Medium with or without DAPI counterstain (hard-setting)
- VECTASHIELD HardSet Antifade Mounting Medium with TRITC—Phalloidin (hard-setting)

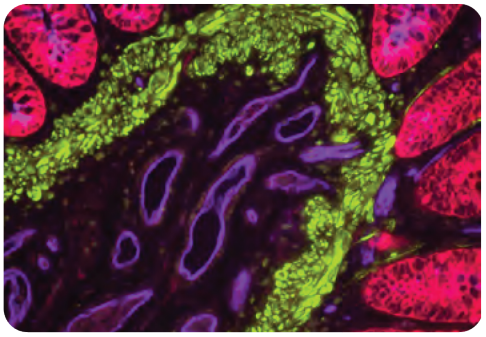
Visualize



VECTASHIELD Antifade Mounting Media

VECTASHIELD Antifade Mounting Media formulations offer unsurpassed protection against fading and photobleaching. The VECTASHIELD, VECTASHIELD PLUS, VECTASHIELD HardSet and VECTASHIELD Vibrance Antifade Mounting Media are well-established, market-leading products that complete the workflow and provide excellent signal retention for image acquisition and specimen archiving.

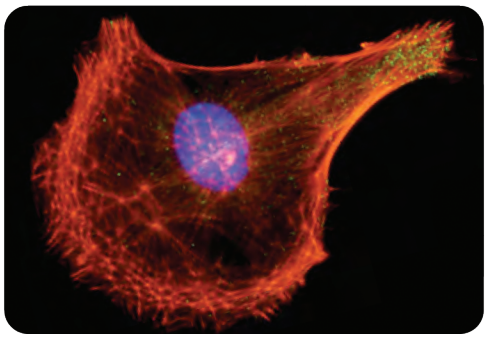
- Inhibits photobleaching of most fluorophores, dyes, fluorescent proteins and stains
- Ideal refractive index
- Ready-to-use, no warming necessary
- Continues to inhibit photobleaching even after prolonged storage
- With or without nuclear or cytoskeletal counterstain
- Hardening or non-hardening formulations and easy-to-use



Human Colon: Rabbit Anti-Cytokeratin (AE1/AE3) and Mouse Anti-Desmin detected simultaneously with VectaFluor Duet Double Labeling Kit; Vasculature detected using DyLight 649 UEA I Lectin (purple). Mounted in VECTASHIELD PLUS Antifade Mounting Medium.

VECTASHIELD Antifade Mounting Medium

VECTASHIELD Antifade Mounting Medium is a glycerol-based, aqueous mountant that remains a viscous liquid on the slide rather than solidifying. After mounting, cover-slipped slides will not readily dry out, enabling you to review them for weeks without the need for sealing. For prolonged storage, coverslips can be permanently sealed with nail polish applied on the coverslip perimeter.



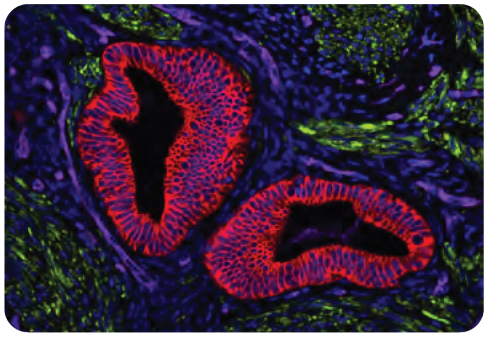
Mouse embryonal fibroblasts: Anti-Integrin (m) detected with DyLight 488 Anti-Mouse IgG, mounted in a 1:1 mixture of VECTASHIELD HardSet Mounting Medium with DAPI and VECTASHIELD HardSet Mounting Medium with TRITC-Phalloidin.

VECTASHIELD PLUS Antifade Mounting Medium

VECTASHIELD PLUS is a new formulation of non-setting media that improves upon the original VECTASHIELD products. Specifically, VECTASHIELD PLUS exhibits no inherent background or toning and provides superior fluorophore signal retention across the spectrum, including far-red wavelengths.

VECTASHIELD HardSet Antifade Mounting Medium

VECTASHIELD HardSet Antifade Mounting Medium is an aqueous mountant that hardens at room temperature in as little as 20 minutes. This mounting medium provides easy slide handling, eliminates the need to secure the coverslip with nail polish, and is convenient for use with oil immersion microscopy. Available with or without DAPI or TRITC-phalloidin counterstain.



Human uterine section (FFPE): Stained for desmin (green) and dytokeratin (red) using VectaFluor Duet Double Labeling Kit, and vasculature using DyLight 649 UEA I lectin (purple). Mounted in VECTASHIELD Vibrance Antifade Mounting Medium with DAPI (blue).

VECTASHIELD Vibrance Antifade Mounting Medium

VECTASHIELD Vibrance Antifade Mounting Medium is an aqueous mountant that hardens at room temperature in as little as 20 minutes. This mounting medium provides easy slide handling, eliminates the need to secure the coverslip with nail polish, and is convenient for use with oil immersion microscopy. Available with or without DAPI counterstain.

Product	No Counterstain	DAPI	PI	TRITC-Phalloidin
VECTASHIELD® Antifade Mounting Medium (non-hardening)	H-1000	H-1200	H-1300	
VECTASHIELD® PLUS Antifade Mounting Medium (non-hardening)	H-1900	H-2000		
VECTASHIELD® HardSet™ Antifade Mounting Medium (hardening)	H-1400	H-1500		H-1600
VECTASHIELD Vibrance® Antifade Mounting Medium (hardening)	H-1700	H-1800		

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. For IF applications special consideration should be given to the presence of autofluorescence.

TrueVIEW Autofluorescence Quenching Kits

TrueVIEW Autofluorescence Quenching Kits provide a novel way to remove unwanted fluorescence in tissue sections due to aldehyde fixation, red blood cells, and structural elements such as collagen and elastin. The unique formulation binds and effectively quenches the autofluorescent elements in even the most problematic tissues, such as kidney, spleen and pancreas.

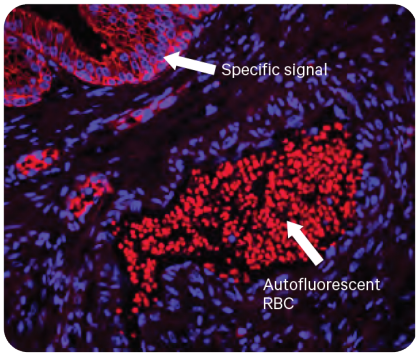
The use of TrueVIEW Quenching reagent leads to significant enhancement in overall signal-to-noise in most immunofluorescence assays.

TrueVIEW Quenching reagent is a unique approach to diminish unwanted autofluorescence from non-lipofuscin sources, that retains the specific fluorescent antigen staining. The quenching action of the kit reagents therefore, provides the investigator with a clear, unambiguous, "true view" visualization of the intended target.

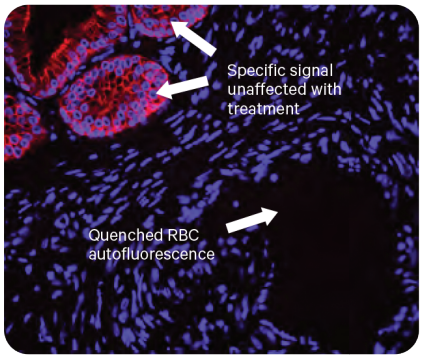
Why TrueVIEW Quencher?

- Specific reduction of autofluorescence from aldehyde fixation
- Improved signal-to-noise ratio
- Effective in even the most challenging tissues
- Easy-to-use, 5 minute incubation step
- Broad fluorophore compatibility
- Compatible with epifluorescence and confocal microscopes
- Antifade mounting medium included (with or without DAPI counterstain)

Serial sections of FFPE Human Prostate stained for epithelium (red)

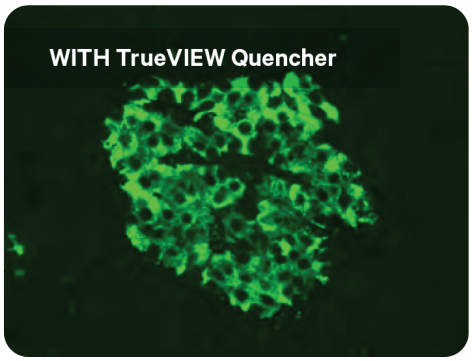


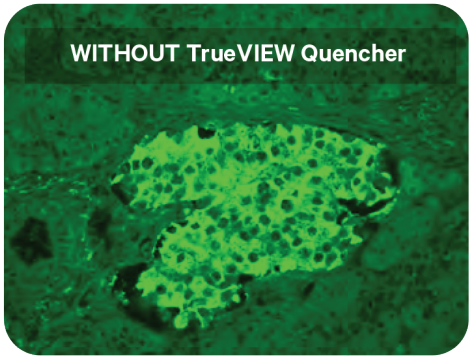
WITHOUT TrueVIEW Quenching*



WITH TrueVIEW Quenching*

*Both sections mounted with VECTASHIELD Vibrance Antifade Mounting Medium with DAPI.





Adjacent human pancreas sections (FFPE) insulin using fluorescein label (green). Mounted with VECTASHIELD Vibrance Antifade Mounting Medium. Note significant reduction of autofluorescence in the TrueVIEW treated section (right) with the retention of specific staining.

> *"I would definitely use this reagent in the future – it is quick and reliable on multiple tissue types."*
>
> *– Dr. K. Sadtler, Postdoctoral Fellow MIT Boston Children's Hospital*

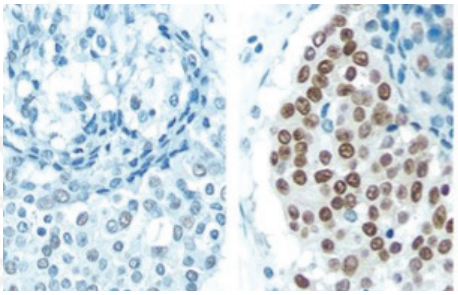
Immunohistochemistry Overview

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.

Step 1

Slide Preparation

- Maximize tissue section retention and adherence on glass slides.
- Demarcate and isolate individual sections to reduce reagent use or for discrete treatment.



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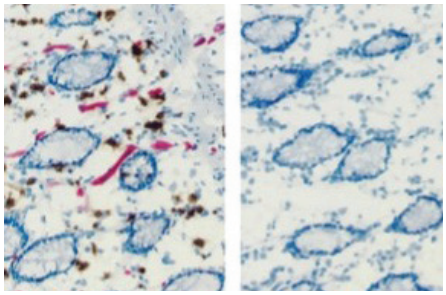
Antigen Retrieval

Increase tissue antigenicity ("immunoreactivity") in aldehyde-fixed preparations with defined pH and salt solutions. The combination of aldehyde fixation (e.g., formalin) and heat exposure from the paraffin-embedding process can make epitopes inaccessible to detection. During antigen retrieval, tissues are treated with a combination of buffered solutions and high temperature, which causes conformational changes in the proteins that expose antigens for detection.



Step 3

Quench / Block

Reduce or eliminate unwanted background (non-specific) staining on tissue sections and cell preparations using a blocking solution. Non-specific staining can result from endogenous enzyme or tissue elements, including endogenous enzyme activity, presence of Fc receptors, or interactions of detection reagents with tissue or cell proteins and other macromolecules. Choose a blocking solution based on the results of negative control sections.

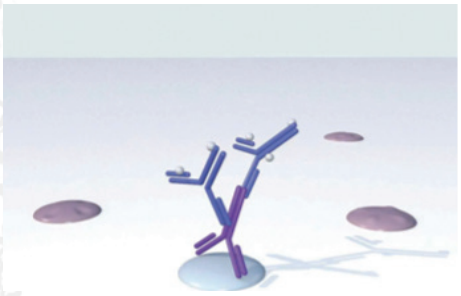
Step 4

Primary Antibody / Lectins

Identify and localize the target protein antigen or glycoprotein moiety in cells or tissue preparations using specific validated markers. When choosing a primary antibody or lectin, consider the tissue species and tissue preparation method (e.g., fixation) to ensure specific binding to your target.

Considerations for Primary Antibody Selection

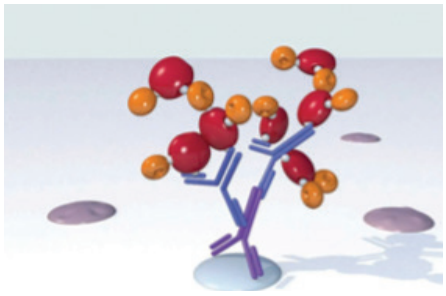
- Specific for antigen of interest
- Consider tissue species and preparation (fixation)
- Consider antigen retrieval requirements



Step 5

Secondary Antibody

Select highly purified and optimally conjugated detection reagents to meet assay conditions, such as primary antibody species, tissue species, target abundance, and ease of use (concentrated or ready-to-use formats).



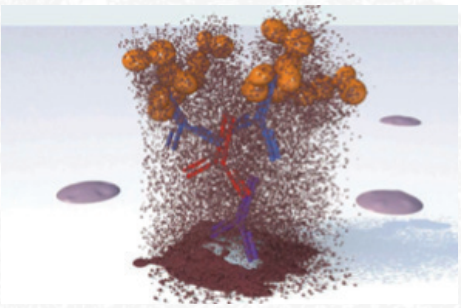
Step 6

Tertiary Reagent

If needed, increase the sensitivity of detection to visualize weakly or transiently expressed, upregulated, or unknown (e.g., gene knock-in studies) antigens.

Considerations for Secondary Antibody and Tertiary Reagent Selection

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



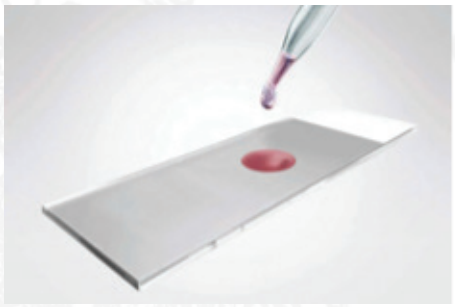
Step 7

Substrate / Chromogen

Visualize your target protein antigen and its cellular and/or extracellular localization and relative expression levels using enzyme-specific chromogenic color development. Choose an enzyme substrate that matches your color preference and that is compatible with other system reagents (e.g., counterstains, mounting media, and other substrates if multiplexing).

Considerations for Substrate Selection

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



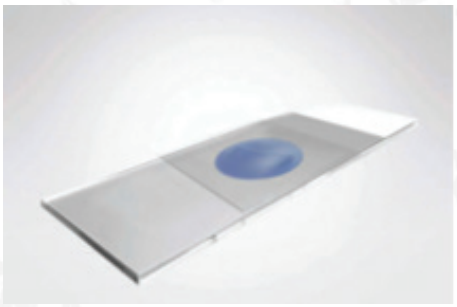
Step 8

Counterstain

Use a contrasting nuclear stain to clarify the target antigen signal within heterogeneous morphological structures. This helps elucidate cellular architecture and specific staining patterns in tissues. Choose a counterstain (blue, green, or red) that contrasts with your enzyme substrate and that is chemically compatible with both the substrate and mounting medium.

Consideration:

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



Step 9

Coverslip / Mount

Preserve the target antigen stain for short-term or indefinite storage and archiving. Choose a mounting medium that is compatible with both your substrate and your counterstain. Use a non-aqueous mounting medium to mount slides to be archived, or an aqueous mounting medium for storage up to a few years.

Consideration:

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

Step 10

Visualize

- Brightfield microscope

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 fluorescent microscopy.

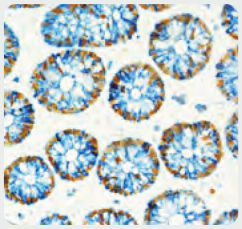
Immunohistochemistry Workflow

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

Tissue Preparation	Antigen Retrieval	Quench/ Block	Primary Antibody/ Lectins*	Secondary Antibody
• VECTABOND Tissue Section Adhesive • ImmPrint™ Histology Pen • ImmEdge Hydrophobic Barrier Pen	• Antigen Unmasking Solutions, Citrate- or Tris-based	• BLOXALL Endogenous HRP and AP Blocking Solution • Avidin/Biotin Blocking Kit • Streptavidin/Biotin Blocking Kit • Normal Sera • Animal-Free Blocker® and Diluent • BSA • Casein Solution • M.O.M. Mouse IgG Blocking Reagent • Carbo-Free™ Block	• Biotinylated lectins • Unconjugated lectins	• ImmPRESS Polymer Reagents (HRP or AP) • ImmPRESS PLUS Polymer Reagents (HRP) • ImmPRESS Duet Polymer Reagents (HRP/AP) • M.O.M. (Mouse on Mouse) ImmPRESS Polymer Kit • M.O.M. (Mouse on Mouse) Basic Kit • H.O.H. (Human on Human) Immunodetection Kit • Biotinylated secondary antibodies • Unconjugated secondary antibodies • Enzyme-conjugated secondary antibodies (HRP or AP) • Biotinylated anti-lectins

* For more information visit: vectorlabs.com/lectins

HRP - Horseradish peroxidase
AP - Alkaline phosphatase

Tertiary Reagent	Substrate/ Chromogen	Counterstain	Coverslip/ Mount	Visualize
• 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	

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

VECTASTAIN ABC Detection Systems

VECTASTAIN ABC detection systems are uniquely formulated with Vector Laboratories' 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

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 Vectors biotinylated, affinity-purified secondary antibodies are designed for use with VECTASTAIN ABC Standard Kits and the appropriate blocking serum. Their 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.

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 Blocker® and Diluent	SP-5030	SP-5035

Enzyme Substrates

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

Substrate	Color	Catalog Number	Microscopy					Mounting	Contrast in Pigmented Tissue	Multiple Labeling	Heat Resistant*
			Bright-field	Darkfield	Electron	Fluore-scence	Spectral Imaging				
Peroxidase											
Vector® DAB	Brown	H-2200	•	•	•		•	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		•	•

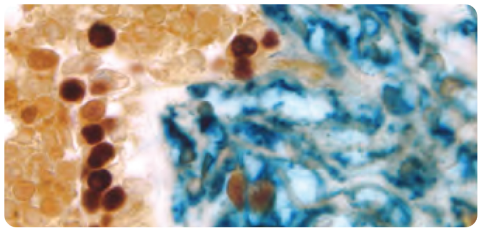
Enzyme Substrate Combinations

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

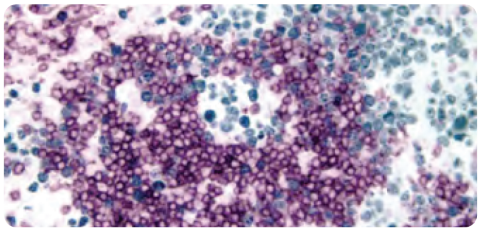
First Substrate \ Second Substrate	Alkaline Phosphatase			Peroxidase					
	ImmPACT Vector Red & Vector Red (magenta) SK-5105 & SK-5100	Vector Blue (blue) SK-5300	BCIP/NBT (indigo) SK-5400	ImmPACT VIP & Vector VIP (purple) SK-4605 & SK-4600	ImmPACT DAB, ImmPACT DAB EqV & DAB (brown) SK-4105, SK-4103, 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

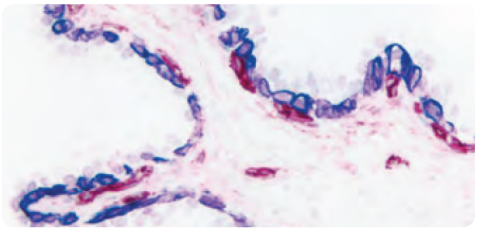
– Indicates incompatibility of substrates for various reasons



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).

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)

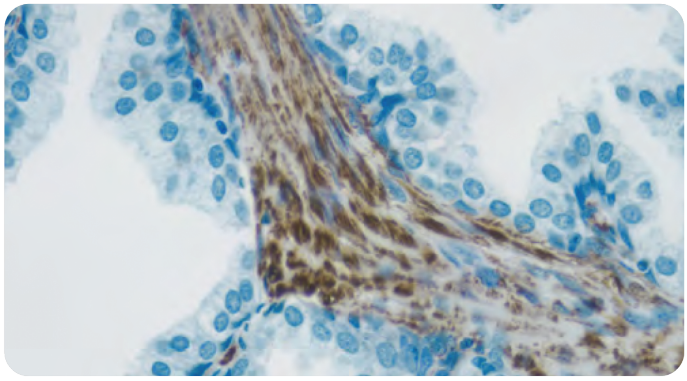
- Modification of Mayer's hematoxylin developed especially for immunocytochemistry
- Ready-to-use without filtration or 'blueing' step
- Stains in less than 45 seconds
- Excellent color contrast with most commonly used peroxidase and alkaline phosphatase substrates
- Suitable for use with non-aqueous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

Methyl Green (light green)

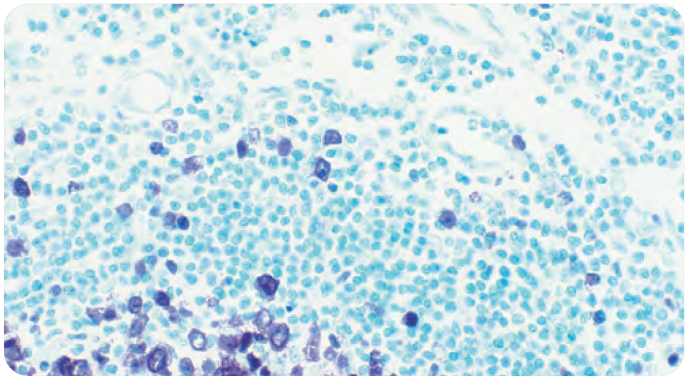
- Superior formulation of methyl green suitable for use with a wide range of enzyme substrates
- Simple, two-step procedure
- Excellent alternative in multiple antigen labeling when hematoxylin obscures the substrate colors
- Suitable for use with non-aqueous mounting media

Nuclear Fast Red (pink)

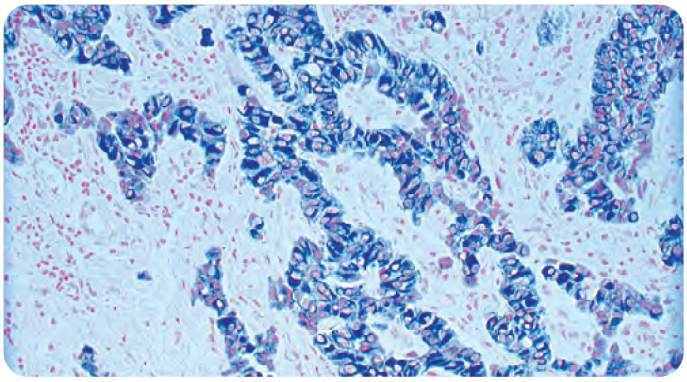
- Fast one-step protocol
- Excellent alternative in multiple antigen labeling when hematoxylin obscures the substrate colors
- Good contrast with a variety of substrates
- Suitable for use with non-aqueous mounting media



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).

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
- Compatable with horseradish peroxidase, alkaline phosphatase, and glucose oxidase substrates
- Refractive index: 1.49 when dry

VectaMount AQ Aqueous Mounting Medium

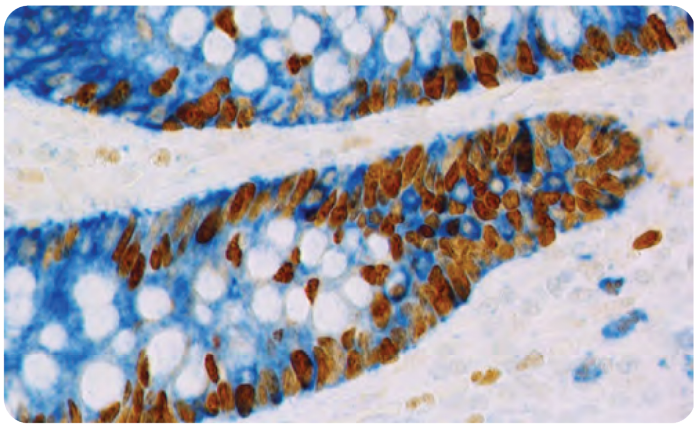
VectaMount AQ Aqueous Mounting Medium preserves the color and clarity of enzyme substrates whose reaction products are soluble in alcohol or other organic solvents. Stained and mounted sections can be stored in a slide box at room temperature for at least two years without fading.

- 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
Peroxidase			
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		•	
ALkaline Phosphatase			
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.

Accessory Reagents for IHC and IF

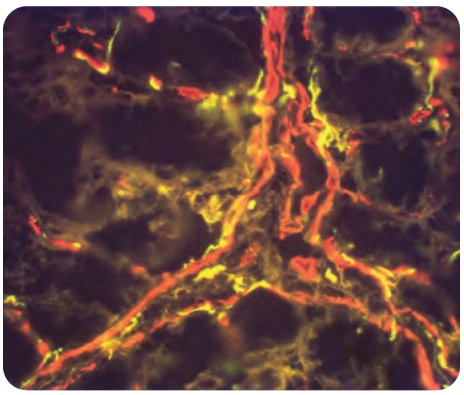
M.O.M. (Mouse on Mouse) Immunodetection Kits

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

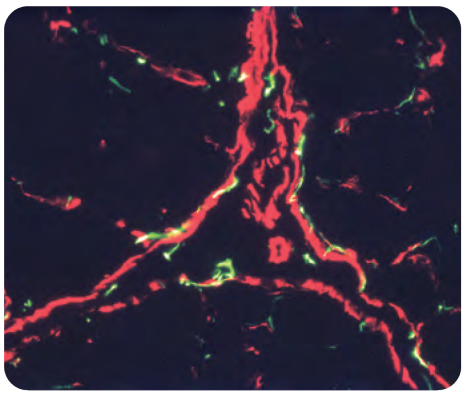
- Significantly reduces endogenous mouse Ig staining when using mouse primary antibodies on mouse tissue
- Simple protocols
- Eliminates tedious calculations
- Eliminates primary antibody prebinding steps
- Clear, crisp, specific staining of antigens of interest
- Compatible with fluorescent or enzyme-based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

Recommended Applications

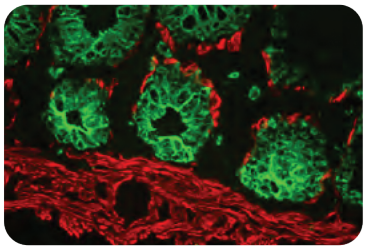
- Studies in genetically engineered mice
- Transgenic and knock-out models
- Mouse xenograft tissue
- Normal mouse tissue

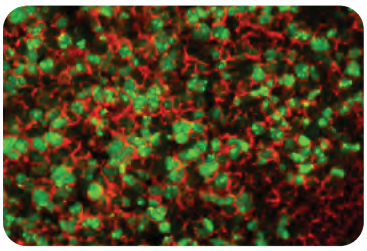


No M.O.M.. Kit: Mouse Intestine (frozen) – Double label • Peripherin (m), Biotinylated Horse Anti-Mouse IgG, Fluorescein Avidin DCS (green) • Desmin (m), Biotinylated Horse Anti-Mouse IgG, Texas Red Avidin DCS (red). Note background and signal mixing.



With M.O.M. Kit: Mouse Intestine (frozen) – Double label • Peripherin (m), M.O.M. Fluorescein Kit (green) • Desmin (m), M.O.M. Basic Kit, Texas Red Avidin DCS (red). Compare with adjacent image prepared without M.O.M. Kit.





Top: Mouse Colon: Multi-cytokeratin (m), M.O.M. Fluorescein Kit (green) • Desmin (m), M.O.M. Basic Kit, Texas Red Avidin DCS (red). Bottom: Mouse Tonsil: Ki67 (m), M.O.M. Fluorescein Kit (green) • CD20 (m), M.O.M. Basic Kit, Texas Red Avidin DCS (red). DCS (red).

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

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

Accessory Reagents for IHC and IF

VECTABOND Reagent Tissue Section Adhesive

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

ImmEdge Hydrophobic Barrier Pen

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

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

ImmPrint Histology Pen

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

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

Control Antibodies

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

Antigen Unmasking Solutions

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

Product	Catalog Number
VECTABOND® Reagent (Tissue Section Adhesive)	SP-1800
ImmEdge® Hydrophobic Barrier PAP Pen	H-4000
ImmPrint™ Histology Pen	H-6100
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

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Labware & Equipment

- Pipette Tips
- Semi Automated Pipettors
- Freezer Racks
- Tubes
- Histology Labware
- Sample Collection and Management
- Sample Storage
- Microwell Plates
- Water Purification Systems
- Benchtop Equipment
- SPR
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