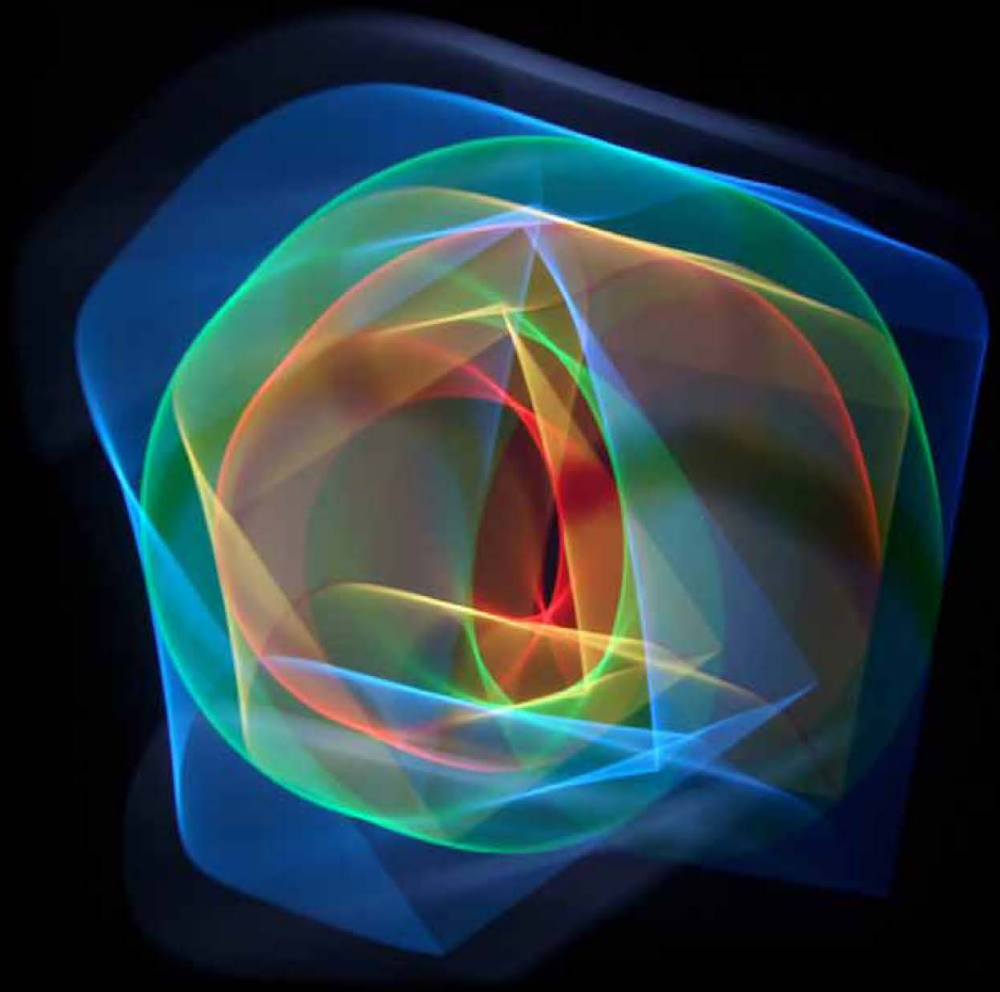

VECTOR

LABORATORIES

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STATE OF THE ART LABELING & DETECTION SYSTEMS

Since 1976 Vector Laboratories has provided innovative labeling and detection solutions to the scientific community. Shortly after introducing a line of purified lectin reagents, we revolutionized the field of histological staining with development of the versatile and sensitive Biotin-Avidin detection method leading to the VECTASTAIN® ABC family of products. These reagents provide consistent, reliable, sensitive, and low background staining for immunohistochemistry and other applications. Over the ensuing years, we have continued to innovate with the development of key reagents, including: our ImmPRESS™ polymer detection systems–peroxidase micropolymers directly coupled to secondary antibodies, which streamline detection procedures; the Vector® M.O.M.™ reagents, enabling the use of mouse primary antibodies on mouse tissue; proprietary enzyme substrates that yield a palette of colors for multiple labeling needs; our acclaimed anti-fade VECTASHIELD® mounting mediums, and our fluorescent products labeled with classic fluors as well as the new DyLight® dyes that allow superior fluorescence capabilities. Other pioneering products include neuronal tracers, ultra-pure immunological products, and novel reagents for labeling, detection and isolation of nucleic acids, fusion proteins and carbohydrates. We also offer a diverse and expanding repertoire of primary antibodies for medical research. Vector Laboratories' substantial presence in the scientific literature is a testament to the widespread use of our products and their quality.

Vector Laboratories is an independent, privately-held California corporation with subsidiaries in the U.K. and Canada. The products listed in this catalog are manufactured at our main 65,000 ft^2 research and development facilities in Burlingame, California. Our products are also available worldwide through a network of independent distributors. Throughout the years we have remained committed to producing superior products and supporting them with prompt and personal service. We have an accessible and knowledgeable technical staff ready to assist in the successful use of our reagents in a myriad of applications. Additional technical information and tutorials are available on our website. The long-term relationships we have established with members of the scientific community reflect a confidence in our record of producing products of unmatched performance and quality. Ours remains the standard by which others are measured.

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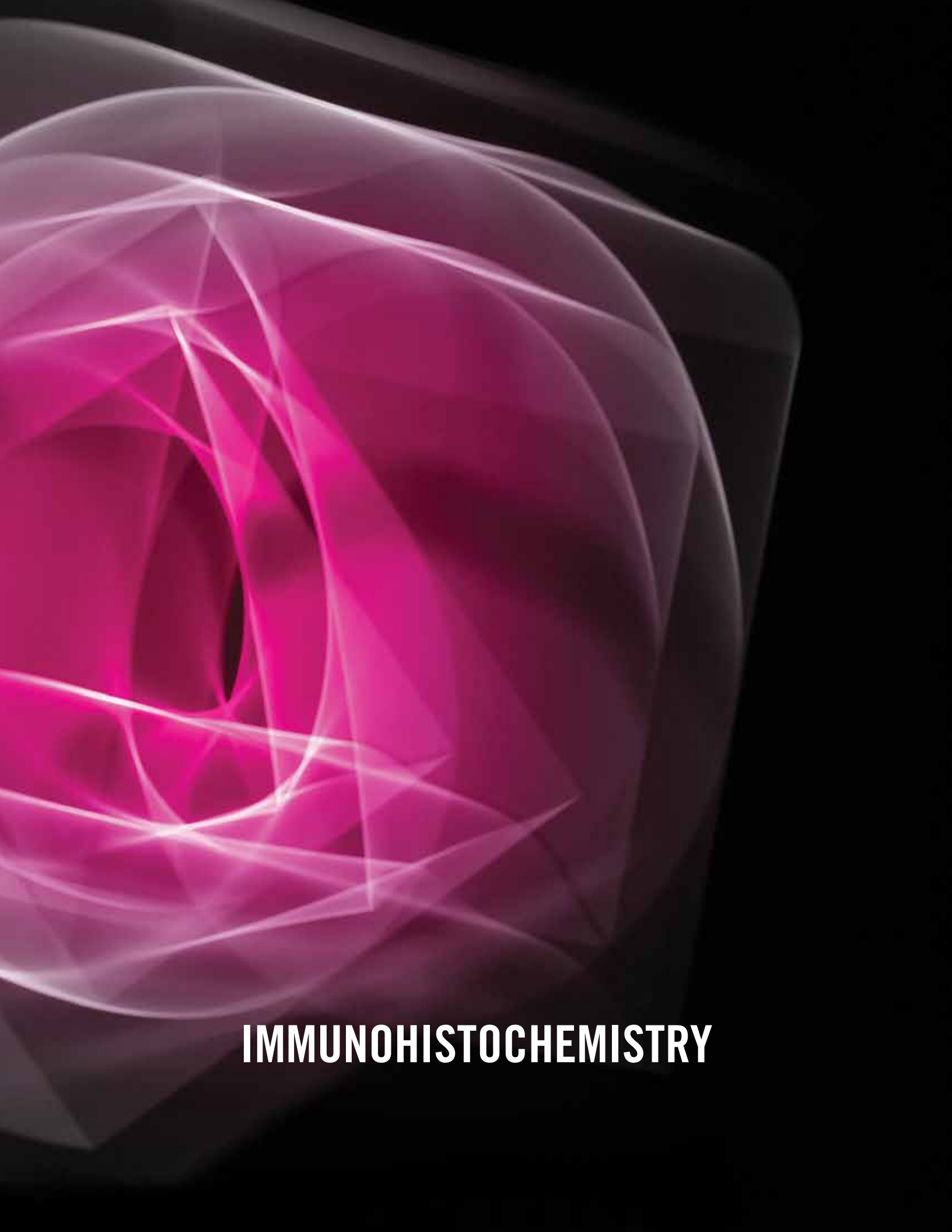
QUICK GUIDE



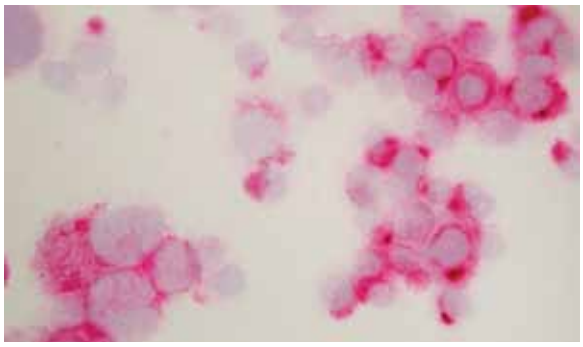
IMMUNOHISTOCHEMISTRY

A

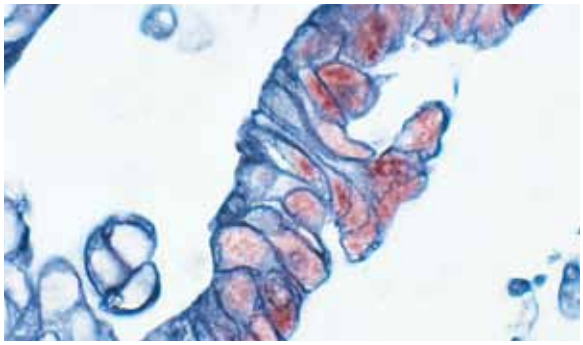
Introduction

Since 1976, Vector Laboratories has been at the forefront of developing innovative detection reagents. The VECTASTAIN® ABC systems established us as the leader in immunohistochemistry. Because of our commitment to developing novel technologies and products of consistent quality, Vector Laboratories remains a trusted name in this field. Our products are well regarded for their high sensitivity, low background, reliability, reproducibility, and value pricing. Many different detection systems are available from us to accommodate the wide range of experimental priorities.

VECTASTAIN® ABC Systems. With more than 40,000 citations to its credit, the VECTASTAIN® ABC kit remains widely popular. Based on the versatile biotin/avidin interaction, the system is modular, and along with our selection of secondary antibodies, can accommodate a wide array of primary antibody and tissue species. Our ABC kits are economical and continue to be a staple product in any immunohistochemistry laboratory. See pages A4-A11.



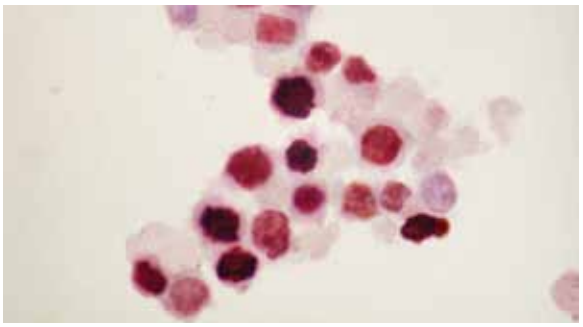
Cytospin of Epstein-Barr virus positive cell line: latent membrane protein-1 (LMP-1; m), VECTASTAIN® ABC-AP Kit, Vector® Red (magenta), Hematoxylin QS (blue). (Image courtesy of Dr. GM Reynolds, Centre for Liver Research, University of Birmingham, U.K.)



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

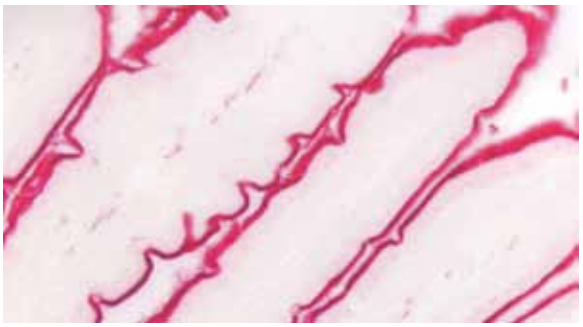
Abbreviations used in figure legends: AP – alkaline phosphatase; HRP – horseradish peroxidase; m – mouse monoclonal antibody; g – goat antibody; rm – rabbit monoclonal antibody; rp – rabbit polyclonal antibody; s – sheep polyclonal antibody

ImmPRESS™ Peroxidase Polymer Reagents. The highly sensitive, one-step, ready-to-use, non-biotin, ImmPRESS™ Polymer Detection reagents are the result of novel conjugation methods and micropolymer chemistry developed at Vector Laboratories. Peroxidase micropolymers allow excellent resolution and crisp, strong staining of antigens, especially nuclear and membrane antigens. ImmPRESS™ reagents avoid the steric problems of other polymer-based reagents, thus providing superior results. These reagents are ideal for multiple antigen labeling because of the simplified one-step protocol. See pages A12-A15.



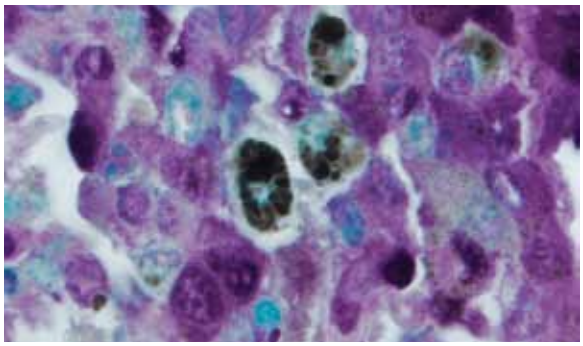
Cytospin of EBV+ cell line: Epstein-Barr virus nuclear antigen 1 (EBNA-1; rat), ImmPRESS™ Reagent (HRP) Anti-Rat Ig, ImmPACT™ NovaRED™ (red). (Image courtesy of Dr. GM Reynolds, Centre for Liver Research, University of Birmingham, U.K.)

Vector® M.O.M.™ (Mouse on Mouse) Immunodetection System. The specific detection of mouse primary antibodies on mouse tissue is difficult due to the presence of endogenous mouse immunoglobulins. A conventional anti-mouse detection system cannot distinguish between the mouse primary antibody and the endogenous mouse immunoglobulins leading to high background. The Vector® M.O.M.™ system is designed for the localization of mouse primary antibodies on mouse tissue. This system uses a VECTASTAIN® ABC staining method but contains a proprietary Mouse Ig Blocking Reagent and a specially modified anti-mouse Ig secondary antibody. Tedious calculations and primary antibody pre-binding steps are not required. Excellent staining results for a once difficult application have now become routine with the Vector® M.O.M.™ system. See pages A16-A17.



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

Proprietary Enzyme Substrates. Along with the traditional chromogenic substrates Vector Laboratories also offers a wide variety of proprietary enzyme substrates. These proprietary substrates provide novel color choices essential for staining antigens in pigmented tissues and melanomas as well as for localizing multiple antigens in the same tissue section. Several substrates can be effectively viewed under darkfield or electron microscopy. Many of our substrates have unique spectral profiles that may be useful in the co-localization of antigens using spectral imaging systems. See pages A18-A21.



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

Multiple Antigen Labeling. Accurate, reliable localization of two or more antigens on the same tissue section is a powerful research tool that can be applied in standard laboratory settings. The high specificity and low background of our reagents and proprietary enzyme substrates provide optimal sensitivities coupled with exquisite color combinations. Many of our substrates allow co-localization of antigens using spectral imaging. See pages A18-A23.

Automated Staining. The detection systems offered in this catalog can be used in automated staining systems to produce the same high sensitivity, low background staining results found with manual staining methods. In addition to greatly expanding the number of detection reagent choices and antigen visualization preferences, the use of Vector® reagents provides substantial cost savings.

Comparison of Detection Systems

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™ Polymer Kit	HRP	•••••	•••	•	•			•	1
VECTASTAIN® Elite® ABC System	HRP	•••••	••			•			2
R.T.U. VECTASTAIN® Elite® Kit	HRP	•••••	••			•		•	2
VECTASTAIN® Universal Quick Kit	HRP	••••	••			•			2
R.T.U. VECTASTAIN® Universal Quick Kit	HRP	••••	••			•		•	2
VECTASTAIN ®ABC System	HRP	•••	•			•			2
VECTASTAIN® ABC Alkaline Phosphatase System	AP	••••	•			•			2
VECTASTAIN® ABC Glucose Oxidase System	GO	•	•			•			2
Mouse On Mouse System	HRP	•••	•••			•	•		2
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
GO - Glucose oxidase

VECTASTAIN® ABC Systems

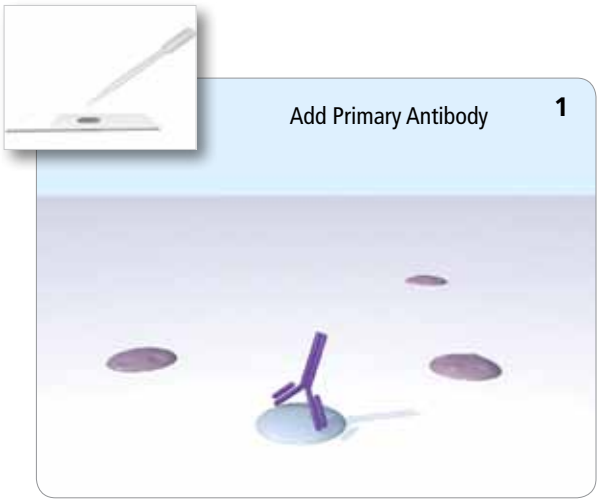
The VECTASTAIN® ABC systems are extremely sensitive due to the form and number of active enzyme molecules associated with the preformed Avidin/Biotinylated enzyme Complex. This complex takes advantage of two important properties of avidin: 1) an extraordinarily high affinity for biotin (over one million times higher than antibody for most antigens), and 2) four biotin-binding sites. These properties allow macromolecular complexes (ABCs) to be formed by mixing Avidin DH (Reagent A) with its paired biotinylated enzyme (Reagent B) prior to use. The ABC, once formed, remains stable for many hours after formation and can be used for several days after preparation.

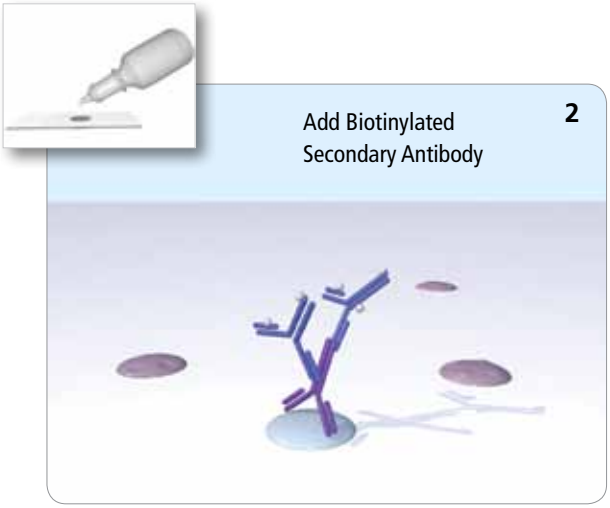
The VECTASTAIN® ABC Reagent can be used to detect any molecule that is biotinylated. This property gives the ABC method great versatility in the types of targets that can be detected as well as the types of applications in which it can be employed. Biotinylated primary antibodies, secondaries, lectins, neuronal tracers, nucleic acids, and ligands can be effectively visualized in applications such as:

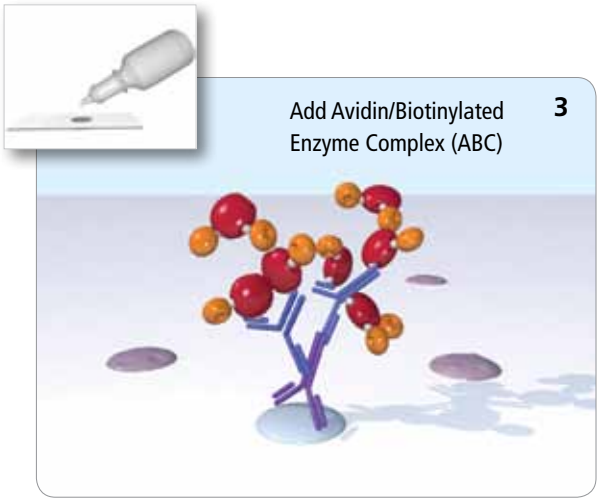
- Tissue staining
- Protein and nucleic acid blot detection
- *In situ* hybridization detection
- Enzyme immunoassays
- Neuronal tracing

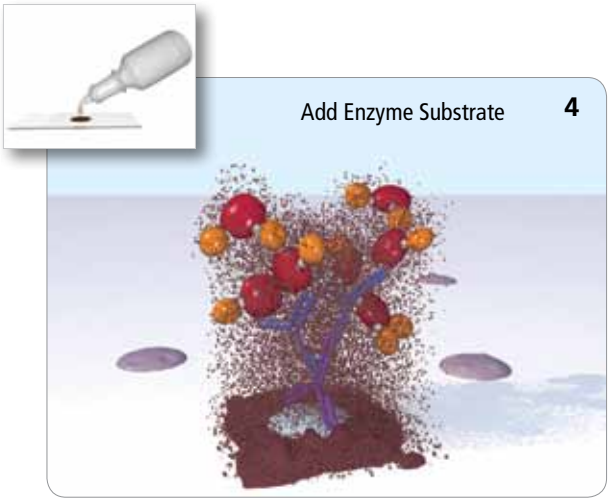
All applications benefit from the high sensitivity, low background, reproducibility, and economy of the VECTASTAIN® ABC system.

Using the ABC System:









Choosing a VECTASTAIN® ABC Kit

1) Choose an enzyme system:
The VECTASTAIN® ABC Kits are available with a choice of three different enzyme systems.

- Peroxidase (ABC, Elite® ABC, RTU or Quick Kits), pages A6-A8
- Alkaline Phosphatase (ABC-AP), page A9
- Glucose Oxidase (ABC-GO), page A10

2) Choose the biotinylated secondary antibody:
To detect a biotinylated target, you will need only the ABC Reagent contained in the Standard Kit, followed by an appropriate substrate.

To detect an unlabeled primary antibody, you will need a biotinylated secondary antibody that binds to the primary antibody species chosen, the ABC Reagent, and an appropriate substrate. For example, to detect a primary antibody made in rabbit, the appropriate choice is a VECTASTAIN® ABC Kit designated "Rabbit IgG".

For additional versatility and convenience, Vector Laboratories offers secondary antibody products that recognize more than one species of primary antibody. Our Universal Biotinylated Anti-Mouse/Rabbit IgG secondary antibody (Cat. No. BA-1400) is a cocktail of biotinylated anti-mouse IgG and anti-rabbit IgG, designed for use with both rabbit and mouse primary antibodies. This antibody should not be used to stain tissues from rodents or rabbits due to its reactivity with IgG present in the tissues of these species. The Universal Pan-Specific Anti-Mouse/Rabbit/Goat secondary antibody (Cat. No. BA-1300) (which is included in our VECTASTAIN® Universal Quick Kits) is a cocktail of biotinylated anti-mouse IgG, anti-rabbit IgG, and anti-goat IgG that recognizes mouse, rabbit, and goat primary antibodies. This secondary antibody should not be used to stain rat, mouse, or other rodent, rabbit, goat, bovine, or sheep tissue due to potential reactivity with endogenous IgG.

3) Choose a convenient format:
Finally, several kits are available in prediluted, stabilized formats for convenience and ease of use. See kits designated R.T.U. or Ready-To-Use.

For information on how to design a custom kit see page A11.

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 (e.g. rat and mouse), the biotinylated secondary antibody may cross react with endogenous IgG in the tissue section leading to background staining. The following three options minimize background staining in these instances:

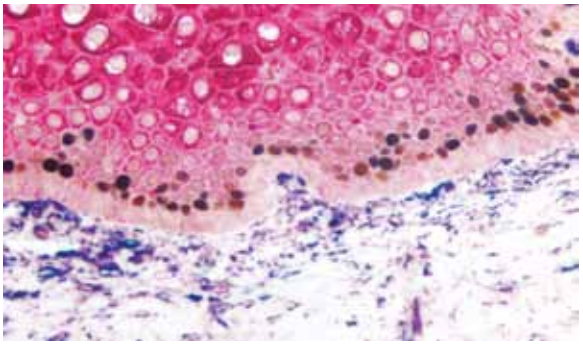
- Biotinylate the primary antibody (ProtOn™ Biotin Labeling Kit, Cat. No. PLK-1202, page F9) and detect with a Standard VECTASTAIN® Elite® ABC Kit, PK-6100, (only includes the ABC reagent).

- Use a biotinylated secondary antibody specifically adsorbed to remove cross-reacting antibodies of closely-related species (e.g. biotinylated anti-mouse IgG, rat adsorbed, Cat. No. BA-2001, page A29).

- Use the M.O.M.™ Immunodetection System for applications of mouse primary antibodies on mouse tissue (see pages A16-A17).

Substrates

After choosing the VECTASTAIN® ABC Kit for your application, select a substrate that matches the enzyme system of the kit (page A18-A21). For additional information on a wide variety of enzyme substrates available please see the descriptions in Section I, "Enzyme Substrates".



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

VECTASTAIN® ABC Systems (continued)

Peroxidase

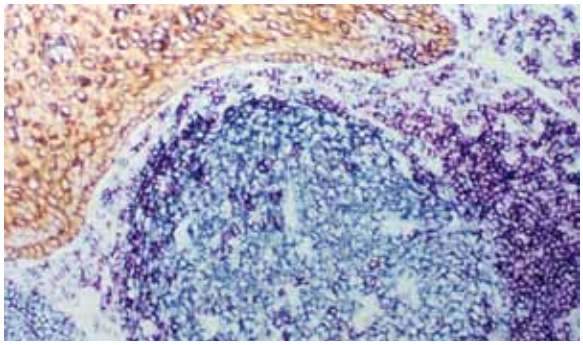
Peroxidase substrates produce sharp, dense precipitates with crisp localization. These characteristics, in conjuction with the high sensitivity and low background of the VECTASTAIN® ABC systems, make the peroxidase enzyme a preferred choice in many applications. (eg. In neural tissue, the peroxidase system is often preferred because it gives more consistent labeling of both cell bodies and processes.) For peroxidase substrates see pages A18-A21.

VECTASTAIN® Elite® ABC System

- Advanced avidin/biotin technology: The Elite® ABC complex is smaller, very uniform, and highly active, allowing more accessibility for binding to a biotinylated target.
- Highest sensitivity, low background: The VECTASTAIN® Elite® ABC system is the most sensitive avidin/biotin-based peroxidase system. The Elite® ABC series is approximately 5 times more sensitive than the original VECTASTAIN® ABC Kit with the same low background.
- Cost effective: Higher sensitivity leads to lower cost per slide.
- Available without ("Standard" kit) or with biotinylated species-specific or universal secondary antibodies. See page A7.
- Available in Ready-To-Use (R.T.U.) formats: Prediluted, stabilized working solutions of Elite® ABC Kit reagents provide the same high sensitivity and low background as the traditional VECTASTAIN® Elite® ABC Kit reagents.

Original VECTASTAIN® ABC Kit

- Good sensitivity/low background
- Original avidin/biotin ABC complex formulation
- Low cost
- Available without ("Standard" kit) or with biotinylated species-specific secondary antibody. See page A8.



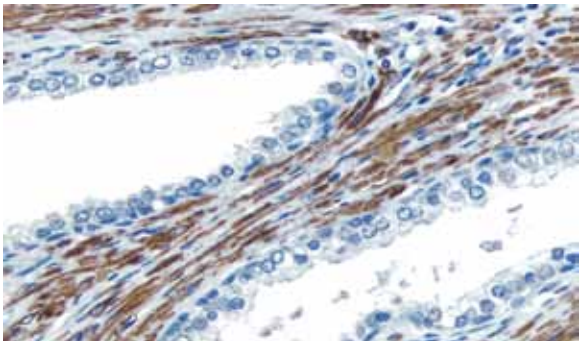
Tonsil: • Multi-Cytokeratin (m), VECTASTAIN® Elite® ABC Kit, Vector® DAB (brown) • CD3 (m), VECTASTAIN® Elite® ABC Kit, Vector® VIP (purple) • CD20 (m), VECTASTAIN® Elite® ABC Kit, Vector® SG (blue-gray).

VECTASTAIN® Universal Quick Kits

The VECTASTAIN® Universal Quick Kit is designed for rapid immunohistochemical staining of tissue sections. The kit relies 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.*
- Available in Ready-To-Use (R.T.U) format: Prediluted, stabilized working solutions of VECTASTAIN® Universal Quick Kit reagents provide the same high sensitivity and low background as the concentrated VECTASTAIN® Universal Quick Kit. See page A8.

** Do not use the pan-specific secondary antibody to stain rat, mouse or other rodent, rabbit, goat, bovine, or sheep tissue due to potential reactivity with endogenous IgG.*



Prostate: Desmin (m), VECTASTAIN® Universal Quick Kit, ImmPACT™ DAB (brown) substrate. Hematoxylin QS (blue) counterstain.

VECTASTAIN® ABC Elite® Kits (Peroxidase)

Product	Catalog Number	Unit Size	ABC Reagent	Secondary Antibody	Normal Blocking Serum	Approximate Number of Sections Stained
VECTASTAIN® Elite® ABC Kit, Standard	PK-6100	1 Kit	2 ml Reagent A 2 ml Reagent B (100 ml working solution)	none	none	500-1000
VECTASTAIN® Elite® ABC Kit, Rabbit IgG*	PK-6101	1 Kit	2 ml Reagent A 2 ml Reagent B (100 ml working solution)	1 ml biotinylated goat anti-rabbit IgG (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	500-1000
VECTASTAIN® Elite® ABC Kit, Mouse IgG*	PK-6102	1 Kit	2 ml Reagent A 2 ml Reagent B (100 ml working solution)	1 ml biotinylated horse anti-mouse IgG (200 ml working solution)	3 ml normal horse serum (200 ml working solution)	500-1000
VECTASTAIN® Elite® ABC Kit, Human IgG*	PK-6103	1 Kit	2 ml Reagent A 2 ml Reagent B (100 ml working solution)	1 ml biotinylated goat anti-human IgG (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	500-1000
VECTASTAIN® Elite® ABC Kit, Rat IgG*	PK-6104	1 Kit	2 ml Reagent A 2 ml Reagent B (100 ml working solution)	1 ml biotinylated rabbit anti-rat IgG (200 ml working solution)	3 ml normal rabbit serum (200 ml working solution)	500-1000
VECTASTAIN® Elite® ABC Kit, Goat IgG*	PK-6105	1 Kit	2 ml Reagent A 2 ml Reagent B (100 ml working solution)	1 ml biotinlyated rabbit anti-goat IgG (200 ml working solution)	3 ml normal rabbit serum (200 ml working solution)	500-1000
VECTASTAIN® Elite® ABC Kit, Sheep IgG*	PK-6106	1 Kit	2 ml Reagent A 2 ml Reagent B (100 ml working solution)	1 ml biotinylated rabbit anti-sheep IgG (200 ml working solution)	3 ml normal rabbit serum (200 ml working solution)	500-1000
VECTASTAIN® Elite® ABC Kit, Universal	PK-6200	1 Kit	2 ml Reagent A 2 ml Reagent B (100 ml working solution)	2 ml biotinylated anti-mouse/rabbit IgG (100 ml working solution)	3 ml normal horse serum (200 ml working solution)	500-1000
R.T.U. VECTASTAIN® Elite® ABC Reagent	PK-7100	50 ml	50 ml ready-to-use stabilized Elite® ABC reagent	none	none	250-500
R.T.U. VECTASTAIN® Elite® ABC Kit, Universal	PK-7200	1 Kit	50 ml ready-to-use stabilized Elite® ABC reagent	50 ml ready-to-use universal, biotinylated anti-mouse/rabbit IgG	50 ml 2.5% normal horse serum	250-500

** These kits contain excess biotinylated antibody.*

VECTASTAIN® ABC Systems (continued)

Original VECTASTAIN® ABC Kits (Peroxidase)

Product	Catalog Number	Unit Size	ABC Reagent	Secondary Antibody	Normal Blocking Serum	Approximate Number of Sections Stained
VECTASTAIN® ABC Kit, Standard	PK-4000	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	none	none	1000-2000
VECTASTAIN® ABC Kit, Rabbit IgG	PK-4001	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated goat anti-rabbit IgG (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC Kit, Mouse IgG	PK-4002	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated horse anti-mouse IgG (200 ml working solution)	3 ml normal horse serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC Kit, Mouse IgM	PK-4010	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated goat anti-mouse IgM (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC Kit, Human IgG	PK-4003	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated goat anti-human IgG (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC Kit, Rat IgG	PK-4004	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated rabbit anti-rat IgG (200 ml working solution)	3 ml normal rabbit serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC Kit, Goat IgG	PK-4005	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated rabbit anti-goat IgG (200 ml working solution)	3 ml normal rabbit serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC Kit, Sheep IgG	PK-4006	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated rabbit anti-sheep IgG (200 ml working solution)	3 ml normal rabbit serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC Kit, Guinea Pig IgG	PK-4007	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated goat anti-guinea pig IgG (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	1000-2000

VECTASTAIN® Universal *Quick* Kits (Peroxidase)

Product	Catalog Number	Unit Size	Preformed Complex	Secondary Antibody	Normal Blocking Serum	Approximate Number of Sections Stained
R.T.U. VECTASTAIN® Universal *Quick* Kit	PK-7800	1 Kit	50 ml prediluted peroxidase-streptavidin complex	50 ml prediluted, biotinylated, universal pan-specific antibody (anti-mouse/rabbit/goat IgG)	50 ml 2.5% normal horse serum	250-500
VECTASTAIN® Universal *Quick* Kit (concentrate)	PK-8800	1 Kit	1.2 ml peroxidase-streptavidin complex	2.2 ml biotinylated, universal pan-specific antibody (anti-mouse/rabbit/goat IgG)	6 ml normal horse serum	250-500

Alkaline Phosphatase

The sensitivity of the VECTASTAIN® ABC-AP system is comparable to that of the peroxidase VECTASTAIN® Elite® ABC system. The VECTASTAIN® ABC-AP Kits may be preferred for tissues that have high endogenous peroxidase activity. The system also offers additional substrate color choices. For alkaline phosphatase substrates see pages A18-A21.

VECTASTAIN® ABC-AP Kits (Alkaline Phosphatase)

Product	Catalog Number	Unit Size	ABC Reagent	Secondary Antibody	Normal Blocking Serum	Approximate Number of Sections Stained
VECTASTAIN® ABC-AP Kit, Standard	AK-5000	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	none	none	1000-2000
VECTASTAIN® ABC-AP Kit, Rabbit IgG	AK-5001	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated goat anti-rabbit IgG (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC-AP Kit, Mouse IgG	AK-5002	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated horse anti-mouse IgG (200 ml working solution)	3 ml normal horse serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC-AP Kit, Mouse IgM	AK-5010	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated goat anti-mouse IgM (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC-AP Kit, Human IgG	AK-5003	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated goat anti-human IgG (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC-AP Kit, Rat IgG	AK-5004	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated rabbit anti-rat IgG (200 ml working solution)	3 ml normal rabbit serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC-AP Kit, Goat IgG	AK-5005	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated rabbit anti-goat IgG (200 ml working solution)	3 ml normal rabbit serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC-AP Kit, Sheep IgG	AK-5006	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated rabbit anti-sheep IgG (200 ml working solution)	3 ml normal rabbit serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC-AP Kit, Guinea Pig IgG	AK-5007	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated goat anti-guinea pig IgG (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC-AP Kit, Universal	AK-5200	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	2 ml biotinylated horse anti-mouse/ rabbit IgG (100 ml working solution)	3 ml normal horse serum (200 ml working solution)	500-1000

VECTASTAIN® ABC Systems (continued)

Glucose Oxidase

Endogenous glucose oxidase activity does not exist in mammalian tissues, so the VECTASTAIN® ABC-GO Kit can be used when endogenous peroxidases or alkaline phosphatases interfere with specific staining and blocking of that endogenous enzyme activity is ineffective or would destroy antigenic determinants. The VECTASTAIN® ABC Glucose Oxidase Kit is the least sensitive of the VECTASTAIN® ABC systems. For glucose oxidase substrates see pages A18-A21.

VECTASTAIN® ABC-GO Kits (Glucose Oxidase)

Product	Catalog Number	Unit Size	ABC Reagent	Secondary Antibody	Normal Blocking Serum	Approximate Number of sections stained
VECTASTAIN® ABC-GO Kit, Standard	OK-3000	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	none	none	1000-2000
VECTASTAIN® ABC-GO Kit, Rabbit IgG	OK-3001	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated goat anti-rabbit IgG (200 ml working solution)	3 ml normal goat serum (200 ml working solution)	1000-2000
VECTASTAIN® ABC-GO Kit, Mouse IgG	OK-3002	1 Kit	2 ml Reagent A 2 ml Reagent B (200 ml working solution)	1 ml biotinylated horse anti-mouse IgG (200 ml working solution)	3 ml normal horse serum (200 ml working solution)	1000-2000

VECTASTAIN® ABC Custom Kits

If a VECTASTAIN® ABC system is not available with a biotinylated secondary antibody of your required specificity, the appropriate reagents can be obtained separately. For example, to make a VECTASTAIN® Elite® ABC Kit for use with a mouse IgG primary antibody on rat tissues:

1) Choose the "Standard" VECTASTAIN® ABC Kit (includes only the reagents to make the ABC complex) containing the desired enzyme for detection (e.g. VECTASTAIN® Elite® ABC Kit, Standard, PK-6100).

2) Choose the biotinylated secondary antibody (e.g. biotinylated horse anti-mouse IgG, rat adsorbed, BA-2001) to detect a mouse primary on rat tissue.

3) Choose the blocking serum. Serum from the same species as the secondary antibody is generally recommended. (In our example, normal horse serum, S-2000).

All of our biotinylated, affinity-purified secondary antibodies are designed for use with the VECTASTAIN® ABC Standard Kits and the appropriate blocking serum. This option to "mix and match" kit components allows users to design a "custom" kit to suit their needs or use kit components interchangeably.

1) Choose Standard VECTASTAIN® Kit with the appropriate detection enzyme

Enzyme	Product	Catalog Number	Unit Size
Peroxidase	VECTASTAIN® Elite® ABC Kit	PK-6100	1 Kit
Peroxidase	R.T.U. VECTASTAIN® Elite® ABC Reagent	PK-7100	50 ml
Peroxidase	VECTASTAIN® ABC Kit	PK-4000	1 Kit
Alkaline Phosphatase	VECTASTAIN® ABC-AP Kit	AK-5000	1 Kit
Glucose Oxidase	VECTASTAIN® ABC-GO Kit	OK-3000	1 Kit

2) Choose the biotinylated secondary antibody

Product	Catalog Number	Unit Size
Anti-Cat IgG (H+L) made in goat, biotinylated	BA-9000	1.5 mg
Anti-Chicken IgG (H+L) made in goat, biotinylated	BA-9010	1.5 mg
Anti-Goat IgG (H+L) made in rabbit, biotinylated	BA-5000	1.5 mg
Anti-Goat IgG (H+L) made in horse, biotinylated	BA-9500	1.5 mg
Anti-Guinea Pig IgG (H+L) made in goat, biotinylated	BA-7000	1.5 mg
Anti-Hamster IgG (H+L) made in goat, biotinylated	BA-9100	1.5 mg
Anti-Horse IgG (H+L) made in goat, biotinylated	BA-8000	1.5 mg
Anti-Human IgG (H+L) made in goat, biotinylated (for chain specific antibodies, see page A29)	BA-3000	1.5 mg
Anti-Mouse IgG (H+L) made in horse, biotinylated	BA-2000	1.5 mg
Anti-Mouse IgG (H+L) made in horse, rat adsorbed, biotinylated	BA-2001	0.5 mg
Anti-Mouse IgG (H+L) made in goat, biotinylated	BA-9200	1.5 mg
Anti-Mouse IgM (H+L) µ chain specific, made in goat, biotinylated	BA-2020	0.5 mg
Anti-Rabbit IgG (H+L) made in goat, biotinylated	BA-1000	1.5 mg
Anti-Rabbit IgG (H+L) made in horse, biotinylated	BA-1100	1.5 mg
Anti-Rat IgG (H+L) made in rabbit, biotinylated	BA-4000	1.5 mg
Anti-Rat IgG (H+L) made in rabbit, mouse adsorbed, biotinylated	BA-4001	0.5 mg
Anti-Rat IgG (H+L) made in goat, biotinylated	BA-9400	1.5 mg
Anti-Rat IgG (H+L) made in goat, mouse adsorbed, biotinylated	BA-9401	0.5 mg
Anti-Sheep IgG (H+L) made in rabbit, biotinylated	BA-6000	1.5 mg
Anti-Swine IgG (H+L) made in goat, biotinylated	BA-9020	1.5 mg
Universal Anti-Mouse/Rabbit IgG (H+L) made in horse, biotinylated	BA-1400	2.1 mg
Universal Pan-Specific Anti-Mouse/Rabbit/Goat IgG (H+L) made in horse, biotinylated	BA-1300	2.2 ml

3) Choose the blocking serum

Product	Catalog Number	Unit Size
Normal Goat Serum	S-1000	20 ml
Normal Rabbit Serum	S-5000	20 ml
Normal Horse Serum	S-2000	20 ml

ImmPRESS™ Polymer Kits (Peroxidase)

The ImmPRESS™ **p**olymerized **r**eporter **e**nzyme **s**taining **s**ystem uses novel conjugation and micropolymer chemistries to create a highly sensitive, ready-to-use, one-step, non-biotin detection system. This unique micropolymer of highly active peroxidase is attached to our affinity purified secondary antibodies, producing reagents with outstanding sensitivity and low background.

The peroxidase micropolymers of the ImmPRESS™ reagent limit steric interference and provide enhanced accessibility to the target, avoiding the disadvantages of other polymer systems that use large dextrans or other macromolecules as backbones. The result is crisp, strong staining of antibody targets, especially nuclear and membrane antigens (such as Ki67, estrogen receptor, bcl-2, CD3, CD8 and CD10) and greater sensitivity than other polymer systems. Key advantages of the ImmPRESS™ Polymer System:

- High sensitivity
- Low background
- Non-biotin
- One-step detection
- Ready-to-use in a convenient dropper bottle
- Shorter assay time
- Simplified multiple labeling
- Blocking solution included

The ImmPRESS™ reagents are supplied prediluted and ready-to-use in convenient dropper bottles along with prediluted blocking serum. No mixing or titering of the ImmPRESS™ reagents is necessary to obtain optimal tissue section staining.

Consider Species Cross-Reactivity

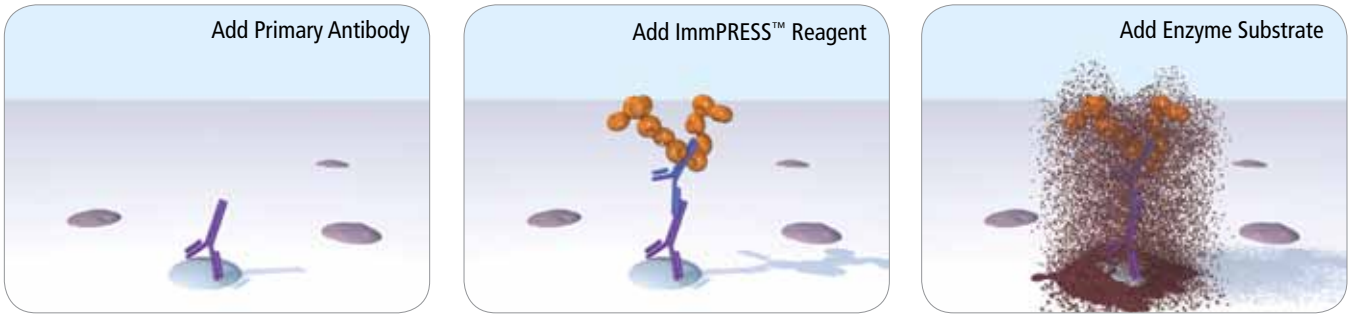
When choosing the optimal detection system for your application, it is important to consider not only the species of the primary antibody but also the species of the tissue. If the species of the primary antibody and the species of the tissue are closely related (e.g. rat and mouse), the secondary antibody may bind to endogenous IgG in the tissue section leading to background. The following options minimize background staining in these instances:

- Use a secondary antibody specifically adsorbed to remove cross-reacting antibodies of closely-related species (e.g. ImmPRESS™ Anti-Mouse IgG, Rat Adsorbed, see page A13).

- Use the M.O.M.™ Mouse Ig Blocking Reagent (Cat. No. MKB-2213) with the ImmPRESS™ Anti-Mouse IgG for applications of mouse primary antibodies on mouse tissue (see pages A16-A17).

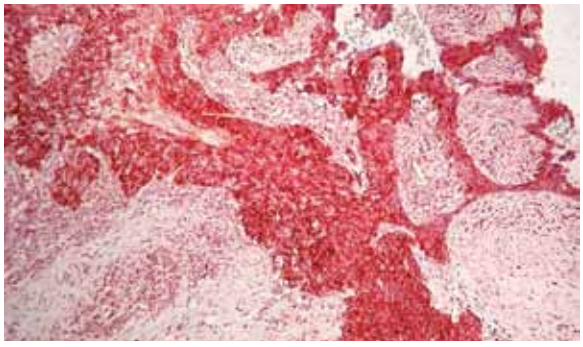
Substrates

After choosing the ImmPRESS™ system for your application, select a peroxidase substrate (A18-A21). For additional information on the wide variety of enzyme substrates available, please see the descriptions in Section I, "Enzyme Substrates".

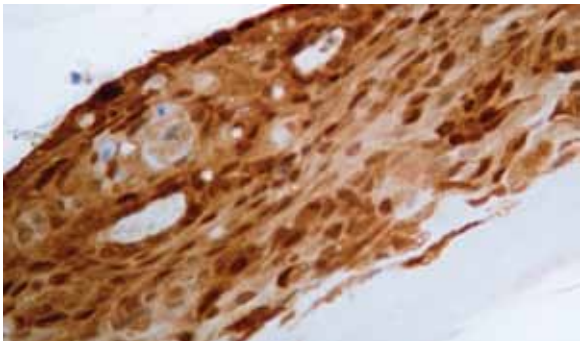


ImmPRESS™ Kits

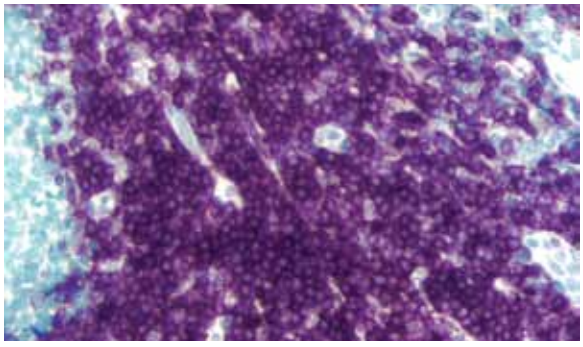
Product	Catalog Number	Unit Size	ImmPRESS™ Reagent	Normal Blocking Serum	Approximate Number of Sections Stained
ImmPRESS™ Anti-Rabbit Ig Kit	MP-7401	15 ml	15 ml ready-to-use solution	15 ml 2.5% normal horse serum	75-150
		50 ml	50 ml ready-to-use solution	50 ml 2.5% normal horse serum	250-500
ImmPRESS™ Anti-Mouse Ig Kit	MP-7402	15 ml	15 ml ready-to-use solution	15 ml 2.5% normal horse serum	75-150
		50 ml	50 ml ready-to-use solution	50 ml 2.5% normal horse serum	250-500
ImmPRESS™ Anti-Mouse Ig, Rat Adsorbed, Kit	MP-7422	15 ml	15 ml ready-to-use solution	15 ml 2.5% normal horse serum	75-150
ImmPRESS™ Anti-Rat Ig Kit	MP-7404	50 ml	50 ml ready-to-use solution	50 ml 2.5% normal goat serum	250-500
ImmPRESS™ Anti-Rat Ig, Mouse Adsorbed, Kit	MP-7444	15 ml	15 ml ready-to-use solution	15 ml 2.5% normal goat serum	75-150
ImmPRESS™ Anti-Goat Ig Kit	MP-7405	50 ml	50 ml ready-to-use solution	50 ml 2.5% normal horse serum	250-500
ImmPRESS™ Universal Antibody Kit, Anti-Rabbit/Mouse Ig	MP-7500	15 ml	15 ml ready-to-use solution	15 ml 2.5% normal horse serum	75-150
		50 ml	50 ml ready-to-use solution	50 ml 2.5% normal horse serum	250-500



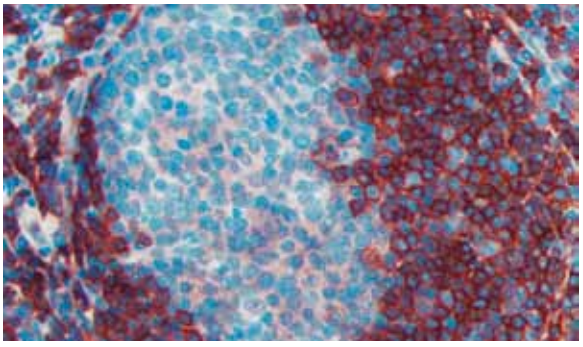
Up-regulation of chemokine CCL20 in nasopharyngeal carcinoma: CCL20 (g), ImmPRESS™ Reagent (HRP) Anti-Goat Ig, ImmPACT™ NovaRED™ (red), Hematoxylin QS (blue). (Image courtesy of Dr. GM Reynolds, Centre for Liver Research, University of Birmingham, U.K.)



Organotypic raft culture model of HPV16 infection showing up-regulation of P16: P16 (m), ImmPRESS™ Reagent (HRP) Anti-Mouse Ig, ImmPACT™ DAB (brown), Hematoxylin QS (blue). (Image courtesy of Dr. GM Reynolds, Centre for Liver Research, University of Birmingham, U.K.)



Tonsil: CD3 (rat), ImmPRESS™ Reagent (HRP) Anti-Rat Ig, ImmPACT™ VIP (purple), Vector® Methyl Green (green).

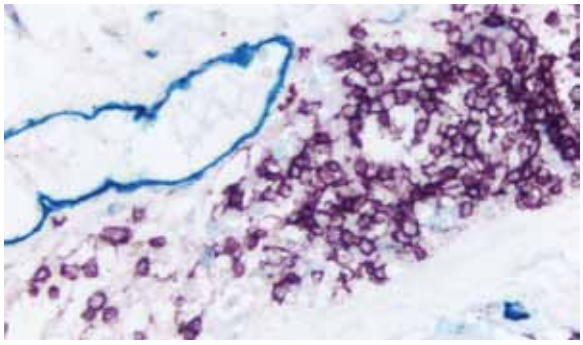


Mouse Tonsil: CD45 (rat), ImmPRESS™ Reagent (HRP) Anti-Rat Ig, Mouse Adsorbed, ImmPACT™ AEC (red), Hematoxylin QS (blue).

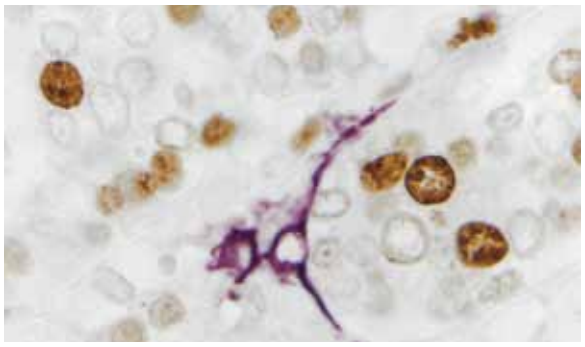
ImmPRESS™ Polymer Kits (continued)

Multiple Antigen Labeling Simplified

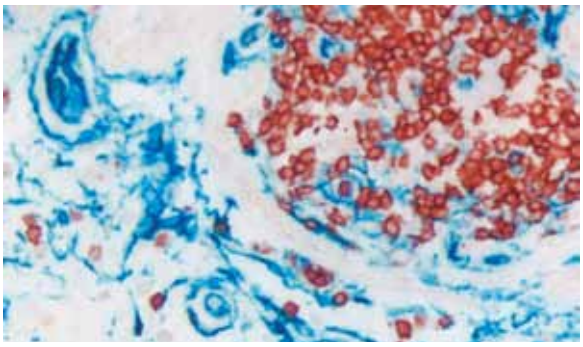
The ready-to-use, one-step, non-biotin, sensitive ImmPRESS™ Reagent significantly shortens staining times for multiple antigen labeling. The reduced number of steps compared to a conventional protocol directly decreases the amount of slide handling. In systems containing endogenous biotin, the ImmPRESS™ Reagent eliminates the need for avidin/biotin blocking steps. Detailed information for multiple antigen labeling can be found in our brochure, " Discovery Through Color: A Guide to Multiple Antigen Labeling", that is available on our website.



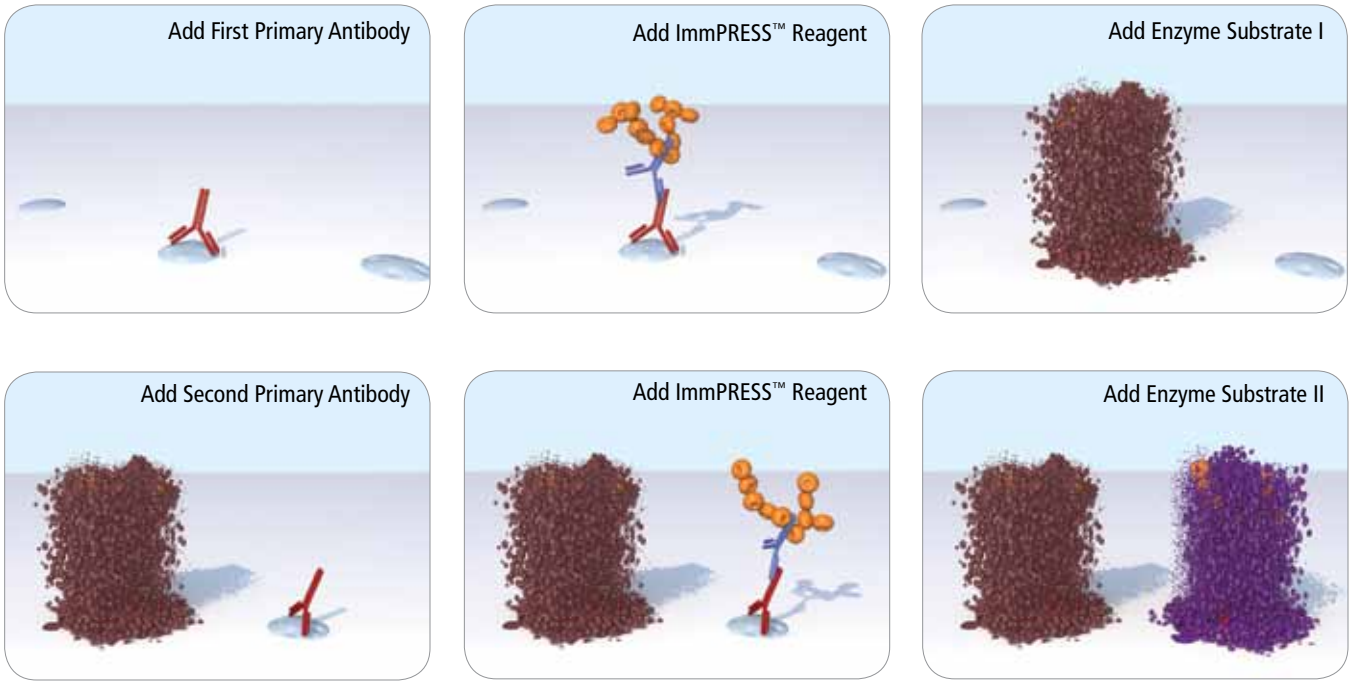
Colon: • M2A antigen (m), VECTASTAIN® ABC-AP Kit (Universal), Vector® Blue (blue) • CD20 (m), ImmPRESS™ Reagent (HRP; Universal), Vector® VIP (purple).

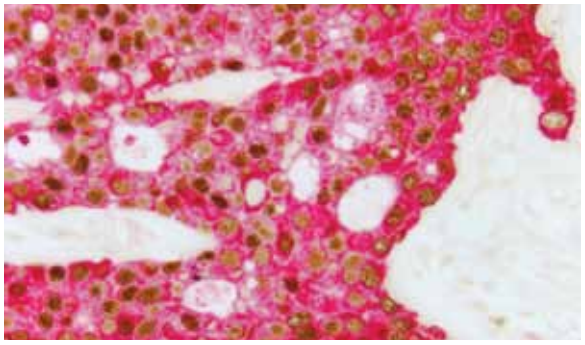


Breast Carcinoma: • Ki67 (rm), ImmPRESS™ Reagent (HRP; Universal), Vector® DAB (brown) • CD34, ImmPRESS™ Reagent (HRP; Universal), Vector® VIP (purple).

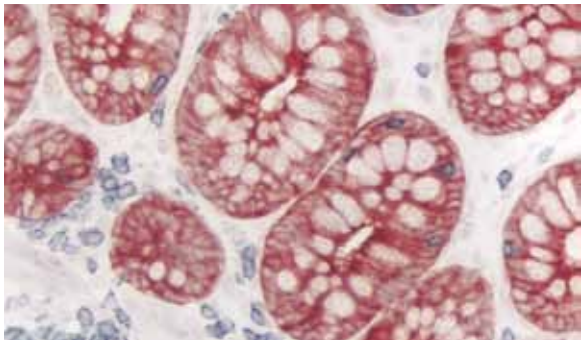


Colon: • CD3 (rm), ImmPRESS™ Reagent (HRP) Anti-Rabbit Ig, ImmPACT™ AMEC Red • CD34 (m), Vector® AP detection system, Vector® Blue (blue).

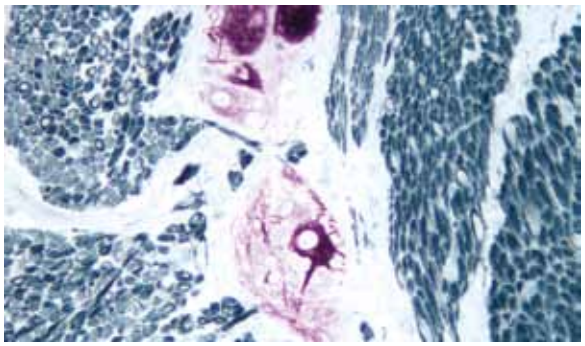




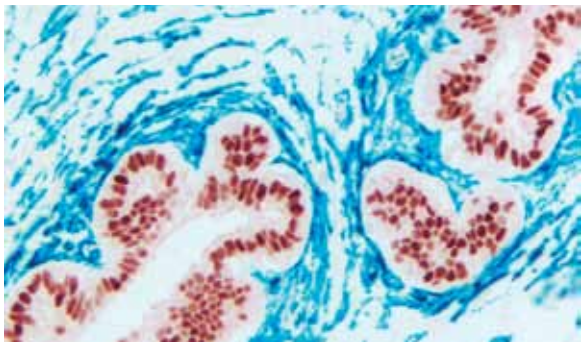
Breast Carcinoma: • Estrogen Receptor (m), ImmPRESS™ Reagent (HRP; Universal), Vector® DAB (brown) • Cytokeratin AE1/AE3 (m), VECTASTAIN® ABC-AP Kit (Universal), Vector® Red (magenta).



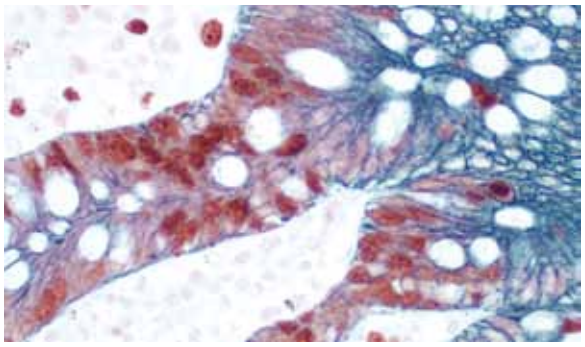
Colon: • CD3 (rm), ImmPRESS™ Reagent (HRP) Anti-Rabbit Ig, ImmPACT™ SG (blue-gray) • Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP) Anti-Mouse Ig, ImmPACT™ AMEC Red (red).



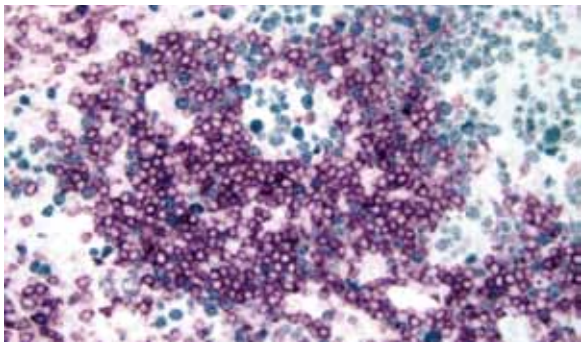
Small Bowel: • Neurofilament 200 kDa (m), ImmPRESS™ Reagent (HRP) Anti-Mouse Ig, Vector® VIP (purple) • Desmin (m), ImmPRESS™ Reagent (HRP) Anti-Mouse Ig, Vector® SG (blue-gray).



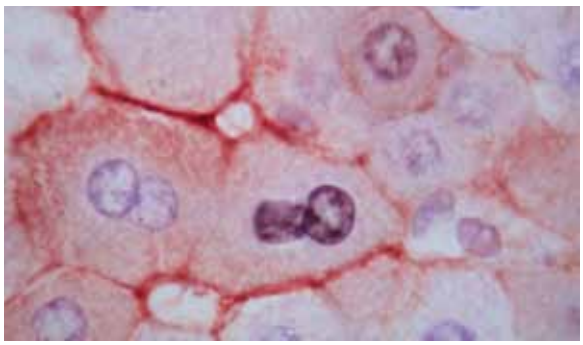
Breast Carcinoma: • Estrogen Receptor (m), ImmPRESS™ Reagent (HRP; Universal), Vector® NovaRED™ (red) • CD34 (m), VECTASTAIN® ABC-AP Kit (Universal), Vector® Blue (blue).



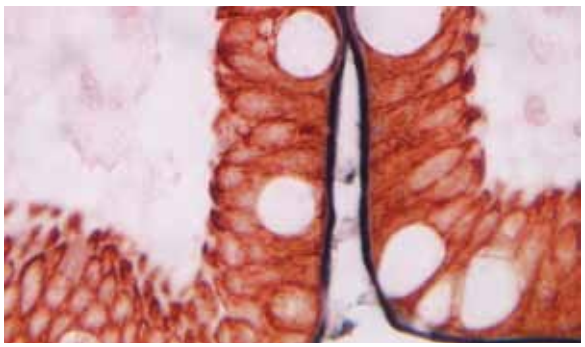
Small Bowel: • Ki67 (rp), ImmPRESS™ Reagent (HRP; Universal), Vector® NovaRED™ (red) • Cytokeratin 8/18 (m), ImmPRESS™ Reagent (HRP; Universal), Vector® SG (blue-gray).



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



Hepatitis B virus infected liver: • HBV core antigen (rp), ImmPRESS™ Reagent (HRP) Anti-Rabbit Ig, ImmPACT™ DAB+Ni (gray-black). • HBV surface antigen (m), ImmPRESS™ Reagent (HRP) Anti-Mouse Ig, ImmPACT™ NovaRED™ (red), Hematoxylin QS (blue). (Image courtesy of Dr. GM Reynolds, Centre for Liver Research, University of Birmingham, U.K.)



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

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

The Vector® M.O.M.™ Immunodetection Kits are specifically designed to localize mouse primary antibodies on mouse tissues. The major problem with using mouse primary antibodies on mouse tissues in immunohistochemistry is the inability of the anti-mouse secondary antibody to distinguish between the mouse primary antibody and endogenous mouse immunoglobulins in the tissue. This can result in high background staining which obscures specific staining. This problem can be essentially eliminated by using a Vector® M.O.M.™ Immunodetection Kit which includes a proprietary M.O.M.™ Mouse Ig Blocking Reagent with a specialized M.O.M.™ Biotinylated Anti-Mouse Ig to significantly reduce undesired binding of the secondary antibody to endogenous tissue immunoglobulin.

Key advantages of the Vector® M.O.M.™ Immunodetection Kits:

- Significant reduction of endogenous mouse Ig staining when using mouse primary antibodies on mouse tissue
- Clear, crisp specific staining of antigens of interest
- Based on VECTASTAIN® ABC reagent protocols
- Procedures are simple and easy to follow
- No tedious calculations or antibody prebinding steps required

The Vector® M.O.M.™ Immunodetection Kits are useful in any mouse-on-mouse application, such as studies in genetically engineered mice, including transgenic and knock-out models, as well as mouse xenografts.

The M.O.M.™ Kits can be also used in multiple antigen staining protocols to localize two different mouse primary antibodies on the same mouse tissue sections using fluorescent or enzyme-based detection systems. Detailed information for multiple antigen labeling can be found in our brochure, " Discovery Through Color: A Guide to Multiple Antigen Labeling", available on our website.

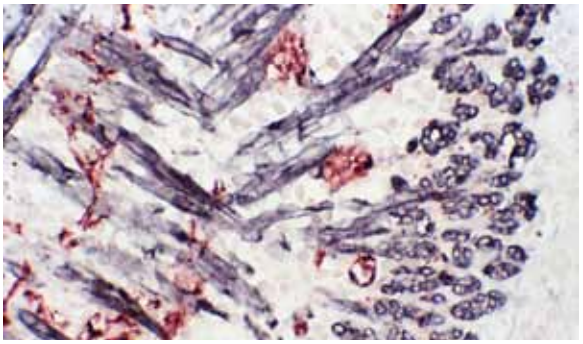
Three Vector® M.O.M.™ Kits are available. All kits use the same proprietary M.O.M.™ Mouse Ig Blocking Reagent and the M.O.M.™ Biotinylated Anti-Mouse Ig Reagent, but offer a choice of an enzyme-based or fluorescent-based visualization method. M.O.M.™ Mouse Ig Blocking Reagent and M.O.M.™ Biotinylated Anti-Mouse Ig Reagent are also available separately.

Vector® M.O.M.™ Peroxidase Kit

Vector® M.O.M.™ Peroxidase Kit (PK-2200) includes the following:

- M.O.M.™ Mouse Ig Blocking Reagent
- M.O.M.™ Biotinylated Anti-Mouse Ig Reagent
- M.O.M.™ Protein Concentrate for general protein blocking and diluent
- VECTASTAIN® Elite® ABC Reagent

Choose from the wide variety of peroxidase substrates available (pages A18-A21) for chromogenic development.

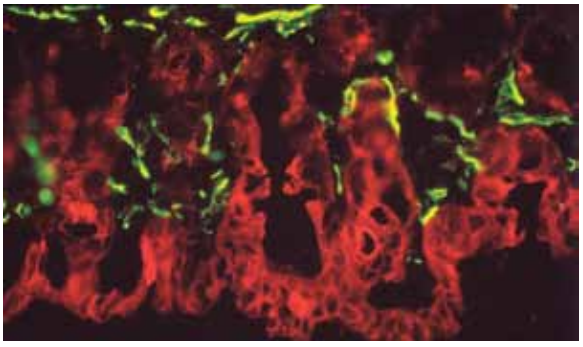


Mouse Newborn Tongue: • Synapsin (m), M.O.M.™ Peroxidase Kit, Vector® NovaRED™ (red) • Desmin (m), M.O.M.™ Peroxidase Kit, Vector® DAB-Ni (black).

Vector® M.O.M.™ Fluorescein Kit

Vector® M.O.M.™ Fluorescein Kit (FMK-2201) includes the following:

- M.O.M.™ Mouse Ig Blocking Reagent
- M.O.M.™ Biotinylated Anti-Mouse Ig Reagent
- M.O.M.™ Protein Concentrate for general protein blocking and diluent
- Fluorescein Avidin DCS (green fluorescence, excitation at 495 nm; emission at 515 nm)



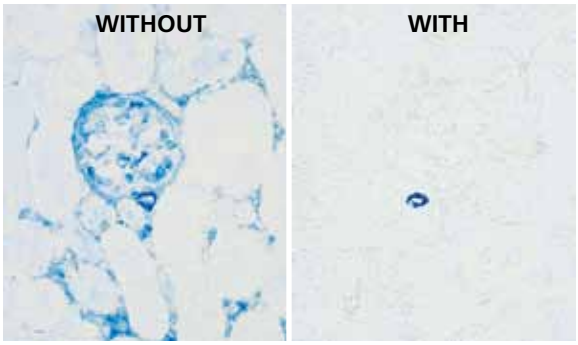
Mouse Intestine: • Desmin (m), M.O.M.™ Fluorescein Kit (green) • Multi-Cytokeratin (m), M.O.M.™ Basic Kit, Texas Red® Avidin DCS.

Vector® M.O.M.™ Basic Kit

Vector® M.O.M.™ Basic Kit (BMK-2202) includes the following:

- M.O.M.™ Mouse Ig Blocking Reagent
- M.O.M.™ Biotinylated Anti-Mouse Ig Reagent
- M.O.M.™ Protein Concentrate for general protein blocking and diluent

An enzyme or fluorochrome conjugate is not included allowing for a choice of any avidin or streptavidin-based detection system. Choose any peroxidase or alkaline phosphatase conjugated avidin or streptavidin for chromogenic detection (for example, VECTASTAIN® ABC-AP Standard Kit followed by a Vector® alkaline phosphatase substrate), or choose a fluorochrome-labeled avidin or streptavidin (see Section B, "Immunofluorescence", page B6).



Sections of mouse kidney stained with mouse antibody against smooth muscle actin using VECTASTAIN® ABC-AP Kit and Vector® Blue substrate. Left photo: Using standard biotinylated anti-mouse antibody and normal blocking serum, confusing background is seen. Right photo: With the Vector® M.O.M.™ Basic kit, clean background and specific staining is achieved.

M.O.M.™ Mouse Ig Blocking Reagent

M.O.M.™ Mouse Ig Blocking Reagent (MKB-2213) is a key component of the M.O.M.™ Kits and is used to block endogenous mouse antibody in the tissue section. Optimal staining results are obtained when this reagent is paired with the special M.O.M.™ Biotinylated Anti-Mouse Ig Reagent. However, if a biotin-free detection system is preferred, the M.O.M.™ Blocking Reagent can be used with the ImmPRESS™ Anti-Mouse Ig Kit (MP-7402). This reagent is supplied as a 1 ml concentrate, sufficient to prepare about 25 ml of working solution or enough to stain about 250 sections.

M.O.M.™ Biotinylated Anti-Mouse Ig Reagent

M.O.M.™ Biotinylated Anti-Mouse Ig Reagent (MKB-2225) is a specially modified secondary antibody that has been optimized specifically for use with the Vector® M.O.M.™ Immunodetection Kit components. This is the same reagent contained in the Vector® M.O.M.™ Kits (Cat. Nos. PK-2200, FMK-2201, and BMK-2202).

Vector® M.O.M™ Kits and Reagents

Product	Catalog Number	Unit Size	Kit Components	Approximate Number of Sections Stained
M.O.M.™ Peroxidase Kit	PK-2200	1 Kit	1 ml M.O.M.™ Mouse Ig Blocking Reagent 0.1 ml M.O.M.™ Biotinylated Anti-Mouse Ig Reagent 6 ml M.O.M.™ Protein Concentrate VECTASTAIN® Elite® ABC Reagents A (1 ml) and B (1 ml)	125-250
M.O.M.™ Fluorescein Kit	FMK-2201	1 Kit	1 ml M.O.M.™ Mouse Ig Blocking Reagent 0.1 ml M.O.M.™ Biotinylated Anti-Mouse Ig Reagent 6 ml M.O.M.™ Protein Concentrate 0.4 ml Fluorescein Avidin DCS	125-250
M.O.M.™ Basic Kit	BMK-2202	1 Kit	1 ml M.O.M.™ Mouse Ig Blocking Reagent 0.1 ml M.O.M.™ Biotinylated Anti-Mouse Ig Reagent 6 ml M.O.M.™ Protein Concentrate	125-250
M.O.M.™ Mouse Ig Blocking Reagent	MKB-2213	1 ml	M.O.M.™ Mouse Ig Blocking Reagent	-
M.O.M.™ Biotinylated Anti-Mouse Ig Reagent*	MKB-2225	0.1 ml	M.O.M.™ Biotinylated Anti-Mouse Ig Reagent	-

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

Enzyme Substrates

A

Vector Laboratories offers many enzyme substrate kits for use with peroxidase, alkaline phosphatase, and glucose oxidase detection systems.

All Vector Laboratories substrate kit reagents are supplied in convenient dropper bottles promoting ease of handling of chromogens and eliminating wait times for mixing and dissolving powders or tablets.

Choose a substrate that matches the enzyme in your detection system. Choosing the optimal substrate for your application will depend on several considerations:

Sensitivity. Substrates provide varying degrees of sensitivities (see sensitivity comparison chart below). For example, ImmPACT™ peroxidase substrates are about 2-4 times more sensitive than the original peroxidase substrate kits, and the alkaline phosphatase substrate BCIP/NBT can achieve increased sensitivities with longer incubation times.

Color. Color contrast is essential in multiple antigen labeling applications, in pigmented tissues such as melanomas, and in counterstained tissues. Color choices also depend on personal preference.

Visualization. Viewing options include brightfield, fluorescence, darkfield, electron microscopy, and spectral imaging.

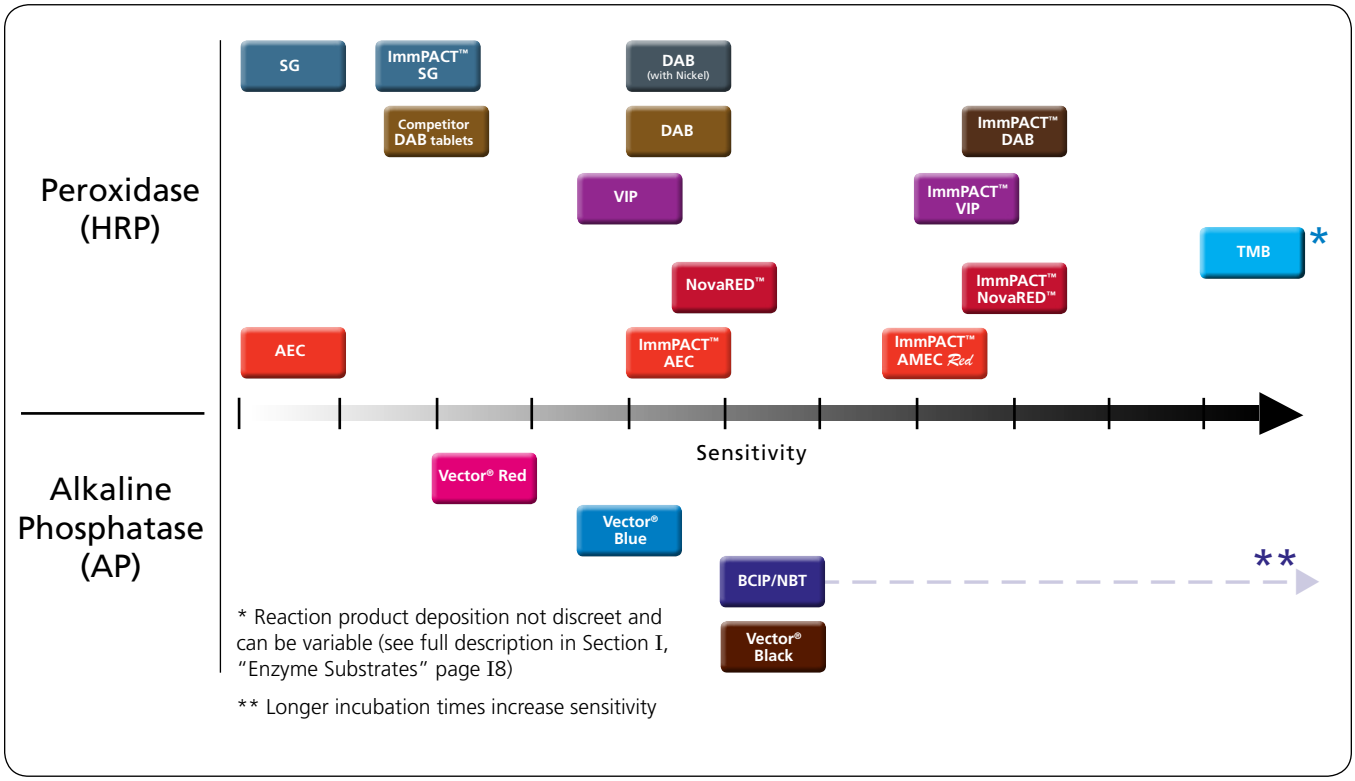
Heat resistance. For IHC/ISH double labeling applications, the heat resistant substrate is applied first in IHC, followed by ISH detection that includes a heat denaturation step.

Additional Information

- **Ordering information** on page A21.
- **Substrate properties** are summarized in the following tables.
- **Photomicrographs** of each precipitated substrate and additional descriptions can be found in Section I, "Enzyme Substrates".
- **Counterstain compatibility of substrates** can be found on page A25.
- **Substrate compatibility in multiple antigen labeling** applications can be found on page A22.

Relative Sensitivity of Substrates in Immunohistochemistry

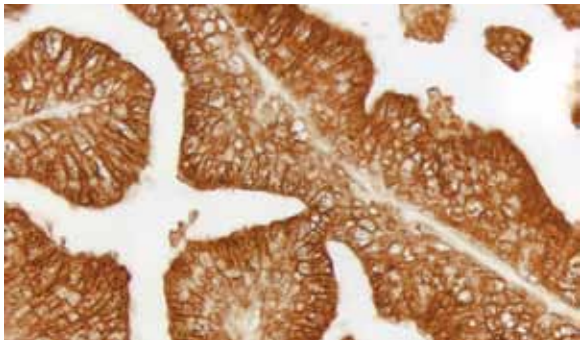
HRP substrates were used with the ImmPRESS™ Polymer Detection Reagent. AP substrates were used with the VECTASTAIN® ABC-AP Kit.



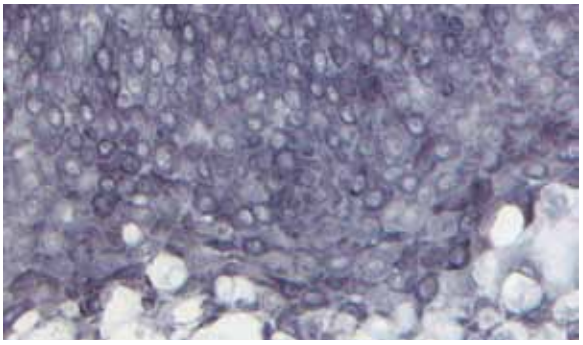
Enzyme Substrate Properties

Substrate	Color	Catalog Number	Microscopy					Mounting	Contrast in Pigmented Tissue	Multiple Labeling	Heat Resistant
			Bright-field	Darkfield	Electron	Fluore-scence	Spectral Imaging				
Peroxidase											
DAB	Brown	SK-4100	●	●	●		●	Non-aqueous or Aqueous		●	●
DAB +Ni	Gray-Black	SK-4100	●	●	●		●	Non-aqueous		●	
ImmPACT™ DAB	Brown	SK-4105	●	●	●		●	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	●	●	
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	●	●	●
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		●	●
Glucose Oxidase											
GO Kit I (NBT)	Indigo	SK-3100	●					Non-aqueous or Aqueous			
GO Kit II (TNBT)	Black	SK-3200	●					Non-aqueous			

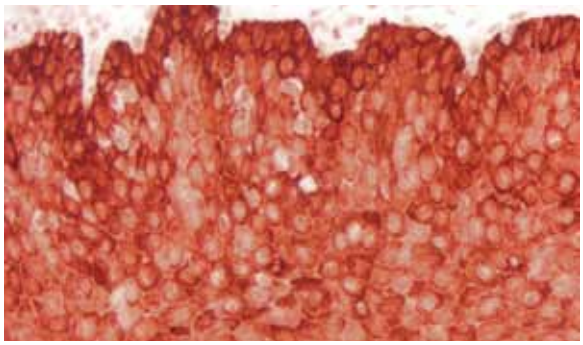
Enzyme Substrates (continued)



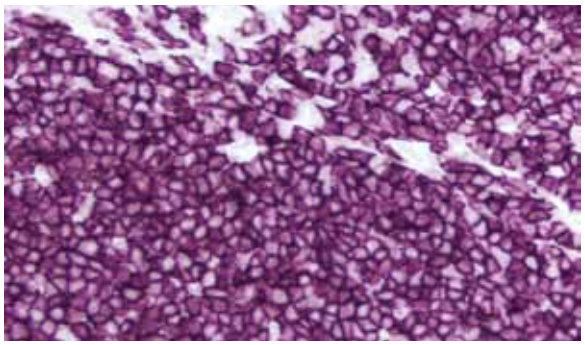
Prostate: Prostate Specific Antigen (m), ImmPRESS™ Reagent (HRP), ImmPACT™ DAB (brown).



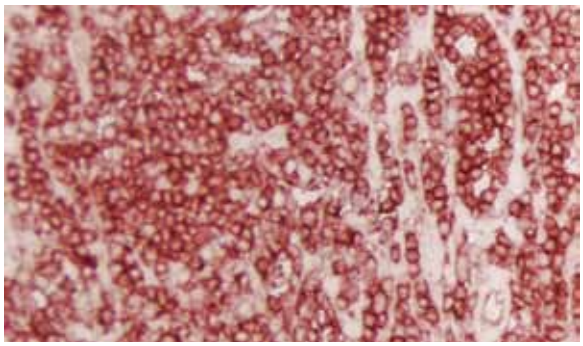
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), Vector® DAB-Ni (gray-black).



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

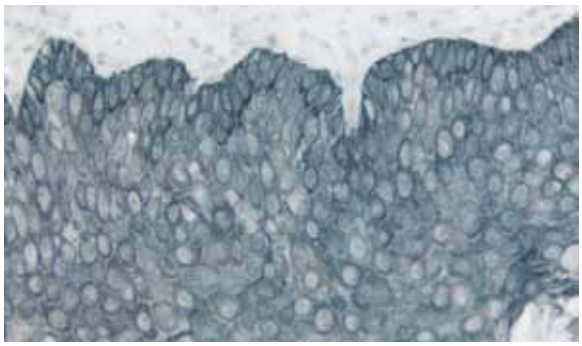


Tonsil: CD20 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ VIP (purple).

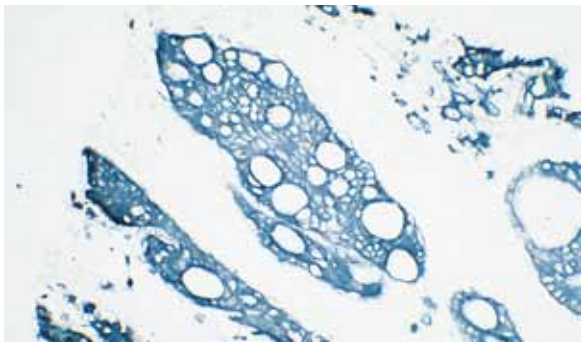


Tonsil: LCA (m), ImmPRESS™ Reagent (HRP), ImmPACT™ AMEC Red (red).

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



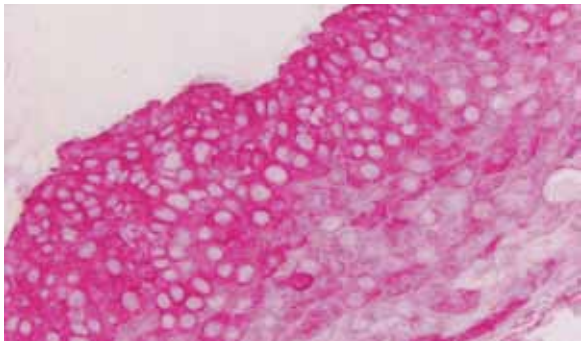
Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ SG (blue-gray).



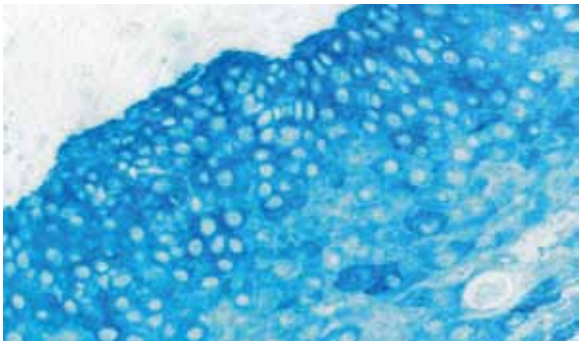
Tumor: Cytokeratin (s), VECTASTAIN® Elite® ABC Kit, TMB (blue).

Enzyme Substrate Kits

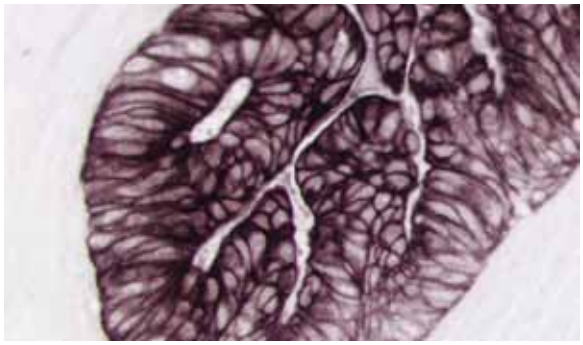
Enzyme	Substrate	Color	Catalog Number	Unit Size	Working Solution
Peroxidase	DAB	Brown	SK-4100	1 Kit	300 ml
	DAB +Ni	Gray-Black	SK-4100	1 Kit	300 ml
	ImmPACT™ DAB	Brown	SK-4105	120 ml	120 ml
	Vector® VIP	Purple	SK-4600	1 Kit	300 ml
	ImmPACT™ VIP	Purple	SK-4605	120 ml	120 ml
	Vector® SG	Blue-Gray	SK-4700	1 Kit	300 ml
	ImmPACT™ SG	Blue-Gray	SK-4705	120 ml	120 ml
	Vector® NovaRED™	Red	SK-4800	1 Kit	300 ml
	ImmPACT™ NovaRED™	Red	SK-4805	120 ml	120 ml
	AEC	Red	SK-4200	1 Kit	300 ml
	ImmPACT™ AEC	Red	SK-4205	120 ml	120 ml
	ImmPACT™ AMEC *Red*	Red	SK-4285	120 ml	120 ml
	TMB	Blue	SK-4400	1 Kit	300 ml
Alkaline Phosphatase	Vector® Red	Magenta	SK-5100	1 Kit	200 ml
	Vector® Blue	Blue	SK-5300	1 Kit	200 ml
	Vector® Black	Brown-Black	SK-5200	1 Kit	200 ml
	BCIP/NBT	Indigo	SK-5400	1 Kit	200 ml
Glucose Oxidase	GO Kit I (NBT)	Indigo	SK-3100	1 Kit	300 ml
	GO Kit II (TNBT)	Black	SK-3200	1 Kit	300 ml



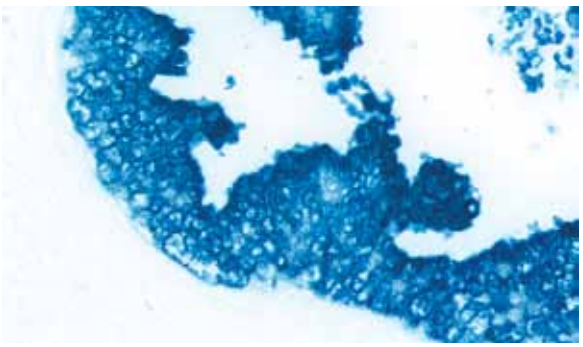
Tonsil: Cytokeratin AE1/AE3 (m), Vector® AP detection system, Vector® Red (magenta).



Tonsil: Cytokeratin AE1/AE3 (m), Vector® AP detection system, Vector® Blue (blue).



Colon Carcinoma: Pan-Cytokeratin (m), VECTASTAIN® ABC-AP Kit, Vector® Black (brown-black).



Prostate: Prostate Specific Antigen (m), VECTASTAIN® ABC-AP Kit, BCIP/NBT (indigo).

Multiple Antigen Labeling

Vector Laboratories is a leader in multiple antigen labeling applications because of our wide range of very sensitive and very low background detection reagents, our proprietary enzyme substrates, and our rigorously tested protocols. These detection systems and unique enzyme substrates offer an extensive choice of color combinations for multiple labeling using either:

- The same enzyme system with different substrates to detect each antigen, *or*
- Different enzyme systems and their substrates to detect each antigen

The photos on the following page illustrate a few possible combinations that give excellent results. The order of labeling, use of control sections, and additional blocking steps may be important to obtain optimal results. For a detailed description of these applications, protocols, and additional images, please visit our website or request a free copy of our brochure, "Discovery Through Color: A Guide to Multiple Antigen Labeling".

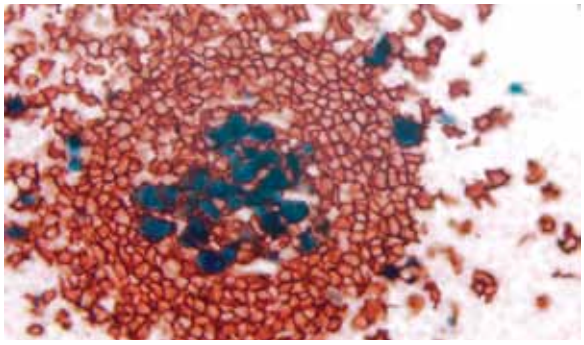
Enzyme Substrate Combinations

This table shows the substrates that are recommended for multiple antigen labeling and the order in which they are optimally used.

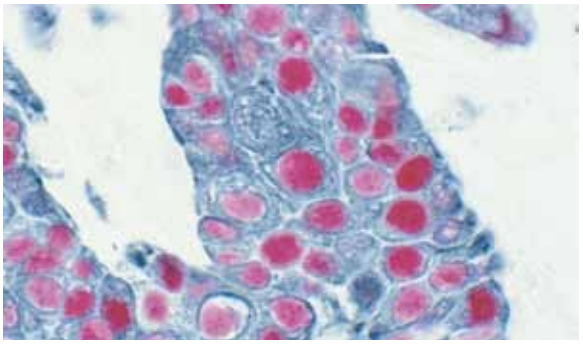
Second Substrate / First Substrate	Vector® Red (magenta) SK-5100	Vector® Blue (blue) SK-5300	BCIP/NBT (indigo) SK-5400	VIP/ ImmPACT™ VIP (purple) SK-4605, SK-4600	DAB/ ImmPACT™ DAB (brown) SK-4105, SK-4100	DAB-Ni (gray-black) SK-4100	NovaRED™/ ImmPACT™ NovaRED™ (red) SK-4805, SK-4800	SG/ ImmPACT™ SG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

Key: Alkaline Phosphatase | Peroxidase | + Indicates good contrast | – Indicates incompatiblity of substrates for various reasons

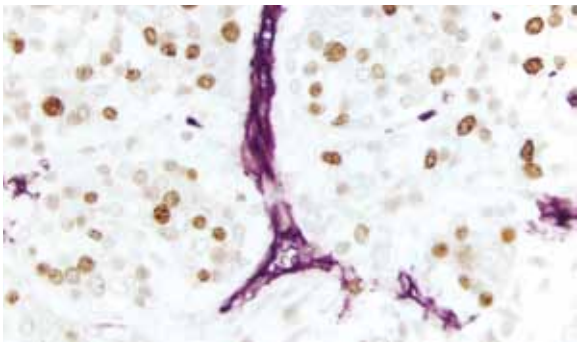
Multiple Label Slides



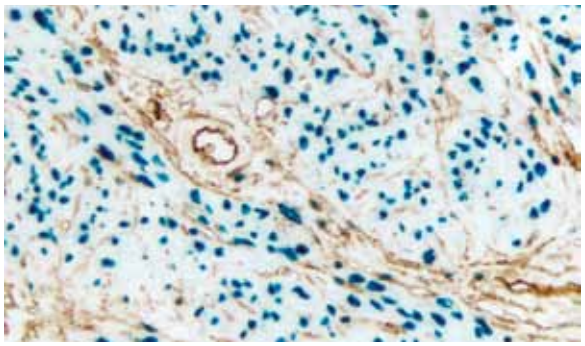
Tonsil: • Cyclin A (m), VECTASTAIN® ABC-AP Kit (Universal), Vector®Blue (blue) • CD20 (m), VECTASTAIN® Elite® ABC Kit (Universal), Vector® NovaRED™ (red).



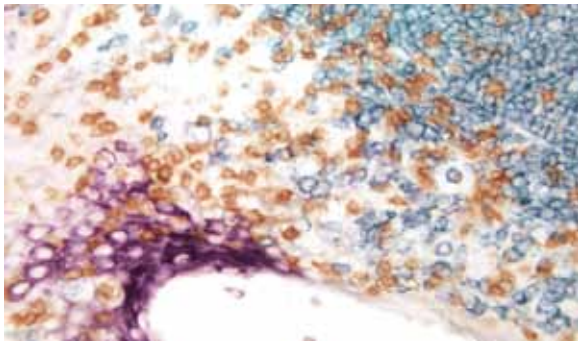
Tumor: • p53 protein (m), VECTASTAIN® ABC-AP Kit, Vector® Red (red) • Pan-Cytokeratin (s), VECTASTAIN® Elite® ABC Kit, Vector® SG (blue-gray).



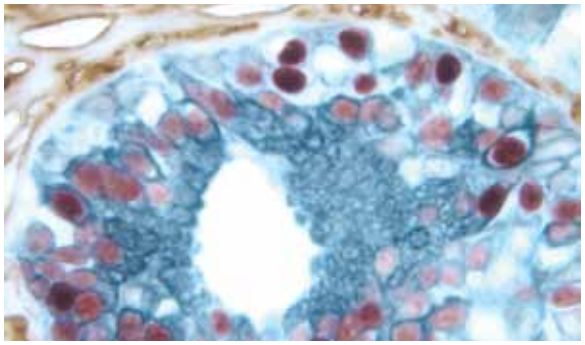
Breast Carcinoma: • Ki67 (rm), ImmPRESS™ Reagent (HRP; Universal), Vector® DAB (brown) • CD34 (m), ImmPRESS™ Reagent (HRP; Universal), Vector® VIP (purple).



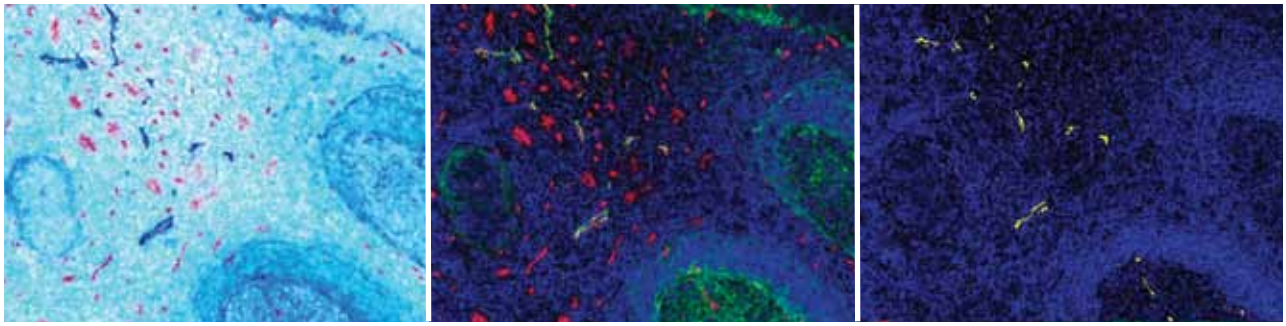
Endometrium: • Progesterone Receptor (rm), VECTASTAIN® ABC-AP Kit (Universal), Vector® Blue (blue) • CD34 (m), VECTASTAIN® Elite® ABC Kit (Universal), Vector® DAB (brown).



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



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



Tonsil: co-localization of D2-40 (m) and CD34 (m). (A) • D2-40, AP-detection system, Vector® Blue (blue) • CD34, AP-detection system, Vector® Red (magenta). Most lymph vessels show D2-40/CD34 co-localization (purple-blue), Vector® Methyl Green counterstain (green). (B) Spectral unmixing of colors applied using Nuance system (Cambridge Research instrumentation, Woburn, Ma.) resulting in pseudo-colored fluorescent composite image - D2-40 (green), CD34 (red). (C) Exclusive co-localization D2-40 and CD34 (yellow), Methyl Green (blue). Note this co-localization correlates with the purple-blue structures in A. Images kindly supplied by Chris M. van der Loos, Ph.D. (Dept of Pathology, AMC Amsterdam, The Netherlands).

Counterstains

Vector Laboratories' counterstains are specially prepared, thoroughly tested and packaged as convenient, ready-to-use solutions for use on individual slides or in staining dishes. Our counterstains stain cell nuclei and are recommended for interpretation of tissue morphology. A straightforward step-by-step protocol is provided with each counterstain.

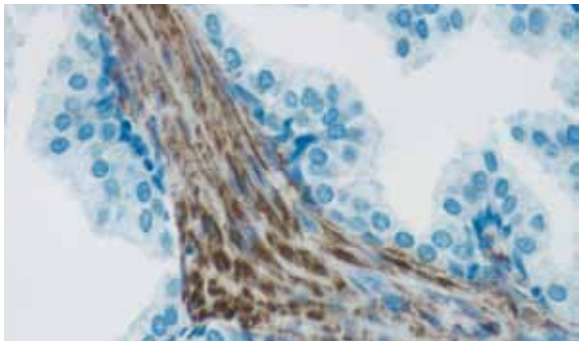
A counterstain/substrate compatibility chart is on page A25.

Vector® Hematoxylin

Vector® Hematoxylin (H-3401) produces blue stained nuclei with crisp detail. This commonly utilized counterstain is based on Gill's formulation and is alcohol and mercury free. As this is a progressive stain formula, the intensity of nuclear staining can be adjusted to achieve the most desirable results either manually or in an automated instrument. This counterstain provides excellent color contrast with most commonly used peroxidase and alkaline phosphatase substrates and is suitable for use with non-aqueous and aqueous mountants.

Vector® Hematoxylin QS

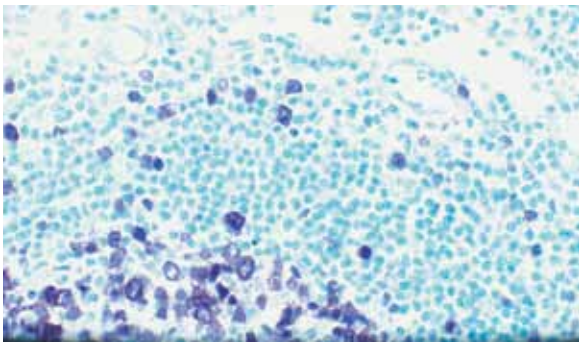
Vector® Hematoxylin QS (H-3404), a modification of Mayer's hematoxylin developed especially for immunocytochemistry, provides crisp blue nuclear staining. This formula is ready-to-use without filtration, requires no separate "blueing" step, and stains in less than 45 seconds. Vector® Hematoxylin QS contains no mercury and provides excellent color contrast with most commonly used peroxidase and alkaline phosphatase substrates. This counterstain is suitable for use with non-aqueous and aqueous mountants.



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

Vector® Methyl Green

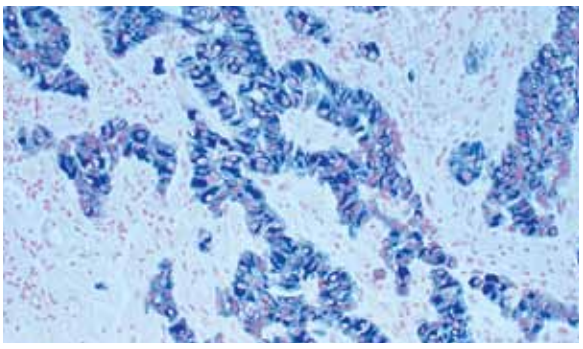
Vector® Methyl Green (H-3402) stains cell nuclei light green. This superior formulation of methyl green is suitable for use with a wide range of enzyme substrates. Optimal tissue staining is achieved by using a simple, two-step procedure. This counterstain provides an excellent alternative in multiple labeling when hematoxylin obscures the substrate colors. This counterstain is suitable for use with non-aqueous mountants.



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

Vector® Nuclear Fast Red

Nuclei stained with Vector® Nuclear Fast Red (H-3402) have a pink appearance. This counterstain provides good contrast with a variety of substrates and is suitable for use with non-aqueous and aqueous mountants. The staining procedure is rapid requiring only one step. This counterstain provides an excellent alternative in multiple labeling when hematoxylin obscures the substrate colors.



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

Counterstain/Substrate Compatibility Table

This table is designed as a reference to determine the optimal counterstain/substrate combination for your application.

Substrate	Substrate Cat. No	VECTOR® Hematoxylin and Hematoxylin QS H-3401 and H-3404	VECTOR® Methyl Green H-3402	VECTOR® Nuclear Fast Red H-3403
ImmPACT™ DAB (brown)	SK-4105	Excellent Contrast	Excellent Contrast	Fair Contrast
DAB (brown)	SK-4100	Excellent Contrast	Excellent Contrast	Fair Contrast
DAB-Ni (gray-black)	SK-4100	Excellent Contrast	Fair Contrast *	Good Contrast
ImmPACT™ AEC (red)	SK-4205	Excellent Contrast	Counterstain Incompatibility **	Color Incompatibility
ImmPACT™ AMEC *Red* (red)	SK-4285	Excellent Contrast	Counterstain Incompatibility **	Color Incompatibility
AEC (red)	SK-4200	Excellent Contrast	Counterstain Incompatibility **	Color Incompatibility
TMB (blue)	SK-4400	Color Incompatibility	Counterstain Incompatibility	Excellent Contrast
ImmPACT™ VIP (purple)	SK-4605	Fair Contrast	Excellent Contrast	Poor Contrast
VECTOR® VIP (purple)	SK-4600	Fair Contrast	Excellent Contrast	Poor Contrast
ImmPACT™ SG (blue-gray)	SK-4705	Poor Contrast	Good Contrast	Excellent Contrast
VECTOR® SG (blue-gray)	SK-4700	Poor Contrast	Good Contrast	Excellent Contrast
ImmPACT™ NovaRED™ (red)	SK-4805	Excellent Contrast	Excellent Contrast ***	Color Incompatibility
VECTOR® NovaRED™ (red)	SK-4800	Excellent Contrast	Excellent Contrast ***	Color Incompatibility
VECTOR® RED (magenta)	SK-5100	Excellent Contrast	Excellent Contrast	Color Incompatibility
VECTOR® BLACK (black)	SK-5200	Excellent Contrast	Excellent Contrast *	Excellent Contrast
VECTOR® BLUE (blue)	SK-5300	Color Incompatibility	Good Contrast	Excellent Contrast
BCIP/NBT (indigo)	SK-5400	Color Incompatibility	Excellent Contrast *	Excellent Contrast
Glucose Oxidase NBT (indigo)	SK-3100	Color Incompatibility	Excellent Contrast	Excellent Contrast
Glucose Oxidase TNBT (black)	SK-3200	Excellent Contrast	Excellent Contrast	Excellent Contrast

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

** Substrate dissolves in acetone wash.

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

Counterstains should be optimized for each tissue type, antigen unmasking protocol, and immunocytochemical staining intensity desired.

Counterstains

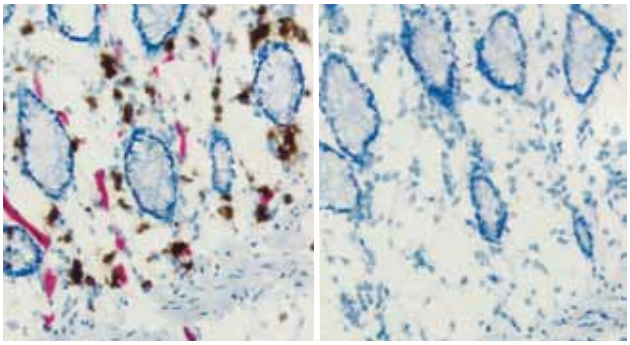
Product	Mountants	Catalog Number	Unit Size
Vector® Hematoxylin	Non-aqueous and Aqueous	H-3401	500 ml
Vector® Hematoxylin QS	Non-aqueous and Aqueous	H-3404	100 ml
Vector® Methyl Green	Non-aqueous	H-3402	500 ml
Vector Nuclear Fast Red	Non-aqueous and Aqueous	H-3403	500 ml

Blocking Reagents

BLOXALL™ Endogenous Peroxidase/Alkaline Phosphatase Blocking Solution

- Inhibits endogenous peroxidase, pseudoperoxidase, and alkaline phosphatase activities
- Ready-to-use in convenient dropper bottle
- Simple one-step protocol
- Compatible with formalin-fixed, paraffin-embedded tissue sections, frozen tissue sections, and cell preparations
- More effective than conventional blocking methods

Tissues can contain endogenous peroxidase, pseudoperoxidase, and/or alkaline phosphatase activity that will produce background staining if an alkaline phosphatase and/or peroxidase detection system and corresponding substrates are used. **BLOXALL™** Endogenous Peroxidase/Alkaline Phosphatase Blocking Solution (SP-6000) inactivates endogenous peroxidase, pseudoperoxidase, and alkaline phosphatase in formalin-fixed, paraffin-embedded tissue sections, frozen tissue sections, and cell preparations in a single 10 minute incubation step. This reagent is provided as a ready-to-use reagent in a convenient dropper bottle.



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

Levamisole Solution

Tissues can contain endogenous alkaline phosphatase activity that will produce background staining, if an alkaline phosphatase detection system and substrate are used. To eliminate this problem, Levamisole Solution (SP-5000), an inhibitor of alkaline phosphatase, is provided in a convenient dropper bottle, and can be added to the alkaline phosphatase substrate solution. Levamisole does not inhibit the isoenzyme used for either the VECTASTAIN® ABC-AP reagents or other alkaline phosphatase conjugates. 100x concentrate.

Avidin/Biotin Blocking Kit

Avidin/Biotin Blocking Kit (SP-2001) blocks all endogenous biotin, biotin receptors, and avidin binding sites present in tissues. This kit is designed for use with biotin/avidin detection systems such as the VECTASTAIN® ABC Kits if avidin or biotinylated reagents bind non-specifically to tissues or proteins. This blocking kit consists of 18 ml of Avidin D and 18 ml of biotin in convenient dropper bottles.

Streptavidin/Biotin Blocking Kit

Streptavidin/Biotin Blocking Kit (SP-2002) blocks all endogenous biotin, biotin receptors, and streptavidin binding sites present in tissues. This kit is designed for use with biotin/streptavidin detection systems such as the VECTASTAIN® Universal *Quick* kits if streptavidin or biotinylated reagents bind non-specifically to tissues or proteins. This blocking kit consists of 18 ml of streptavidin and 18 ml of biotin in convenient dropper bottles.

Normal Sera

Our Normal Sera (S-1000, S-2000, S-3000, S-4000 and S-5000) 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 are supplied undiluted with 0.08% sodium azide as a preservative. When diluted, this reagent can be used for blocking non-specific binding or as an antibody diluent.

2.5% Normal Horse Serum

Our 2.5% Normal Horse Serum (S-2012) is a pooled sample collected from healthy adult animals. The serum is heat-treated and centrifuged to remove precipitates and then filtered. This serum is tested with the appropriate antibody to check for possible cross-reactivities. This product is a 2.5% solution (v/v) in buffer containing 0.08% sodium azide. This is the same serum preparation that is included in the ImmPRESS™ Detection Systems and the R.T.U. VECTASTAIN® ABC Kits. This reagent can be used for blocking non-specific binding or as an antibody diluent.

Bovine Serum Albumin (BSA)

Immunohistochemical Grade

This ultrapure grade of bovine serum albumin (BSA; SP-5050) can be used as a diluent or a blocking agent. It is free of impurities present in other grades of BSA which can introduce artifacts or increase background staining in immunohistochemical staining, ELISAs, or blot development.

10x Casein Solution

10x Casein Solution (SP-5020) is a general blocking agent for histochemical, nucleic acid, protein blotting, and other applications. It is supplied as 250 ml of a 10x concentrate.

Carbo-Free™ Blocking Solution

Carbo-Free™ Blocking Solution (SP-5040) is a protein-based agent used as a general blocking or diluent solution. Unlike serum, nonfat dry milk, casein, or other common protein-containing blocking agents, this product is essentially free of glycoproteins. This solution is ideal for applications using lectins in which glycoprotein contamination could generate background staining or false positive results. It is supplied as 125 ml of a 10x concentrate.

Animal-Free Blocker™

Animal-Free Blocker™ (SP-5030) is a plant-derived blocking agent and diluent for immunohistochemistry, nucleic acid, protein blotting, and for 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. It is supplied as 250 ml of a 5x concentrate.

Blocking Reagents

Product	Catalog Number	Unit Size
BLOXALL™ Blocking Solution	SP-6000	100 ml
Levamisole Solution	SP-5000	18 ml
Avidin/Biotin Blocking Kit	SP-2001	1 kit
Streptavidin/Biotin Blocking Kit	SP-2002	1 kit
Normal Goat Serum	S-1000	20 ml
Normal Horse Serum	S-2000	20 ml
Normal Chicken Serum	S-3000	20 ml
Normal Swine Serum	S-4000	20 ml
Normal Rabbit Serum	S-5000	20 ml
2.5 % Normal Horse Serum	S-2012	50 ml
Bovine Serum Albumin (BSA)	SP-5050	500 mg
10x Casein Solution	SP-5020	250 ml
Carbo-Free™ Blocking Solution	SP-5040	125 ml
Animal -Free Blocker™	SP-5030	250 ml

Enzyme Conjugated Secondary Antibodies

Our high affinity antibodies are purified by affinity chromatography and adsorbed to remove any cross-reactivities that are likely to interfere with specific labeling. These antibodies are then cross-linked with the highest specific activity alkaline phosphatase or horseradish peroxidase. A proprietary procedure for conjugation allows the maximum preservation of enzyme activity and antibody specificity. These antibodies are subjected to rigorous quality control assays and can be used for tissue staining, ELISAs, and blots.

For maximum sensitivity and convenience, see also the one-step ImmPRESS™ Peroxidase Polymer Reagents (pages A12-A15).

Enzyme Conjugated Secondary Antibodies

Product	Catalog Number	Unit Size
Alkaline Phosphatase		
Anti-Mouse IgG (H+L) made in horse Alkaline Phosphatase labeled	AP-2000	1.0 ml
Anti-Rabbit IgG (H+L) made in goat Alkaline Phosphatase labeled	AP-1000	1.0 ml
Anti-Human IgG (H+L) made in goat Alkaline Phosphatase labeled	AP-3000	1.0 ml
Anti-Goat IgG H+L) made in horse Alkaline Phosphatase labeled	AP-9500	1.0 ml
Peroxidase		
Anti-Mouse IgG (H+L) made in horse Peroxidase labeled	PI-2000	1.0 mg
Anti-Rabbit IgG (H+L) made in goat Peroxidase labeled	PI-1000	1.0 mg
Anti-Human IgG (H+L) made in goat Peroxidase labeled	PI-3000	1.0 mg
Anti-Goat IgG (H+L) made in horse Peroxidase labeled	PI-9500	1.0 mg

Avidin and Streptavidin Enzyme Conjugates

Avidin and streptavidin reagents are powerful tools to detect or purify biotinylated proteins, nucleic acids, and other macromolecules.

Vector Laboratories' enzyme-conjugated avidin and streptavidin are produced with the highest specific activity enzymes in optimal ratios. Specific covalent linkages are chosen to provide stable, highly active conjugates.

These enzyme conjugates are suitable for use in solid-phase assays, tissue/cell staining systems, and blotting applications.

Enzyme Conjugated Avidin and Streptavidin

Product	Catalog Number	Unit Size
Alkaline Phosphatase		
Alkaline Phosphatase Streptavidin	SA-5100	1 ml
Alkaline Phosphatase Avidin D[a]	A-2100	100 U
Peroxidase		
Horseradish Peroxidase Streptavidin, concentrate	SA-5004	1 mg
Horseradish Peroxidase Streptavidin, R.T.U.	SA-5704	100 ml
Horseradish Peroxidase Avidin D, concentrate	A-2004	5 mg
Horseradish Peroxidase Avidin D, R.T.U.	A-2704	100 ml
Beta-Galactosidase		
Beta-Galactosidase Avidin D[b]	A-2300	100 U
Glucose Oxidase		
Glucose Oxidase Avidin D	A-2200	5 mg

[a] *One unit is 1 µmole p-nitrophenylphosphate hydrolyzed per min. at 37 ºC, pH 9.8.*

[b] *One unit is 1 µmole o-nitrophenyl-ß-D-galactopyranoside hydrolyzed per min. at 37 ºC, pH 7.3.*

Biotinylated Secondary Antibodies

Vector Laboratories' affinity-purified antibodies are of unmatched quality. These antibodies are prepared using proprietary immunization schedules that produce high affinity antibodies. The antibodies are then purified by affinity chromatography, and cross-reactivities that are likely to interfere with specific labeling are removed by solid phase adsorption techniques. The biotinylated secondary antibodies are conjugated to ensure the maximum degree of labeling without compromising the specificity or affinity of the antibody. These antibodies are subjected to rigorous quality control assays and can be used for tissue and cell staining, ELISAs, and blots.

Most of these antibodies are supplied in lyophilized form and can be reconstituted with 1 ml water. With some exceptions the recommended dilution for most applications is 1:200.

(H+L) indicates the antibody recognizes both heavy and light chains.

Product	Catalog Number	Unit Size
Anti-Cat IgG (H+L) biotinylated, made in goat	BA-9000	1.5 mg
Anti-Chicken IgG (H+L) biotinylated, made in goat	BA-9010	1.5 mg
Anti-Goat IgG (H+L) *a, d* biotinylated, made in rabbit	BA-5000	1.5 mg
Anti-Goat IgG (H+L) *a* biotinylated, made in horse	BA-9500	1.5 mg
Anti-Guinea Pig IgG (H+L) *d* biotinylated, made in goat	BA-7000	1.5 mg
Anti-Hamster IgG (H+L) biotinylated, made in goat	BA-9100	1.5 mg
Anti-Horse IgG (H+L) biotinylated, made in goat	BA-8000	1.5 mg
Anti-Human IgG (H+L) *d* biotinylated, made in goat	BA-3000	1.5 mg
Anti-Human IgA, biotinylated, α chain specific, made in goat	BA-3030	0.5 mg
Anti-Human IgE, biotinylated, ε chain specific, made in goat	BA-3040	0.5 mg
Anti-Human IgG, biotinylated, γ chain specific, made in goat	BA-3080	0.5 mg
Anti-Human IgM, biotinylated, μ chain specific, made in goat	BA-3020	0.5 mg
Anti-Human Kappa Chain biotinylated, κ chain specific, made in goat	BA-3060	0.5 mg
Anti-Human Lambda Chain biotinylated, λ chain specific, made in goat	BA-3070	0.5 mg

Product	Catalog Number	Unit Size
Anti-Mouse IgG (H+L) *d* biotinylated, made in horse	BA-2000	1.5 mg
Anti-Mouse IgG (H+L) *b* biotinylated, rat adsorbed, made in horse	BA-2001	0.5 mg
Anti-Mouse IgG (H+L) biotinylated, made in goat	BA-9200	1.5 mg
Anti-Mouse IgM *d*, biotinylated, μ chain specific, made in goat	BA-2020	0.5 mg
Anti-Rabbit IgG (H+L) *d* biotinylated, made in goat	BA-1000	1.5 mg
Anti-Rabbit IgG (H+L) biotinylated, made in horse	BA-1100	1.5 mg
Anti-Rat IgG (H+L) *d* biotinylated, made in rabbit	BA-4000	1.5 mg
Anti-Rat IgG (H+L) *c*, biotinylated, mouse adsorbed, made in rabbit	BA-4001	0.5 mg
Anti-Rat IgG (H+L) biotinylated, made in goat	BA-9400	1.5 mg
Anti-Rat IgG (H+L) *c*, biotinylated, mouse adsorbed, made in goat	BA-9401	0.5 mg
Anti-Sheep IgG (H+L) *a, d* biotinylated, made in rabbit	BA-6000	1.5 mg
Anti-Swine IgG (H+L) biotinylated, made in goat	BA-9020	1.5 mg
Universal Anti-Mouse/Rabbit IgG (H+L) *d, e* biotinylated, made in horse	BA-1400	2.1 mg
Universal Pan-Specific Anti-Mouse/ Rabbit/Goat IgG (H+L) *f, g* biotinylated, made in horse	BA-1300	2.2 ml

a - Suitable for use with goat, sheep, and bovine IgG primary antibodies.

b - Designed for use in rat tissues.

c - Designed for use in mouse tissues.

d - Antibodies included in VECTASTAIN® ABC Kits.

e - Universal Anti-Mouse/Rabbit IgG (BA-1400) should be reconstituted with 2 ml water and diluted 1:50 for use. Do not use the Universal antibody to stain rodent or rabbit tissue because of cross reactivity with endogenous IgG.

f - Universal Pan-Specific Anti-Mouse/Rabbit/Goat IgG (BA-1300) should be diluted 1:20. Do not use this Pan-Specific antibody to stain rodent, rabbit, goat, sheep, or bovine tissue because of cross reactivity with endogenous IgG.

g - Antibody used in the VECTASTAIN® Universal Quick Kits.

Accessory Reagents

ImmEdge™ Hydrophobic Barrier Pen

The ImmEdge™ Pen (H-4000) is a hydrophobic barrier pen for immunohistochemistry and *in situ* hybridization. It is designed to provide a heat-stable, water-repellent barrier that keeps reagents localized on tissue specimens and prevents mixing of reagents when multiple sections are mounted on the same slide.

The pale blue barrier is insoluble in alcohol and acetone but is completely removed by all commonly used xylene and "xylene substitute" clearing agents. The ImmEdge™ Pen contains no ozone-depleting solvents and is compatible with enzyme or fluorescence-based detection systems.

ImmPrint™ Histology Pen

The ImmPrint™ Histology Pen (H-6100) is a solvent-resistant permanent marking pen designed for writing on glass microscope slides, tissue cassettes, and most hard surfaces. The high density, fast-drying, black ink is resistant to most organic solvents encountered in histological applications such as alcohols and clearing agents. Unlike other pens commonly used for histology, the ImmPrint™ pen has a smooth writing tip that resists drying out.

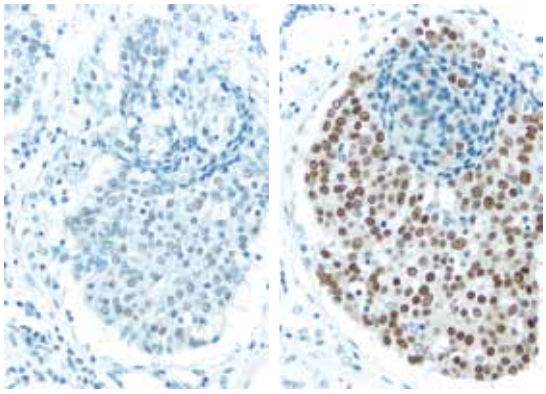
Control Antibodies

These IgG preparations (I-1000, I-2000, I-4000, and I-5000) are intended for use as controls for primary antibodies made in rabbit, mouse, rat, or goat. Supplied as lyophilized powders, these antibodies have been purified from pooled serum of healthy adult animals and contain a spectrum of the IgG subclasses present in serum. They should be applied to the control tissue section at the same concentrations as the primary antibody. These control sections will help determine whether staining with the primary antibody is specific for the antigen or whether staining is the result of nonspecific adsorption of primary antibody to tissue sites.

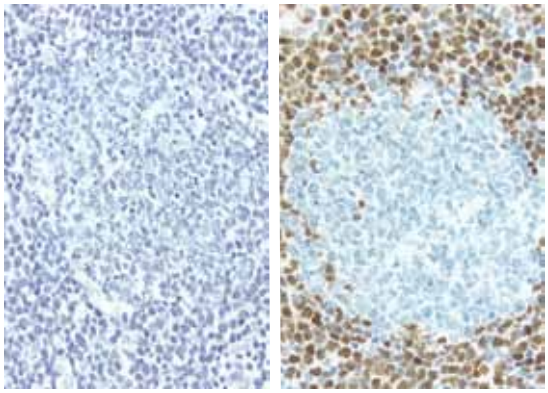
Product	Catalog Number	Unit Size
ImmEdge™ Pen	H-4000	2-pen set
ImmPrint™ Histology Pen	H-6100	5-pen set
Control Antibodies		
Rabbit IgG	I-1000	5 mg
Mouse IgG	I-2000	1 mg
Rat IgG	I-4000	1 mg
Goat IgG	I-5000	5 mg
Antigen Unmasking Solution, Citrate-based	H-3300	250 ml
Antigen Unmasking Solution, Tris-based	H-3301	250 ml

Antigen Unmasking Solutions

Vector Laboratories' 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. These Antigen Unmasking Solutions are available in two formulations. The citrate-based solution (H-3300) is pH 6.0; the Tris-based solution (H-3301) is pH 9.0. Both solutions are supplied as convenient 100x concentrated stocks, sufficient for preparation of 25 L of working solution.



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



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

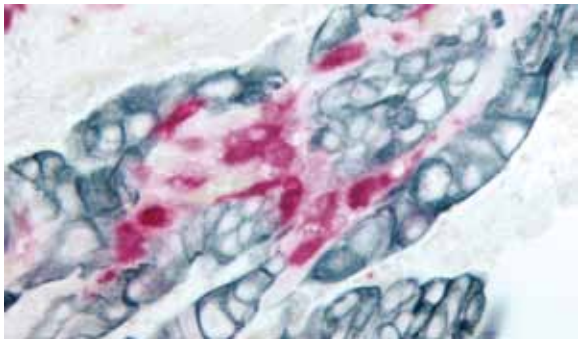
VectaMount™ Mounting Media

Permanent Mounting Medium

VectaMount™

- Permanent, non-aqueous mounting
- Toluene- and xylene-free
- Low hazard
- Odorless
- Compatible with horseradish peroxidase, alkaline phosphatase, and glucose oxidase substrates
- Dries with an ideal refractive index

VectaMount™ Mounting Medium (H-5000) is an optically clear and odorless formula for permanently preserving histochemical stains or precipitable enzyme substrates in tissue sections or cell preparations. ***VectaMount***™ Mounting Medium contains no toluene or xylene. It has a viscosity which provides for easy application and uniform spreading over the tissue section. Mounted sections are clear with an ideal refractive index suitable for high resolution oil immersion microscopy. ***VectaMount***™ Mounting Medium preserves the color and intensity of preparations stained with enzyme substrates such as DAB, TMB, and BCIP/NBT, as well as our proprietary substrates Vector® NovaRED™, Vector® VIP, Vector® SG, ImmPACT™ DAB, ImmPACT™ NovaRED™, ImmPACT™ VIP, ImmPACT™ SG, Vector® Red, Vector® Blue, and Vector® Black. The crystal formation that frequently occurs with the alkaline phosphatase substrate BCIP/NBT using other permanent mounting media is essentially eliminated.



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

Aqueous Mounting Medium

VectaMount™ AQ

- Aqueous mounting
- Compatible with many horseradish peroxidase and alkaline phosphatase substrates

VectaMount™ **AQ** Mounting Medium (H-5501) is a hard-setting mounting medium developed for use with enzymatic substrates, such as AEC, ImmPACT™ AEC, and ImmPACT™ AMEC *Red*, whose reaction products are soluble in alcohol or other organic solvents. In applications where aqueous mounting is preferred, ***VectaMount***™ **AQ** is suitable for use with other substrates such as DAB, Vector® SG, BCIP/NBT, Vector® Red, Vector® Blue, ImmPACT™ DAB, and ImmPACT™ SG. ***VectaMount***™ **AQ** is simple to use, requires no mixing, and preserves the color and clarity of the substrates. Stained sections mounted with ***VectaMount***™ **AQ** can be stored in a slide box at room temperature for at least 2 years without fading.

Tissue Section Adhesive

VECTABOND™ Reagent

VECTABOND™ Reagent (SP-1800) is designed to significantly increase the adherence of both frozen and paraffin-embedded tissue sections and cell preparations to glass slides and coverslips. Tissue sections will remain attached even when subjected to the most extreme conditions, such as high temperature antigen unmasking techniques and *in situ* hybridization. VECTABOND™ Reagent treated slides can be stored for long periods. This product chemically modifies the glass to form a highly adherent surface. VECTABOND™ Reagent is provided as 7 ml of concentrate that dilutes to 350 ml of treatment solution, sufficient for at least 500 standard slides.

Product	Catalog Number	Unit Size
VectaMount™ Permanent Mounting Medium	H-5000	60 ml
VectaMount™ **AQ** Aqueous Mounting Medium	H-5501	60 ml
VECTABOND™ Reagent (Tissue Section Adhesive)	SP-1800	7 ml

IMMUNOFLUORESCENCE

Introduction

With over 35 years of research, development, and manufacturing experience Vector Laboratories has acquired considerable expertise in the production of immunofluorescence reagents. Our extensive range of fluorescent reagents accommodates a variety of experimental designs and levels of signal amplification.

Fluorochrome-Labeled Secondary Antibodies. Our affinity purified antibodies are unmatched in quality for immunological techniques. All antibodies are prepared using proprietary immunization schedules that produce high affinity antibodies. The antibodies are purified by affinity chromatography, and cross-reactivities that are likely to interfere with specific labeling are removed by solid phase adsorption techniques. Antibodies are conjugated to ensure the optimal degree of labeling while not compromising the specificity or affinity of the antibody. See Figure 1.

Fluorochrome-Labeled Streptavidin and Avidin Systems. Fluorescent signals can be amplified using our biotinylated secondary antibodies followed by our highly purified fluorescent streptavidin and avidin conjugates. These fluorescent conjugates possess very low non-specific binding properties and extremely high affinity for biotin. Using a biotin/avidin or biotin/streptavidin detection system results in an additional layer of amplification over a directly conjugated secondary antibody. See Figure 2.

Amplification of Fluorescent Signal using Biotinylated Anti-Streptavidin or Biotinylated Anti-Avidin. Biotinylated Anti-Streptavidin and Biotinylated Anti-Avidin antibodies provide an ideal method to further increase sensitivity. These antibodies bind to streptavidin or avidin, respectively, through both their antigen binding sites and also through the covalently attached biotin residues. After the first application of a fluorochrome-labeled streptavidin or avidin, the signal is amplified by incubation with Biotinylated Anti-Streptavidin or a Biotinylated Anti-Avidin antibody followed by a second incubation with fluorochrome-labeled streptavidin or avidin. This procedure results in the introduction of more fluorochromes at the target site. See Figure 3.

The figures below illustrate different experimental setups used to achieve increasing levels of sensitivity.

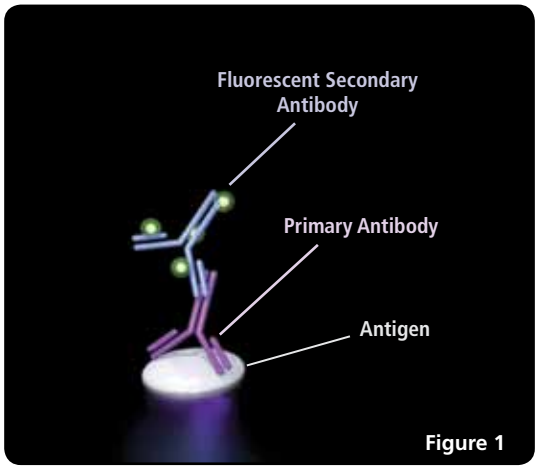


Figure 1

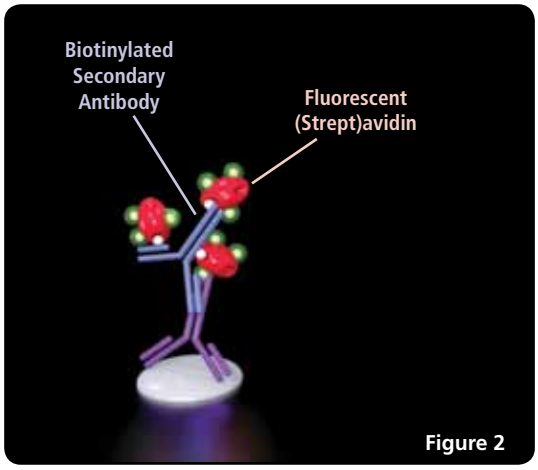


Figure 2

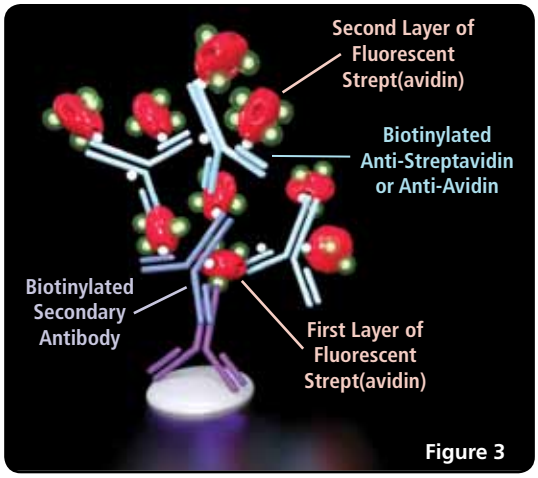


Figure 3

Choice of Fluorophores. We offer secondary antibodies and avidin and streptavidin conjugated to traditional fluorochromes such as fluorescein, rhodamine, Texas Red®, AMCA, phycoerythrin – as well as the newer DyLight® dyes.

Fluorochromes	Color	Excitation Max (nm)	Emission Max (nm)
AMCA	Blue	350	450
DyLight® 488	Green	493	518
Fluorescein	Green	495	515
Rhodamine	Red	550	575
DyLight® 549	Orange	556	571
Phycoerythrin (PE)	Red-Orange	565	574
$Rhodamine_{600}$	Red	575	600
DyLight® 594	Red	592	617
Texas Red®	Red	595	615
DyLight® 649	Far Red	655	670

Species Cross-Reactivity Considerations. It is important to consider not only the species of the primary antibody but also the species of the tissue when choosing the optimal detection system for your application. If the species of the primary antibody and the species of the tissue are closely related (e.g. rat and mouse), the secondary antibody may bind to endogenous immunoglobulins in the tissue section leading to background. The following are options to minimize background staining in these instances:

- Use directly labeled primary antibodies. They can be labeled with either fluorescein (ProtOn™ Fluorescein Labeling Kit, PLK-1201, page F9) or with biotin (ProtOn™ Biotin Labeling Kit, PLK-1202, page F9). Biotinylated primary antibodies can be detected with a flurochrome-labeled avidin or streptavidin.

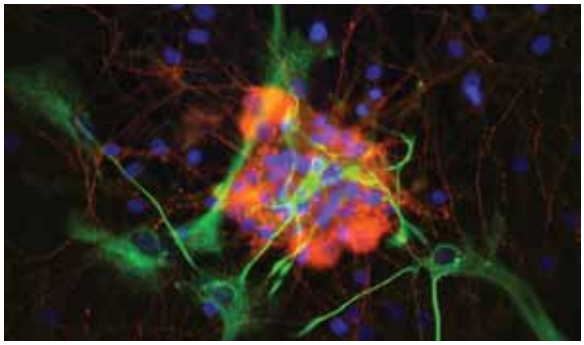
- Use a secondary antibody specifically adsorbed to remove cross-reacting antibodies of closely-related species (e.g. fluorescein conjugated anti-mouse IgG, rat adsorbed, FI-2001, page G28-G29).

- Use the M.O.M.™ Immunodetection System for applications of mouse primary antibodies on mouse tissue (see pages B8).

Multiple Antigen Labeling. Our affinity purified, highly-adsorbed secondary antibodies and our avidin and streptavidin conjugates allow specific and crisp labeling of multiple antigens in the same section, as well as co-localization of antigens in the same cellular compartment of a section. When choosing detection systems for double antigen labeling, the species of each primary and secondary antibody, as well as the tissue species, must be considered.

If the two primary antibodies are both made in mouse, directly label and detect the second mouse primary antibody, or use the M.O.M.™ Immunodetection System. The M.O.M™ system contains a proprietary Mouse Ig Blocking Reagent and a specially modified biotinylated anti-mouse secondary antibody. Together, these reagents minimize cross-reactivity of the detection system for endogenous mouse IgG. Used in a double-label application with two mouse primaries, this system will minimize cross-reactivity of the second detection system with the first, resulting in specific and discreet staining of the two antigens. For more information, request or download our free brochure on multiple antigen labeling, "Discovery Through Color".

VECTASHIELD® Mounting Media. VECTASHIELD® Mounting Media are unsurpassed in preventing photobleaching. These ready-to-use mounting media are stored at 4 °C, available in non-hardening and hardening versions, and with and without nuclear counterstains (see page B9).



Dorsal root ganglia cells (neurons and satellite glia) and astrocytes: • βIII Tubulin (m), DyLight® 549 anti-mouse IgG (red) • GFAP (rp), DyLight® 488 anti-rabbit IgG (green). Mounted in VECTASHIELD® Hard•Set™ Mounting Medium with DAPI. (Image courtesy of Dr. Emma East, Department of Life Sciences, The Open University, U.K.)

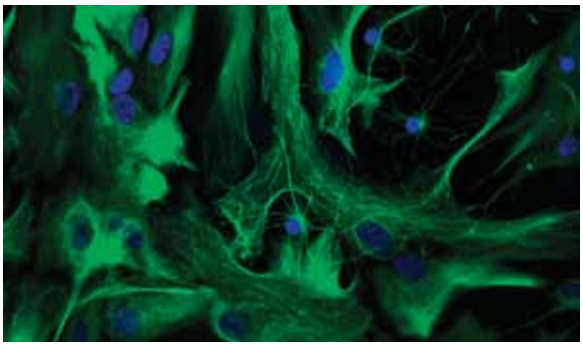
Fluorochrome-Labeled Secondary Antibodies

Our affinity purified antibodies are of unmatched quality for use in immunological techniques. All antibodies are prepared using proprietary immunization schedules that produce high affinity antibodies. The antibodies are then purified by affinity chromatography, and cross-reactivities that are likely to interfere with specific labeling are removed by solid phase adsorption techniques. Antibodies are conjugated in a manner that ensures the maximum degree of labeling without compromising the specificity or affinity of the antibody.

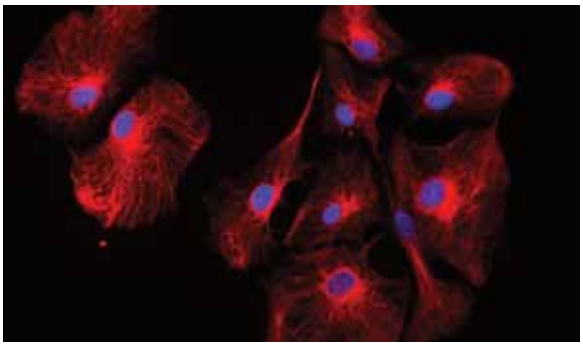
Vector Laboratories produces fluorochrome-labeled secondary antibodies conjugated using traditional fluors – fluorescein, rhodamine, Texas Red®, AMCA, phycoerythrin – as well as the newer DyLight® dyes.

DyLight® dyes offer several advantages including greater photostability, pH independence and brighter fluorescence. Vector Laboratories offers DyLight® conjugated anti-mouse IgG and anti-rabbit IgG antibodies for use in a variety of applications, in particular, cell- and tissue-based immunofluorescent antigen detection/visualization. The DyLight® conjugates are completely stable from pH 4 to pH 9, making them compatible with many buffers and diluents.

Phycoerythrin is a fluorescent protein that produces a bright red-orange fluorescence. Our newly improved Phycoerythrin conjugates are substantially brighter than previous conjugates. Phycoerythrin-conjugated antibodies can be used in cell sorting and in tissue staining.



Astrocytes: GFAP (rp), DyLight® 488 anti-rabbit IgG, mounted in VECTASHIELD® HardSet™ Mounting Medium with DAPI. (Image courtesy of Dr. Emma East, Department of Life Sciences, The Open University, U.K.)



Astrocytes: GFAP (rp), DyLight® 594 anti-rabbit IgG, mounted in VECTASHIELD® HardSet™ Mounting Medium with DAPI. (Image courtesy of Dr. Emma East, Department of Life Sciences, The Open University, U.K.)

Secondary Antibodies

Unless otherwise specified, our antibodies will recognize both heavy and light chains (H+L).

Antibody Description	Conjugate	Color (Ex/Em)	Catalog Number	Unit Size	Stock Concentration	Recommended Dilution*
Mouse						
Anti-Mouse IgG (H+L), made in horse	AMCA	Blue (350/450)	CI-2000	1.5 mg	1.5 mg/ml	1:150
	DyLight® 488	Green (493/518)	DI-2488	1.5 mg	1.5 mg/ml	1:150
	Fluorescein	Green (495/515)	FI-2000	1.5 mg	1.5 mg/ml	1:150
	DyLight® 549	Orange (556/571)	DI-2549	1.5 mg	1.5 mg/ml	1:150
	R-Phycoerythrin	Red-Orange (565/574)	EI-2007	1.0 mg	1.0 mg/ml	1:100
	DyLight® 594	Red (592/617)	DI-2594	1.5 mg	1.5 mg/ml	1:150
	Texas Red®	Red (595/615)	TI-2000	1.5 mg	1.5 mg/ml	1:150
	DyLight® 649	Far Red (655/670)	DI-2649	1.5 mg	1.5 mg/ml	1:150
Anti-Mouse IgG Kit, made in horse - 500 µg AMCA Anti-Mouse IgG - 500 µg Fluorescein Anti-Mouse IgG - 500 µg Texas Red® Anti-Mouse IgG	AMCA, Fluorescein, Texas Red®	Blue (350/450) Green (495/515) Red (595/615)	FI-2100	1 kit	0.5 mg/ml 0.5 mg/ml 0.5 mg/ml	1:50 1:50 1:50
Anti-Mouse IgG (H+L), rat adsorbed, made in horse	Fluorescein	Green (495/515)	FI-2001	0.5 mg	1.0 mg/ml	1:50
Anti-Mouse IgM, mu chain specific, made in goat	AMCA	Blue (350/450)	CI-2020	0.5 mg	0.5 mg/ml	1:25
	Fluorescein	Green (495/515)	FI-2020	0.5 mg	1.0 mg/ml	1:100
	Texas Red®	Red (595/615)	TI-2020	0.5 mg	1.0 mg/ml	1:100

** The dilution factors shown are recommended for most applications.*

Antibody Description	Conjugate	Color (Ex/Em)	Catalog Number	Unit Size	Stock Concentration	Recommended Dilution*
Rabbit						
Anti-Rabbit IgG (H+L), made in goat	AMCA	Blue (350/450)	CI-1000	1.5 mg	1.5 mg/ml	1:150
	DyLight® 488	Green (493/518)	DI-1488	1.5 mg	1.5 mg/ml	1:150
	Fluorescein	Green (495/515)	FI-1000	1.5 mg	1.5 mg/ml	1:150
	DyLight® 549	Orange (556/571)	DI-1549	1.5 mg	1.5 mg/ml	1:150
	R-Phycoerythrin	Red-Orange (565/574)	EI-1007	1.0 mg	1.0 mg/ml	1:100
	DyLight® 594	Red (592/617)	DI-1594	1.5 mg	1.5 mg/ml	1:150
	Texas Red®	Red (595/615)	TI-1000	1.5 mg	1.5 mg/ml	1:150
	DyLight® 649	Far Red (655/670)	DI-1649	1.5 mg	1.5 mg/ml	1:150
Anti-Rabbit IgG Kit, made in goat - 500 µg AMCA Anti-Rabbit IgG - 500 µg Fluorescein Anti-Rabbit IgG - 500 µg Texas Red® Anti-Rabbit IgG	AMCA, Fluorescein, Texas Red®	Blue (350/450) Green (495/515) Red (595/615)	FI-1200	1 kit	0.5 mg/ml 0.5 mg/ml 0.5 mg/ml	1:50 1:50 1:50
Human						
Anti-Human IgG (H+L), made in goat	AMCA	Blue (350/450)	CI-3000	1.5 mg	1.5 mg/ml	1:150
	Fluorescein	Green (495/515)	FI-3000	1.5 mg	1.5 mg/ml	1:150
	Texas Red®	Red (595/615)	TI-3000	1.5 mg	1.5 mg/ml	1:150
Anti-Human IgE, epsilon chain specific, made in goat	Fluorescein	Green (495/515)	FI-3040	0.5 mg	1.0 mg/ml	1:100
Anti-Human IgG, gamma chain specific, made in goat	Fluorescein	Green (495/515)	FI-3080	0.5 mg	1.0 mg/ml	1:100
Anti-Human IgM, mu chain specific, made in goat	Fluorescein	Green (495/515)	FI-3020	0.5 mg	1.0 mg/ml	1:100
Anti-Human Kappa Chain, made in goat	AMCA	Blue (350/450)	CI-3060	0.5 mg	1.0 mg/ml	1:50
	Fluorescein	Green (495/515)	FI-3060	0.5 mg	1.0 mg/ml	1:100
Anti-Human Lambda Chain, made in goat	AMCA	Blue (350/450)	CI-3070	0.5 mg	1.0 mg/ml	1:50
	Fluorescein	Green (495/515)	FI-3070	0.5 mg	1.0 mg/ml	1:100
Rat						
Anti-Rat IgG (H+L), made in rabbit	Fluorescein	Green (495/515)	FI-4000	1.5 mg	1.5 mg/ml	1:150
Anti-Rat IgG (H+L), mouse adsorbed, made in rabbit	Fluorescein	Green (495/515)	FI-4001	0.5 mg	1.0 mg/ml	1:100
Anti-Rat IgG, made in goat	Texas Red®	Red (595/615)	TI-9400	1.5 mg	1.5 mg/ml	1:150
Goat						
Anti-Goat IgG (H+L), made in rabbit	AMCA	Blue (350/450)	CI-5000	1.5 mg	1.5 mg/ml	1:150
	Fluorescein	Green (495/515)	FI-5000	1.5 mg	1.5 mg/ml	1:150
	Texas Red®	Red (595/615)	TI-5000	1.5 mg	1.5 mg/ml	1:150
Sheep						
Anti-Sheep IgG (H+L), made in rabbit	Fluorescein	Green (495/515)	FI-6000	1.5 mg	1.5 mg/ml	1:150
	Texas Red®	Red (595/615)	TI-6000	1.5 mg	1.5 mg/ml	1:150
Hamster						
Anti-Hamster IgG (H+L), made in goat	Fluorescein	Green (495/515)	FI-9100	1.5 mg	1.5 mg/ml	1:150
	Texas Red®	Red (595/615)	TI-9100	1.5 mg	1.5 mg/ml	1:150
Guinea Pig						
Anti-Guinea Pig IgG (H+L), made in goat	Fluorescein	Green (495/515)	FI-7000	1.5 mg	1.5 mg/ml	1:150
	Texas Red®	Red (595/615)	TI-7000	1.5 mg	1.5 mg/ml	1:150

** The dilution factors shown are recommended for most applications.*

Fluorochrome-Labeled Biotin-Streptavidin/Avidin Systems

Vector Laboratories' fluorochrome-conjugated streptavidin and avidin reagents are highly purified and possess very low non-specific binding properties. They have extremely high affinity for biotin. These fluorescent conjugates can be used to detect biotinylated secondary antibodies and other macromolecules in applications such as immunofluorescence, *in situ* hybridization, or flow cytometry.

Amplification of fluorescent signals can be easily achieved with our biotinylated secondary antibodies followed by our highly purified fluorochrome-labeled streptavidin or avidin. Using a biotin/avidin or biotin/streptavidin detection system results in an additional layer of amplification over a directly conjugated secondary antibody.

Our streptavidin and avidin are conjugated using traditional fluors such as AMCA, fluorescein, phycoerythrin, Texas Red®, as well as the new DyLight® dyes.

DyLight® dyes offer several advantages including greater photostability, pH independence, and brighter fluorescence. We offer DyLight® conjugated streptavidin for use in a variety of applications, in particular, cell- and tissue-based immunofluorescent detection. The DyLight® conjugates are completely stable from pH 4 to pH 9, making them compatible with many aqueous-based buffers and diluents.

Phycoerythrin is a protein that produces a bright red-orange fluorescence. Our newly improved Phycoerythrin Streptavidin (SA-5207) is substantially brighter than previous conjugates. Phycoerythrin Streptavidin can be used on gene chips, in cell sorting, and in tissue staining.

A complete listing of biotin-labeled reagents and further descriptions of streptavidin and different "forms" of avidin can be found in Section H, "Biotin and Avidin/Streptavidin Reagents" pages H7-H8.

Fluorochrome-Labeled Streptavidin Conjugates

Product Description	Color (Ex/Em)	Catalog Number	Unit Size	Stock Concentration	Recommended Dilution*
AMCA Streptavidin	Blue (350/450)	SA-5008	1 mg	1 mg/ml	1:100
DyLight® 488 Streptavidin	Green (493/518)	SA-5488	1 mg	1 mg/ml	1:100
Fluorescein Streptavidin	Green (495/515)	SA-5001	1 mg	1 mg/ml	1:100
DyLight® 549 Streptavidin	Orange (556/571)	SA-5549	1 mg	1 mg/ml	1:100
Phycoerythrin Streptavidin	Orange (565/574)	SA-5207	1 mg	1 mg/ml	1:100
DyLight® 594 Streptavidin	Red (592/617)	SA-5594	1 mg	1 mg/ml	1:100
Texas Red® Streptavidin	Red (595/615)	SA-5006	1 mg	1 mg/ml	1:100
DyLight® 649 Streptavidin	Far Red (655/670)	SA-5649	1 mg	1 mg/ml	1:100
Fluorescent Streptavidin Kit - 250 µg AMCA Streptavidin - 250 µg Fluorescein Streptavidin - 250 µg Texas Red®Streptavidin	 Blue (350/450) Green (495/515) Red (595/615)	SA-1200	1 Kit	 0.5 mg/ml 0.5 mg/ml 0.5 mg/ml	 1:50 1:50 1:50

Fluorochrome-Labeled Avidin Conjugates

Product Description	Color (Ex/Em)	Catalog Number	Unit Size	Stock Concentration	Recommended Dilution*
AMCA Avidin D	Blue (350/450)	A-2008	5 mg	5 mg/ml	1:250
Fluorescein Avidin D	Green (495/515)	A-2001	5 mg	5 mg/ml	1:500
Fluorescein Avidin DN	Green (495/515)	A-3101	1 mg	1 mg/ml	1:100
Fluorescein Avidin DCS	Green (495/515)	A-2011	1 mg	2 mg/ml	1:200
Rhodamine Avidin D	Red (550/575)	A-2002	5 mg	5 mg/ml	1:333
Rhodamine Avidin DCS	Red (550/575)	A-2012	1 mg	2 mg/ml	1:200
$Rhodamine_{600}$ Avidin D	Red (575/600)	A-2005	5 mg	5 mg/ml	1:250
Texas Red® Avidin D	Red (595/615)	A-2006	5 mg	2.5 mg/ml	1:250
Texas Red® Avidin DCS	Red (595/615)	A-2016	1 mg	2 mg/ml	1:200
Fluorescent Avidin Kit - 0.5 mg AMCA Avidin D - 0.5 mg Fluorescein Avidin DCS - 0.5 mg Texas Red® Avidin D	 Blue (350/450) Green (495/515) Red (595/615)	A-1100	1 kit	 0.5 mg/ml 0.5 mg/ml 0.5 mg/ml	 1:25 1:50 1:50

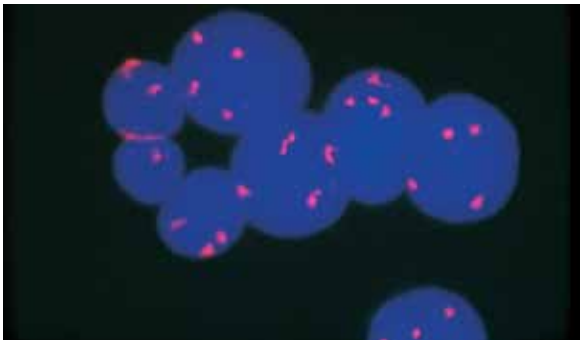
** The dilution factors shown are recommended for most applications.*

Anti-Streptavidin and Anti-Avidin Antibody Reagents

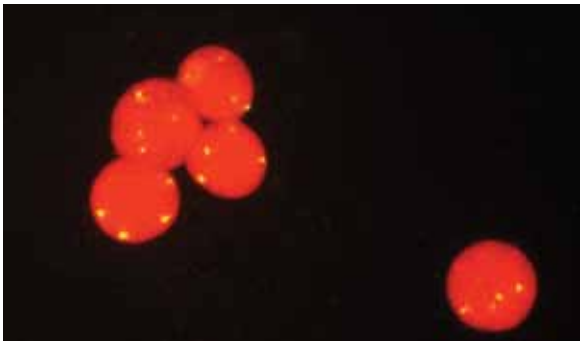
Our antibodies to avidin and streptavidin are produced in goats using our highly purified avidin or streptavidin and isolated by affinity chromatography. Anti-Avidin does not bind streptavidin and Anti-Streptavidin does not recognize avidin. These antibodies provide opportunities to significantly amplify signals in many applications.

Biotinylated Anti-Avidin and Biotinylated Anti-Streptavidin have been widely used as amplifying reagents in immunohistochemistry, *in situ* hybridization, microarray assays, ELISAs, blots, and many other applications. The capability of binding avidin or streptavidin via either biotin binding sites or through antigen binding sites, makes these biotinylated antibodies unique. These antibodies can be used either as part of preformed complexes or in sequence to amplify fluorescent signals. When used in sequence, the target is first labeled with fluorochrome-conjugated avidin or streptavidin, followed by incubation with Biotinylated Anti-Avidin or Biotinylated Anti-Streptavidin, followed by a second layer of fluorochrome-conjugated avidin or streptavidin. This sequence can be repeated. This multi-layered approach introduces more fluorochromes at the target site and can provide a multi-fold amplification over a single layer.

These affinity purified antibodies are also available unconjugated or fluorescein-labeled.



FastTag® Biotin-labeled human chromosome 1 centromere-specific probe detected with Texas Red® Avidin DCS, Biotinylated Anti-Avidin and Texas Red® Avidin DCS (red). Mounted in VECTASHIELD® Mounting Medium with DAPI (blue).



FastTag® Biotin-labeled human chromosome 1 centromere-specific probe detected with Fluorescein Avidin DCS, Biotinylated Anti-Avidin and Fluorescein Avidin DCS (yellow-green). Mounted in VECTASHIELD® Mounting Medium with Propidium Iodide (red).

Anti-Streptavidin Antibodies

Antibody Description	Conjugate	Catalog Number	Unit Size	Stock Concentration	Recommended Dilution*
Anti-Streptavidin, made in goat	Biotin	BA-0500	0.5 mg	0.5 mg/ml	1:100
	Unconjugated	SP-4000	1 mg	2 mg/ml	1:400
	Fluorescein	SP-4040	0.5 mg	1 mg/ml	1:50

Anti-Avidin Antibodies

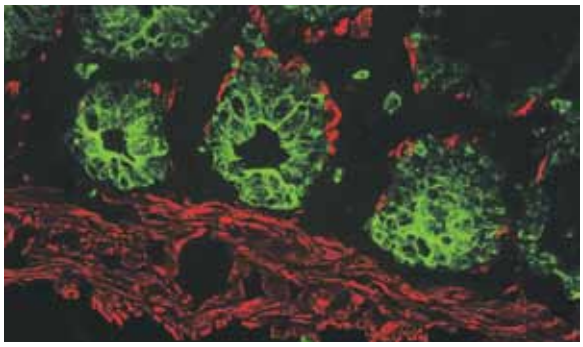
Antibody Description	Conjugate	Catalog Number	Unit Size	Stock Concentration	Recommended Dilution*
Anti-Avidin, made in goat	Biotin	BA-0300	0.5 mg	0.5 mg/ml	1:100
	Unconjugated	SP-2000	1 mg	1 mg/ml	1:200
	Fluorescein	SP-2040	0.5 mg	1 mg/ml	1:50

** The dilution factors shown are recommended for most applications.*

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

The Vector® M.O.M.™ Immunodetection kits are specifically designed to localize mouse primary antibodies on mouse tissues. The major problem with using mouse primary antibodies on mouse tissues is the inability of the anti-mouse secondary antibody to distinguish between the mouse primary antibody and endogenous mouse immunoglobulins in the tissue. This can result in high background staining that obscures specific staining. This problem can be essentially eliminated by using a Vector® M.O.M.™ Immunodetection Kit which includes a proprietary M.O.M.™ Mouse Ig Blocking Reagent and a specialized M.O.M.™ Biotinylated Anti-Mouse Ig Reagent that significantly reduce undesired binding of the secondary antibody to endogenous tissue immunoglobulin.

Using the M.O.M.™ technology, it is now possible to fluorescently detect several mouse primary antibodies on the same tissue section regardless of the species of the tissue. Two or more different fluorescent streptavidin or avidin labels can be introduced using a multiple antigen labeling protocol.



Mouse Colon: • Multi-Cytokeratin (m), M.O.M.™ Fluorescein Kit (green) • Desmin (m), M.O.M.™ Basic Kit, Texas Red® Avidin DCS (red).

Vector® M.O.M.™ Fluorescein Kit

Vector® M.O.M.™ Fluorescein Kit (FMK-2201) includes the following:

- M.O.M.™ Mouse Ig Blocking Reagent
- M.O.M.™ Biotinylated Anti-Mouse Ig Reagent
- M.O.M.™ Protein Concentrate for general protein blocking and diluent
- Fluorescein Avidin DCS (green fluorescence, excitation at 495 nm; emission at 515 nm)

Vector® M.O.M.™ Basic Kit

Vector® M.O.M.™ Basic Kit (BMK-2202) includes the following:

- M.O.M.™ Mouse Ig Blocking Reagent
- M.O.M.™ Biotinylated Anti-Mouse Ig Reagent
- M.O.M.™ Protein Concentrate for general protein blocking and diluent

Vector® M.O.M.™ Basic Kit does not include enzyme or fluorochrome conjugates allowing a choice of any avidin- or streptavidin-based detection system (see page B6).

For chromogenic detection, a M.O.M.™ Peroxidase Kit is available. See Section A, "Immunohistochemistry", pages A16-A17. For additional information on multiple antigen labeling, please request a copy of "Discovery Through Color" brochure.

M.O.M.™ Immunodetection Kits

Product	Catalog Number	Unit Size	Kit Components	Approximate Number of Sections Stained
M.O.M.™ Fluorescein Kit	FMK-2201	1 Kit	6 ml M.O.M.™ Protein Concentrate 1 ml Mouse Ig Blocking Reagent 0.1 ml M.O.M.™ Biotinylated Anti-Mouse Ig Reagent 0.4 ml Fluorescein Avidin DCS	250
M.O.M.™ Basic Kit	BMK-2202	1 Kit	6 ml M.O.M.™ Protein Concentrate 1 ml Mouse Ig Blocking Reagent 0.1 ml M.O.M.™ Biotinylated Anti-Mouse Ig Reagent	250
M.O.M.™ Mouse Ig Blocking Reagent [b]	MKB-2213	1 ml	Mouse Ig Blocking Reagent	-
M.O.M.™ Biotinylated Anti-Mouse Ig Reagent [a, b]	MKB-2225	0.1 ml	M.O.M.™ Biotinylated Anti-Mouse Ig Reagent	-
M.O.M.™ Peroxidase Kit [b]	PK-2200	1 Kit	6 ml M.O.M.™ Protein Concentrate 1 ml Mouse Ig Blocking Reagent 0.1 ml M.O.M.™ Biotinylated Anti-Mouse Ig Reagent VECTASTAIN® Elite® ABC Reagents A (1 ml) and B (1 ml)	250

[a] *This reagent must be used with the M.O.M.™ Mouse Ig Blocking Reagent (MKB-2213). This is not a stand-alone reagent for mouse-on-mouse applications.*
[b] *For more information, see Section A, pages A16-A17.*

VECTASHIELD® Mounting Media for Fluorescence

VECTASHIELD® Mounting Media are unsurpassed in preventing photobleaching. The different formulations of VECTASHIELD® Mounting Media all offer the same outstanding anti-fade and anti-photobleaching properties. They are all compatible with fluorescein, Texas Red®, AMCA, DyLight® dyes, Alexa Fluor® dyes, fluorescent nuclear stains, fluorescent proteins, fluorescent tracers, histochemical stains, and most fluorochromes.

Features of VECTASHIELD® Mounting Media:

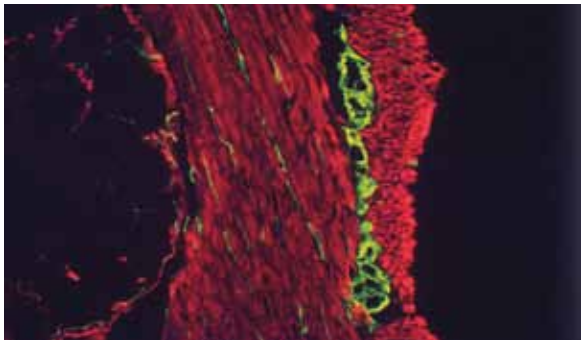
- Inhibit photobleaching of most fluorochromes
- Available in non-hardening or hardening formulations
- Available with or without DAPI or propidium iodide
- No warming necessary
- Slides can be viewed after prolonged storage
- Continues to inhibit photobleaching after prolonged storage of mounted slides
- Easy to use
- Optically clear
- Ideal refractive index

The original **VECTASHIELD® Mounting Medium** is a glycerol-based, aqueous mountant that does not solidify but remains a viscous liquid on the slide. After mounting, coverslipped slides will not readily dry out and can be reviewed for weeks afterwards without sealing. For prolonged storage, coverslips can be permanently sealed around the perimeter with nail polish. Mounted slides should be stored at 4 °C.

VECTASHIELD® Hard✦Set™ Mounting Medium is an aqueous mountant that hardens at room temperature in as little as 20 minutes. This facilitates handling of the slide, eliminates the need to secure the coverslip, and is more convenient for use with oil immersion microscopy. Stained and mounted slides may be stored for several weeks at 4 °C; for prolonged storage, place slides at -20 °C.

Both the VECTASHIELD® Hard✦Set™ and the original VECTASHIELD® Mounting Media are available with or without the counterstain DAPI (4′, 6-diamidino-2-phenylindole). DAPI produces a blue fluorescence when bound to DNA with excitation at about 360 nm and emission at 460 nm. The original VECTASHIELD® Mounting Medium is also available with the counterstain propidium iodide (PI). PI is a reddish nuclear counterstain with a broad excitation range at around 535 nm and emission at about 615 nm when bound to DNA.

See page A31 for other mounting media.



Mouse Intestine: • Peripherin (m), M.O.M.™ Basic Kit, Fluorescein Avidin DCS (green) • Desmin (m), M.O.M.™ Basic Kit, Texas Red® Avidin DCS (red). Mounted in VECTASHIELD® Mounting Medium.

VECTASHIELD® Mounting Media

Product	Counterstain	Cat. Number	Unit Size	Hardening	Refractive Index
VECTASHIELD® Mounting Medium	none	H-1000	10 ml	no	1.44
	DAPI	H-1200	10 ml	no	1.44
	PI	H-1300	10 ml	no	1.44
VECTASHIELD® Hard✦Set™ Mounting Medium	none	H-1400	10 ml	yes	1.36*
	DAPI	H-1500	10 ml	yes	1.36*

**Measured after hardening.*

Accessory Reagents

Control Antibodies

These IgG preparations (I-1000, I-2000, I-4000, and I-5000) are intended for use as controls for primary antibodies made in rabbit, mouse, rat, or goat. Supplied as lyophilized powders, these antibodies have been purified from pooled serum of healthy adult animals and contain a spectrum of the IgG subclasses present in serum. They should be applied to the control tissue section at the same concentrations as the primary antibody. These control sections will help determine whether staining with the primary antibody is specific for the antigen or whether staining is the result of nonspecific adsorption of primary antibody to tissue sites.

VECTABOND™ Reagent

VECTABOND™ Reagent (SP-1800) is designed to significantly enhance the adherence of both frozen and paraffin-embedded tissue sections and cell preparations to glass slides and coverslips. Tissue sections will remain attached even when subjected to the most extreme conditions, such as high temperature antigen unmasking techniques and *in situ* hybridization applications. VECTABOND™ Reagent-treated slides can be stored for long periods. This product chemically modifies the glass to form a highly adherent surface. VECTABOND™ Reagent is provided as a 7 ml concentrate that dilutes to 350 ml of treatment solution, sufficient for at least 500 standard slides.

ImmEdge™ Hydrophobic Barrier Pen

The ImmEdge™ Pen (H-4000) is a hydrophobic barrier pen for immunohistochemistry and *in situ* hybridization. It is designed to provide a heat-stable, water-repellent barrier that keeps reagents localized on tissue specimens and prevents mixing of reagents when multiple sections are mounted on the same slide.

The pale blue barrier is insoluble in alcohol and acetone but is completely removed by all commonly used xylene and "xylene substitute" clearing agents. The ImmEdge™ Pen contains no ozone-depleting solvents and is compatible with enzyme- or fluorescence-based detection systems.

ImmPrint™ Histology Pen

The ImmPrint™ Histology Pen (H-6100) is a solvent-resistant permanent marking pen, designed for writing on glass microscope slides, tissue cassettes, and most hard surfaces. The high density, fast-drying black ink is resistant to most organic solvents encountered in histological applications, such as alcohols or clearing agents. Unlike other pens commonly used for histology, the ImmPrint™ pen has a smooth writing tip that resists drying out.

Antigen Unmasking Solutions

Vector Laboratories' 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. The Antigen Unmasking Solutions are available in two formulations. The citrate-based solution (H-3300) is pH 6.0; the Tris-based solution (H-3301) is pH 9.0. Both solutions are supplied as convenient 100x concentrated stocks, sufficient for preparation of 25 L of working solution.

Accessory Reagents

Product	Catalog Number	Unit Size
Control Antibodies		
Rabbit IgG	I-1000	5 mg
Mouse IgG	I-2000	1 mg
Rat IgG	I-4000	1 mg
Goat IgG	I-5000	5 mg
VECTABOND™ Reagent	SP-1800	7 ml
ImmEdge™ Pen	H-4000	2 pens
ImmPrint™ Histology Pen	H-6100	5 pens
Antigen Unmasking Solution, Citrate-based (100x)	H-3300	250 ml
Antigen Unmasking Solution, Tris-based (100x)	H-3301	250 ml

Blocking Reagents

Normal Sera

Our Normal Sera (S-1000, S-2000, S-3000, S-4000 and S-5000) 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 are supplied undiluted with 0.08% sodium azide as a preservative. When diluted, these reagents can be used for blocking non-specific binding or as an antibody diluent.

2.5% Normal Horse Serum

Our 2.5% Normal Horse Serum (S-2012) is a pooled sample collected from healthy adult animals. The serum is heat-treated, centrifuged, and filtered to remove precipitates. This serum is tested with the appropriate antibody to check for possible cross-reactivity. This product is a 2.5% solution (v/v) in buffer containing 0.08% sodium azide. This is the same serum preparation that is included in the ImmPRESS™ Detection Systems and the R.T.U. VECTASTAIN® ABC Kits. This reagent can be used for blocking non-specific binding or as an antibody diluent.

Bovine Serum Albumin (BSA)

Immunohistochemical Grade

This ultrapure grade of bovine serum albumin (BSA; SP-5050) can be used as a diluent or a blocking agent. It is free of impurities present in other grades of BSA which can introduce artifacts or increase background staining in immunohistochemical staining, ELISAs, or blot development.

10x Casein Solution

10x Casein Solution (SP-5020) is a general blocking agent for immunohistochemical, nucleic acid, protein blotting, and other applications. It is supplied as 250 mls of a 10x concentrate.

Carbo-Free™ Blocking Solution

Carbo-Free™ Blocking Solution (SP-5040) is a protein-based agent intended for use as a general blocking or diluent solution. Unlike serum, nonfat dry milk, casein, or other common protein-containing blocking agents, this product is essentially free of glycoproteins. This solution is ideal for applications using lectins in which glycoprotein contamination could generate background staining or false positive results. It is supplied as 125 ml of a 10x concentrate.

Animal-Free Blocker™

Animal-Free Blocker™ (SP-5030) is a plant-derived blocking agent and diluent for immunohistochemistry, nucleic acid or protein blotting, and for 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. It is supplied as 250 ml of a 5x concentrate.

Avidin/Biotin Blocking Kit

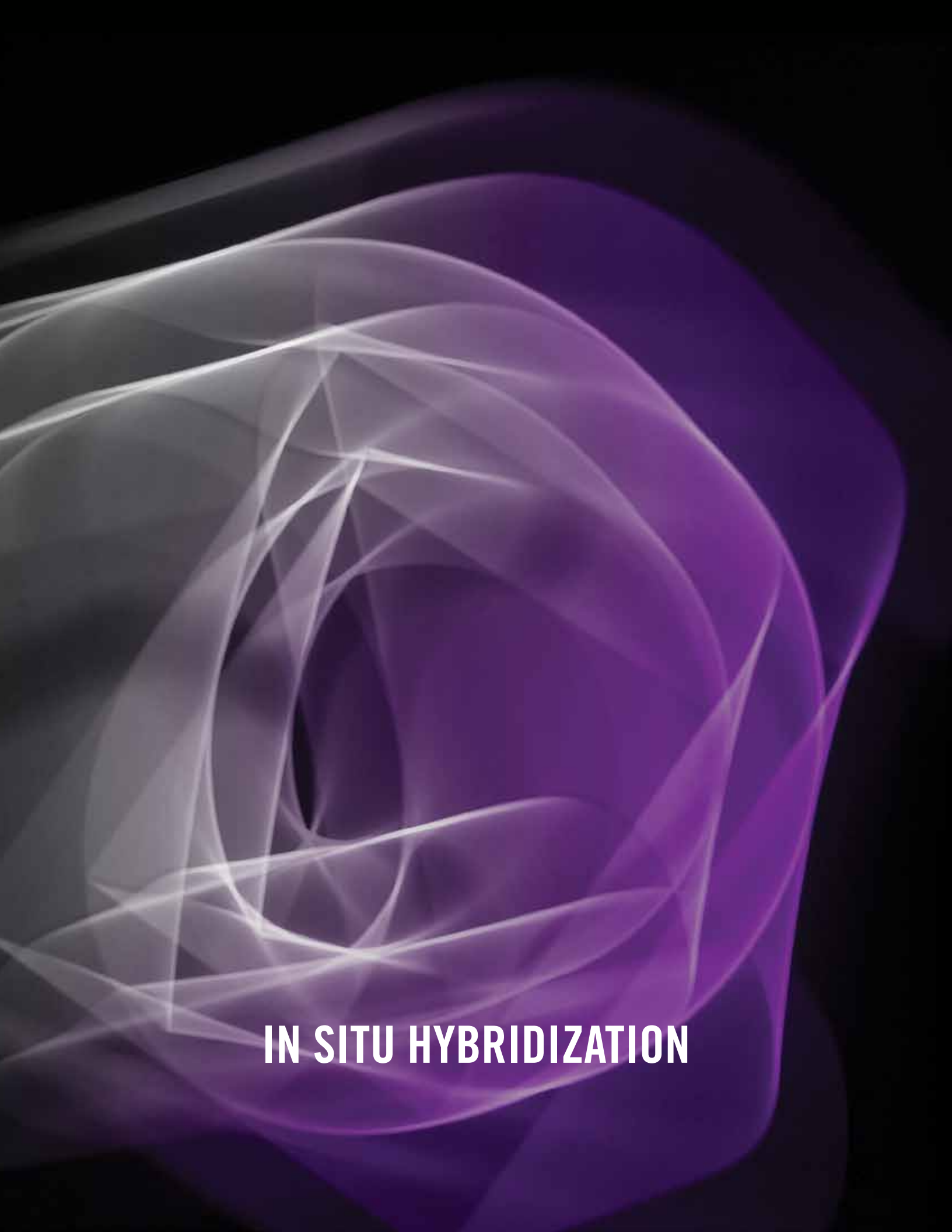
Avidin/Biotin Blocking Kit (SP-2001) blocks all endogenous biotin, biotin receptors, and avidin binding sites present in tissues. This kit is designed for use with biotin/avidin detection systems such as the VECTASTAIN® ABC Kits if avidin or biotinylated reagents bind nonspecifically to tissues or proteins. This blocking kit consists of 18 ml of Avidin D and 18 ml of biotin in convenient dropper bottles.

Streptavidin/Biotin Blocking Kit

Streptavidin/Biotin Blocking Kit (SP-2002) blocks all endogenous biotin, biotin receptors, and streptavidin binding sites present in tissues. This kit is designed for use with biotin/streptavidin detection systems such as the VECTASTAIN® Universal *Quick* kits if streptavidin or biotinylated reagents bind nonspecifically to tissues or proteins. This blocking kit consists of 18 ml of streptavidin and 18 ml of biotin in convenient dropper bottles.

Blocking Reagents

Product	Catalog Number	Unit Size
Normal Goat Serum	S-1000	20 ml
Normal Horse Serum	S-2000	20 ml
Normal Chicken Serum	S-3000	20 ml
Normal Swine Serum	S-4000	20 ml
Normal Rabbit Serum	S-5000	20 ml
2.5 % Normal Horse Serum	S-2012	50 ml
Bovine Serum Albumin (BSA)	SP-5050	500 mg
10x Casein Solution	SP-5020	250 ml
Carbo-Free™ Blocking Solution (10x)	SP-5040	125 ml
Animal-Free Blocker™ (5x)	SP-5030	250 ml
Avidin/Biotin Blocking Kit	SP-2001	1 kit
Streptavidin/Biotin Blocking Kit	SP-2002	1 kit


IN SITU HYBRIDIZATION

C

Introduction

Fluorescence and enzyme-based detection reagents from Vector Laboratories are ideal for *in situ* hybridization (ISH) applications, because of their high affinity, high sensitivity, and low background.

Fluorescence ISH. Choosing a detection method frequently depends on the abundance and accessibility of the target. Some targets, such as repetitive sequences, can be directly visualized with a fluorescently labeled probe. See Figure 1.

Less abundant targets may require a method with greater sensitivity. In these cases, the fluorescent label on the probe can serve as a tag. Signal amplification is achieved by binding a biotinylated antibody to this tag followed by a fluorochrome-labeled streptavidin or avidin. See Figure 2.

Additional signal amplification can be achieved using Biotinylated Anti-Streptavidin or Biotinylated Anti-Avidin antibodies. These antibodies bind to streptavidin or avidin, respectively, through their antigen binding sites and also through the biotin residues. After the application of the fluorochrome-labeled streptavidin or avidin, the signal is enhanced by incubation with either Biotinylated Anti-Streptavidin or Biotinylated Anti-Avidin antibody, respectively. This step is followed by a second incubation with fluorochrome-labeled streptavidin or avidin. This procedure results in the introduction of a greater number of fluorochromes at the target site. See Figure 3.

Enzyme-based ISH. Probes labeled with biotin, fluorochrome, or DNP can be detected with an antibody that is directly conjugated to either peroxidase or alkaline phosphatase in a "one-step" detection procedure. A wide choice of substrates is available for these enzyme conjugates (see page C16). For a significant increase in sensitivity, an ImmPRESS™ peroxidase polymer, biotin/streptavidin or biotin/avidin based systems can also be employed for detecting these labels.

Multiple Label ISH and IHC/ISH. Multiple probes with different labels can be hybridized simultaneously. After hybridization, the labeled probes can be detected sequentially using the same strategies employed for single probe detection.

It is also possible to perform ISH and IHC (immunohistochemistry) in the same tissue section. This is usually done sequentially. Antigen detection is usually performed first due to possible loss of antigenicity from the harsh conditions of hybridization. After localization of the antigen with a precipitating substrate, the ISH probe is hybridized and detected. The peroxidase substrates, DAB (SK-4100) or ImmPACT™ DAB (SK-4105), or the alkaline phosphatase substrates, Vector® Red (SK-5100) or BCIP/NBT (SK-5400) can be used first for localization of the antigen because the reaction products of these substrates remain stable throughout subsequent ISH procedures. For enzyme substrates see page C16.

For a detailed description of nucleic acid labels and labeling methods, please see Section F, "Labeling Reagents", pages F2-F7.

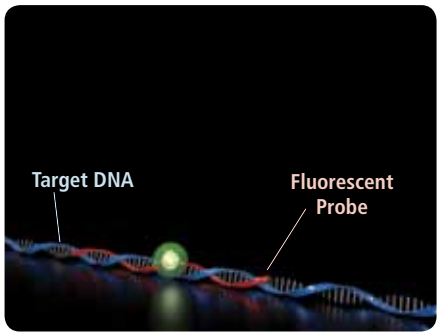


Fig. 1

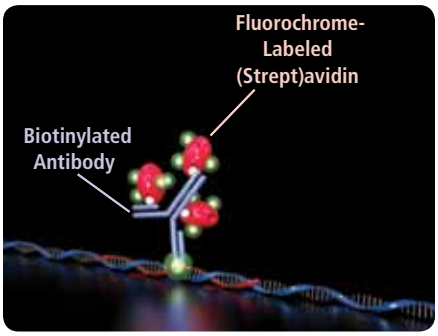


Fig. 2

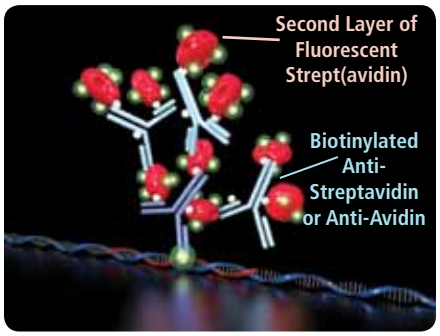


Fig. 3

Detection and Amplification. Factors such as tissue fixation, endogenous biotin or enzyme activity, desired sensitivity, and permanency of record should be considered when choosing either the optimal probe label or subsequent detection system. Protocols for fluorescence and enzyme based *in situ* detection are available in our brochure entitled, "A Guide to Nucleic Acid Labeling and Detection" (also available online).

Various options are available for fluorescent or chromogenic visualization of *in situ* probes. When choosing the appropriate reagents, it is important to consider:

1. The label on the probe (e.g. biotin, fluorescein, etc.)
2. The visualization method (for fluorescence see pages C4-C9; for chromogenic detection, see pages C10-C17)
3. The level of sensitivity required.

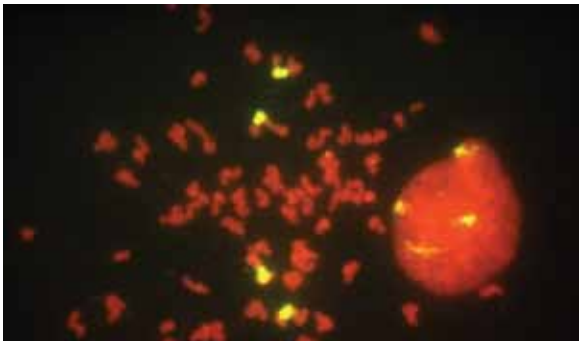
Several streptavidin and avidin reagents, as well as antibodies to labels, are available directly conjugated to the detection moiety (fluorochrome or enzyme). These "one-step" reagents are convenient and sensitive enough for many applications.

If additional sensitivity or flexibility is needed, many of the antibodies to labels can be detected with additional layers of amplification. For streptavidin- and avidin-based systems, use Biotinylated Anti-Streptavidin or Biotinylated Anti-Avidin reagents to amplify signal intensity in FISH applications (see page C5). For non-biotin/streptavidin or non-biotin/avidin strategies, affinity-purified secondary antibody conjugates can be used for signal amplification.

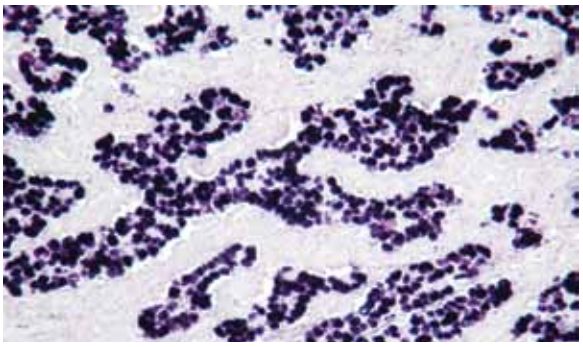
Each "method" or detection strategy listed on the following pages consists of the antibody to the label and matching secondary detection reagents. The flow charts in this section offer guides to various available options. Choose the option that best fits your requirements for sensitivity and cross-reactivity (e.g. in multiple labeling applications). The dilution factors in the tables are suggested guidelines; optimization may be required.

Associated Reagents. Increasing the accessibility of the labeled probe and detection reagents to the target sequence enhances sensitivity and specificity of an ISH signal. This can be achieved by reducing the size of DNA probes and pretreating tissues with a digestive enzyme. **NicKit™ p.s.o.** (MB-1905, page C18) has been developed for reducing the size of DNA probes to an optimal length for ISH without the problem of over- or under- digestion encountered with other methods. **Proteinase K** (VP-Y179, page C18) is used to digest proteins on tissue sections in order to enhance probe and detection reagent accessibility to the target. This enzyme unmasks nucleic acids from associated proteins. This step is often required when tissues have been treated with cross-linking fixatives like formaldehyde or glutaraldehyde.

A protein blocking reagent is imperative to reduce background when detecting hybridized ISH probes. **5x *In Situ* Blocking Solution** (MB-1220, page C18) is designed to be used as a general blocking agent and diluent for fluorescent and enzyme-based ISH.



HL-60 Chromosome Spread: FastTag® Biotin-labeled human chromosome 1 centromere-specific probe detected with Biotinylated Anti-Fluorescein and Fluorescein Avidin DCS. Mounted in VECTASHIELD® with Propidium Iodide (red).



In situ hybridization detection of EBV-encoded RNAs (EBERs) in Epstein-Barr virus positive gastric carcinoma. Visualized with BCIP/NBT substrate (indigo). (Image courtesy of Dr. GM Reynolds, Centre for Liver Research, University of Birmingham, U.K.)

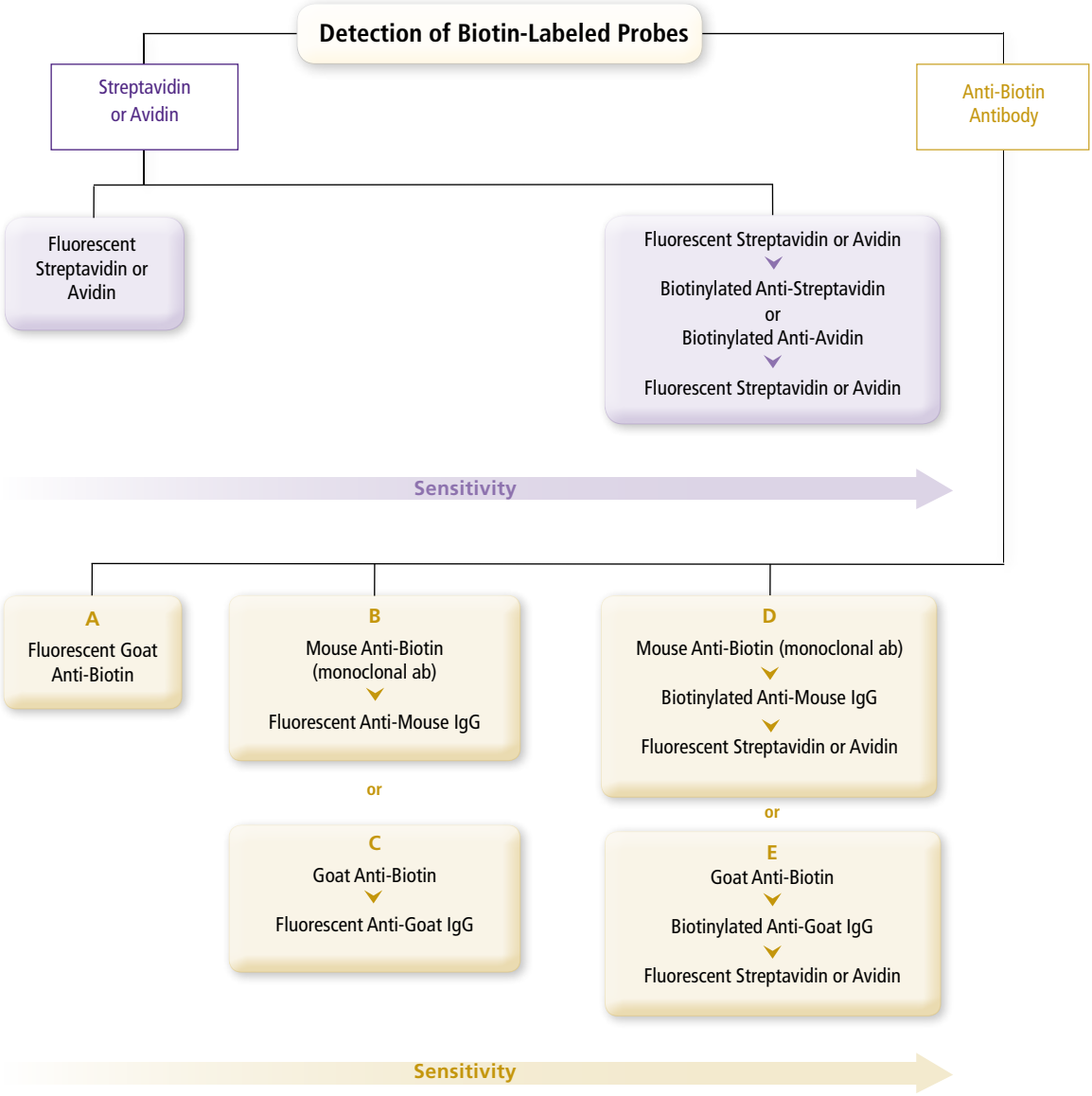
Fluorescent Detection Reagents

Detection of Biotin-Labeled Probes

Several options are available to detect a biotinylated ISH probe using fluorescent detection. These probes can either be detected with a fluorochrome streptavidin or avidin conjugate, or an anti-biotin antibody conjugate (see below).

Probes detected with a fluorochrome streptavidin or avidin conjugate can be further amplified using Biotinylated Anti-Streptavidin or Biotinylated Anti-Avidin antibodies followed by a second incubation with the fluorochrome streptavidin or avidin conjugate, respectively. Biotinylated Anti-Streptavidin and Biotinylated Anti-Avidin have the unusual property of binding to streptavidin or avidin either through the antigen-binding site or through the biotin residues. This procedure results in the introduction of more fluorochromes at the target site.

Biotinylated ISH probes can also be detected with anti-biotin antibodies, produced in either mouse or goat. Several different detection reagents can be used to amplify the signal in an anti-biotin antibody detection system. In general, the number of amplification steps correlates with the level of sensitivity achieved. These options are summarized in the flow chart below with specific detection strategies listed on page C6.



Fluorochrome-Labeled Streptavidin and Avidin

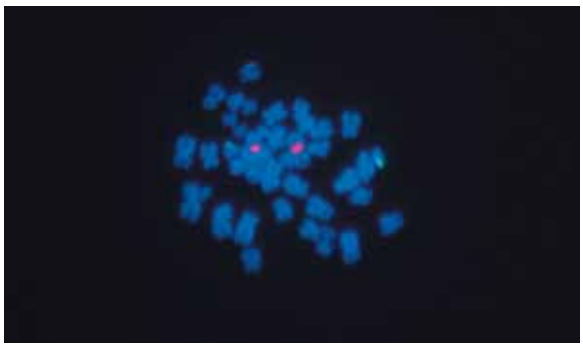
(See Section H, "Biotin and Avidin/Streptavidin Reagents" for additional product descriptions.)

Product Description	Color (λ, Ex/Em)	Catalog Number	Unit Size	Stock Concentration	Dilution
AMCA Streptavidin	Blue (350/450)	SA-5008	1 mg	1 mg/ml	1:100
DyLight® 488 Streptavidin	Green (493/518)	SA-5488	1 mg	1 mg/ml	1:100
Fluorescein Streptavidin	Green (495/515)	SA-5001	1 mg	1 mg/ml	1:100
DyLight® 549 Streptavidin	Orange (556/571)	SA-5549	1 mg	1 mg/ml	1:100
DyLight® 594 Streptavidin	Red (592/617)	SA-5594	1 mg	1 mg/ml	1:100
Texas Red® Streptavidin	Red (595/615)	SA-5006	1 mg	1 mg/ml	1:100
DyLight® 649 Streptavidin	Far Red (655/670)	SA-5649	1 mg	1 mg/ml	1:100
AMCA Avidin D	Blue (350/450)	A-2008	5 mg	5 mg/ml	1:250
Fluorescein Avidin DCS	Green (495/515)	A-2011	1 mg	2 mg/ml	1:200
Rhodamine Avidin DCS	Red (550/575)	A-2012	1 mg	2 mg/ml	1:200
Texas Red® Avidin DCS	Red (595/615)	A-2016	1 mg	2 mg/ml	1:200

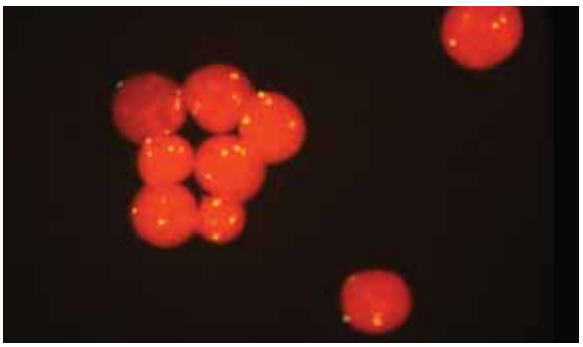
Biotinylated Anti-Streptavidin and Biotinylated Anti-Avidin

(See Section H, "Biotin and Avidin/Streptavidin Reagents" for additional product descriptions.)

Product Description	Catalog Number	Unit Size	Stock Concentration	Dilution
Anti-Streptavidin, made in goat, biotinylated	BA-0500	0.5 mg	0.5 mg/ml	1:100
Anti-Avidin, made in goat, biotinylated	BA-0300	0.5 mg	0.5 mg/ml	1:100



HL-60 Chromosome Spread: Digoxigenin-labeled human chromosome 18 centromere-specific probe, DyLight® 488 Anti-Digoxigenin/Digoxin • Biotinylated human chromosome 1 centromere-specific probe, Dylight® 594 Streptavidin. Mounted in Vectashield® Mounting Medium with DAPI.



HL-60 Chromosome Spread: FastTag® Biotin-labeled human chromosome 1 centromere-specific probe detected with Fluorescein Avidin DCS, Biotinylated Anti-Avidin, and Fluorescein Avidin DCS (yellow-green). Mounted in VECTASHIELD® Mounting Medium with Propidium Iodide (red).

Fluorescent Detection Reagents (continued)

Antibodies to Biotin - Detection Options

Fluorescent detection options using anti-biotin reagents are outlined in the flow chart (page C4) and detailed in the table.

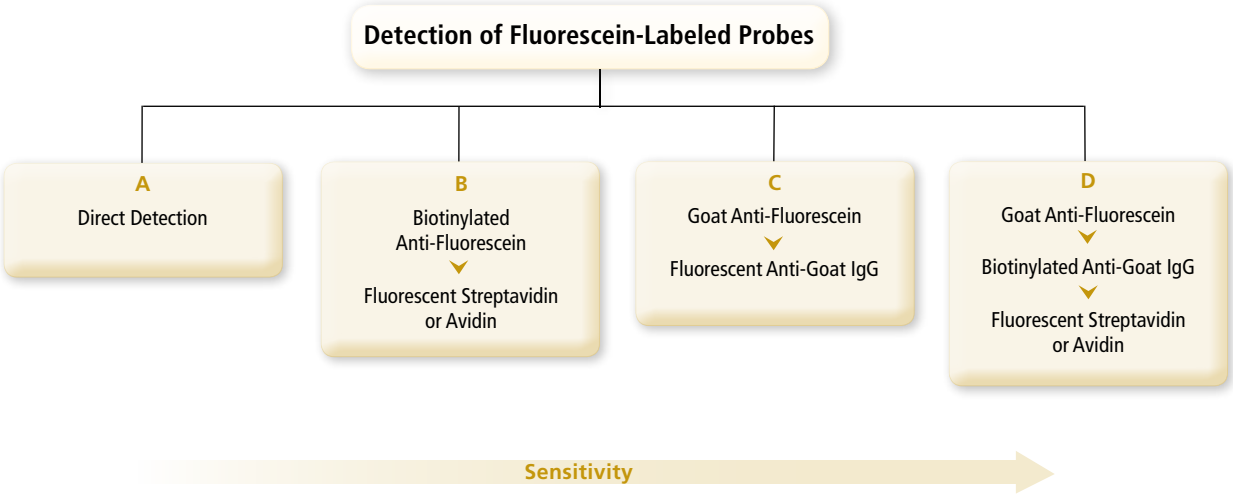
Additional product descriptions for these reagents can be found in Section B, " Immunofluorescence".

Method	Product Description	Color (λ, Ex/Em)	Catalog Number	Unit Size	Stock Concentration	Dilution
A	Anti-Biotin, made in goat, Fluorescein labeled	Green (495/515)	SP-3040	0.5 mg	1 mg/ml	1:100
B	Anti-Biotin-M, mouse monoclonal	N/A	MB-9100	1 ml	N/A	1:200
	Plus choose one of the following:					
	Anti-Mouse IgG (H+L), made in horse, AMCA labeled	Blue (350/450)	CI-2000	1.5 mg	1.5 mg/ml	1:150
	Anti-Mouse IgG (H+L), made in horse, DyLight® 488 labeled	Green (493/518)	DI-2488	1.5 mg	1.5 mg/ml	1:150
	Anti-Mouse IgG (H+L), made in horse, Fluorescein labeled	Green (495/515)	FI-2000	1.5 mg	1.5 mg/ml	1:150
	Anti-Mouse IgG (H+L), made in horse, DyLight® 549 labeled	Orange (556/571)	DI-2549	1.5 mg	1.5 mg/ml	1:150
	Anti-Mouse IgG (H+L), made in horse, DyLight® 594 labeled	Red (592/617)	DI-2594	1.5 mg	1.5 mg/ml	1:150
	Anti-Mouse IgG (H+L), made in horse, Texas Red® labeled	Red (595/615)	TI-2000	1.5 mg	1.5 mg/ml	1:150
	Anti-Mouse IgG (H+L), made in horse, DyLight® 649 labeled	Far Red (655/670)	DI-2649	1.5 mg	1.5 mg/ml	1:150
C	Anti-Biotin, made in goat	N/A	SP-3000	1 mg	1 mg/ml	1:2000
	Plus choose one of the following:					
	Anti-Goat IgG (H+L), made in rabbit, AMCA labeled	Blue (350/450)	CI-5000	1.5 mg	1.5 mg/ml	1:150
	Anti-Goat IgG (H+L), made in rabbit, Fluorescein labeled	Green (495/515)	FI-5000	1.5 mg	1.5 mg/ml	1:150
	Anti-Goat IgG (H+L), made in rabbit, Texas Red® labeled	Red (595/615)	TI-5000	1.5 mg	1.5 mg/ml	1:150
D	Anti-Biotin-M, mouse monoclonal	N/A	MB-9100	1 ml	N/A	1:200
	Plus choose one of the following:					
	Biotinylated Anti-Mouse IgG (H+L), made in horse	N/A	BA-2000	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Mouse IgG (H+L), made in goat	N/A	BA-9200	1.5 mg	1.5 mg/ml	1:200
	Plus choose a fluorochrome-labeled streptavidin or avidin (page C5)					
E	Anti-Biotin, made in goat	N/A	SP-3000	1 mg	1 mg/ml	1:2000
	Plus choose one of the following:					
	Biotinylated Anti-Goat IgG (H+L), made in horse	N/A	BA-9500	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Goat IgG (H+L), made in rabbit	N/A	BA-5000	1.5 mg	1.5 mg/ml	1:200
	Plus choose a fluorochrome-labeled streptavidin or avidin (page C5)					

Detection of Fluorescein-Labeled Probes

Fluorescein is not found endogenously in biological systems. It can be directly visualized or used as a specific tag and detected with our high affinity, purified antibody reagents.

Detection options are outlined in the flow chart and detailed in the table. Additional product descriptions for these reagents can be found in Section B, "Immunofluorescence".



Method	Product Description	Color (λ, Ex/Em)	Catalog Number	Unit Size	Stock Concentration	Dilution
B	Biotinylated Anti-Fluorescein, made in goat	N/A	BA-0601	0.5 mg	1 mg/ml	1:100
	Plus choose a fluorochrome-labeled streptavidin or avidin (page C5)					
C	Anti-Fluorescein, made in goat	N/A	SP-0601	1 mg	1 mg/ml	1:100
	Plus choose one of the following:					
	Anti-Goat IgG (H+L), made in rabbit, AMCA labeled	Blue (350/450)	CI-5000	1.5 mg	1.5 mg/ml	1:150
	Anti-Goat IgG (H+L), made in rabbit, Fluorescein labeled	Green (495/515)	FI-5000	1.5 mg	1.5 mg/ml	1:150
	Anti-Goat IgG (H+L), made in rabbit, Texas Red® labeled	Red (595/615)	TI-5000	1.5 mg	1.5 mg/ml	1:150
D	Anti-Fluorescein, made in goat	N/A	SP-0601	1 mg	1 mg/ml	1:100
	Plus choose one of the following:					
	Biotinylated Anti-Goat IgG (H+L), made in horse	N/A	BA-9500	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Goat IgG (H+L), made in rabbit	N/A	BA-5000	1.5 mg	1.5 mg/ml	1:200
	Plus choose a fluorochrome-labeled streptavidin or avidin (page C5)					

Fluorescent Detection Reagents (continued)

Detection of Digoxigenin-Labeled Probes

ISH probes are frequently labeled with digoxigenin (DIG), a steroid found in plants. This is particularly common in double-label applications with a biotin-labeled probe. Digoxigenin-labeled probes can be detected using our high affinity, purified antibody raised against digoxigenin and digoxin. In Method A below, detection is achieved using DyLight® labeled anti-digoxigenin/digoxin. In Method B below, further amplification may be obtained using unconjugated anti-digoxigenin/digoxin followed by affinity-purified secondary antibody conjugates.

Method	Product Description	Color (λ, Ex/Em)	Catalog Number	Unit Size	Stock Concentration	Dilution
A	Anti-Digoxigenin/Digoxin, made in goat, DyLight® 488 labeled	Green (493/518)	DI-7488	0.5 mg	1 mg/ml	1:100
	Anti-Digoxigenin/Digoxin, made in goat, DyLight® 594 labeled	Red (592/617)	DI-7594	0.5 mg	1 mg/ml	1:100
B	Anti-Digoxigenin/Digoxin, made in goat, unconjugated	N/A	MB-7000	1 mg	1 mg/ml	1:100
	Plus choose one of the following:					
	Anti-Goat IgG (H+L), made in rabbit, AMCA labeled	Blue (350/450)	CI-5000	1.5 mg	1.5 mg/ml	1:150
	Anti-Goat IgG (H+L), made in rabbit, Fluorescein labeled	Green (495/515)	FI-5000	1.5 mg	1.5 mg/ml	1:150
	Anti-Goat IgG (H+L), made in rabbit, Texas Red® labeled	Red (595/615)	TI-5000	1.5 mg	1.5 mg/ml	1:150

Detection of DNP-Labeled Probes

DNP is not found endogenously in tissue and is an excellent alternative to biotin. It can also be used as a second label in double-label ISH applications. A high affinity, purified antibody is available to detect the signal.

Product Description	Catalog Number	Unit Size	Stock Concentration	Dilution
Anti-DNP, made in rabbit, biotinylated	BA-0603	0.5 mg	1 mg/ml	1:100
Plus choose a fluorochrome-labeled streptavidin or avidin (page C5)				

Detection of Texas Red®-Labeled Probes

Texas Red® is not found endogenously in biological systems. It is a high quantum yield rhodamine derivative that can be directly visualized. The label can also be detected using our high affinity, purified anti-rhodamine antibodies.

Method	Antibody Description	Color (λ, Ex/Em)	Catalog Number	Unit Size	Stock Concentration	Dilution
A	Anti-Rhodamine[a], made in goat, biotinylated	N/A	BA-0605	0.5 mg	1 mg/ml	1:100
	Plus choose a fluorochrome-labeled streptavidin or avidin (page C5)					
B	Anti-Rhodamine[a], made in goat, unconjugated	N/A	SP-0602	1 mg	1 mg/ml	1:100
	Plus choose one of the following:					
	Anti-Goat IgG (H+L), made in rabbit, AMCA labeled	Blue (350/450)	CI-5000	1.5 mg	1.5 mg/ml	1:150
	Anti-Goat IgG (H+L), made in rabbit, Fluorescein labeled	Green (495/515)	FI-5000	1.5 mg	1.5 mg/ml	1:150
	Anti-Goat IgG (H+L), made in rabbit, Texas Red® labeled	Red (595/615)	TI-5000	1.5 mg	1.5 mg/ml	1:150

[a] *binds most rhodamines including Texas Red®*

VECTASHIELD® Mounting Media for Fluorescence

VECTASHIELD® Mounting Media are unsurpassed in preventing photobleaching. The different formulations of VECTASHIELD® Mounting Media all offer the same outstanding anti-fade and anti-photobleaching properties. They are all compatible with fluorescein, Texas Red®, AMCA, DyLight® dyes, Alexa Fluor® dyes, fluorescent nuclear stains, fluorescent proteins, fluorescent tracers, histochemical stains, and most fluorochromes.

Features of VECTASHIELD® Mounting Medium:

- Inhibits photobleaching of most fluorochromes
- Available in non-hardening or hardening formulations
- Available with or without DAPI or propidium iodide
- No warming necessary
- Slides can be viewed after prolonged storage
- Continues to inhibit photobleaching after prolonged storage of mounted slides
- Easy to use
- Optically clear
- Ideal refractive index

The original **VECTASHIELD® Mounting Medium** is a glycerol-based, aqueous mountant that does not solidify but remains a viscous liquid on the slide. After mounting, coverslipped slides will not readily dry out and can be reviewed for weeks afterwards without sealing. For prolonged storage, coverslips can be permanently sealed around the perimeter with nail polish. Mounted slides should be stored at 4 °C.

VECTASHIELD® Hard✦Set™ Mounting Medium is an aqueous mountant that hardens when allowed to set at room temperature in as little as 20 minutes. This facilitates handling of the slide, eliminates the need to secure the coverslip, and is more convenient for use with oil immersion microscopy. Stained and mounted slides may be stored for several weeks at 4 °C; for prolonged storage, place slides at -20 °C.

Both the VECTASHIELD® Hard✦Set™ and the original VECTASHIELD® Mounting Media are available with or without the counterstain DAPI (4′,6 diamidino-2-phenylindole). DAPI produces a blue fluorescence when bound to DNA with excitation at about 360 nm and emission at 460 nm. The original VECTASHIELD® Mounting Medium is also available with the counterstain propidium iodide (PI). PI is a reddish nuclear counterstain with a broad excitation range at around 535 nm and emission at about 615 nm when bound to DNA.

See page C17 for other mounting media.

VECTASHIELD® Mounting Media

Product	Counterstain	Cat. Number	Unit Size	Hardening	Refractive Index
VECTASHIELD® Mounting Medium	none	H-1000	10 ml	no	1.44
	DAPI	H-1200	10 ml	no	1.44
	PI	H-1300	10 ml	no	1.44
VECTASHIELD® Hard✦Set™ Mounting Medium	none	H-1400	10 ml	yes	1.36*
	DAPI	H-1500	10 ml	yes	1.36*

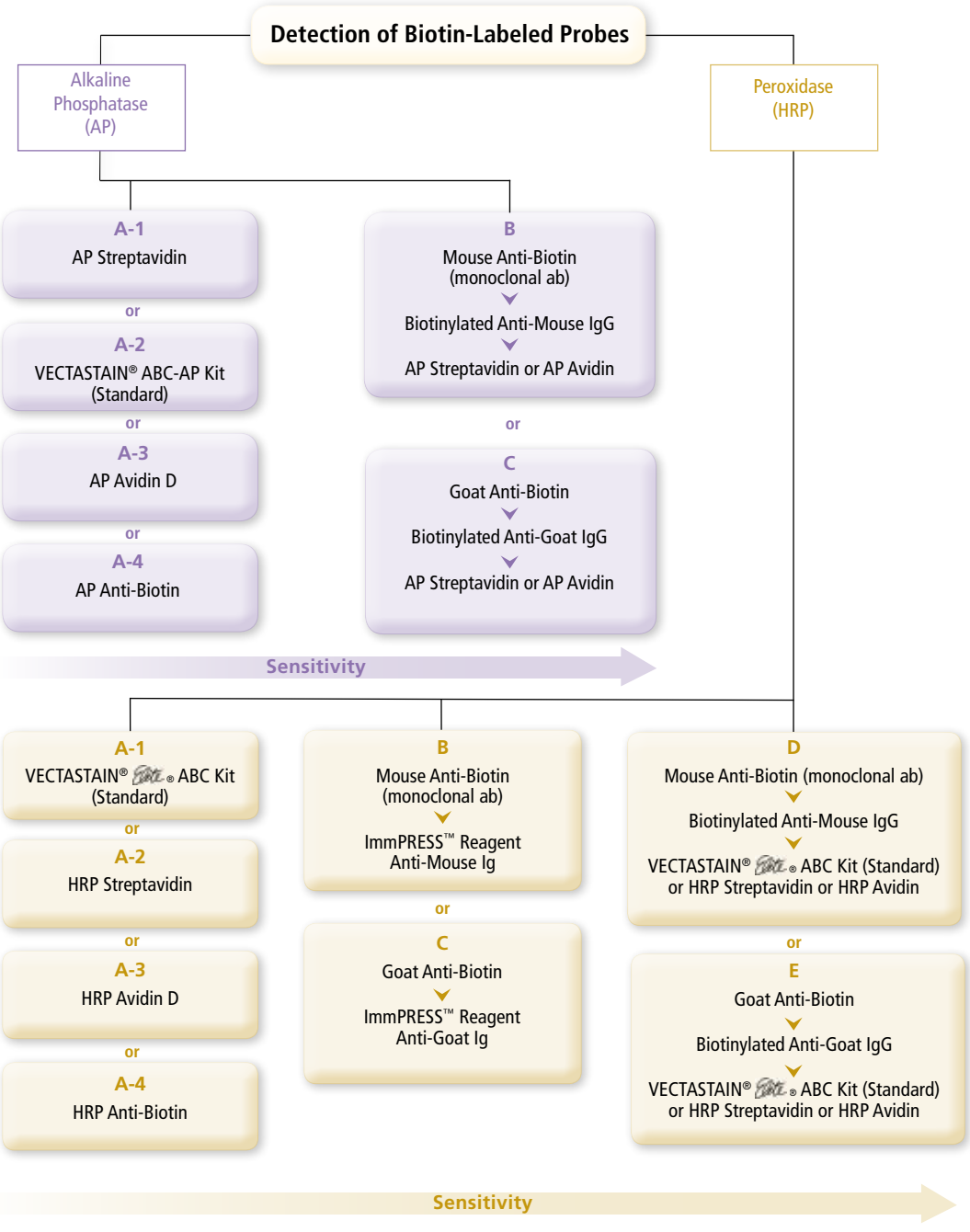
** Measured after hardening.*

Chromogenic Detection Reagents

Enzyme-based detection systems are available for the following labels: biotin, fluorescein, digoxigenin (DIG), dinitrophenyl (DNP), Texas Red®, and rhodamine. After selecting an appropriate enzyme system (peroxidase or alkaline phosphatase), choose a corresponding peroxidase or alkaline phosphatase chromogenic substrate for visualization (page C16). Additional product descriptions for the VECTASTAIN® ABC reagents, the ImmPRESS™ Peroxidase polymer reagents, as well as other enzyme conjugates can be found in Section A, "Immunohistochemistry".

Detection of Biotin-Labeled Probes

Several options are available for the chromogenic detection of a biotinylated ISH probe. These probes can either be detected with a streptavidin or avidin enzyme conjugate, an anti-biotin antibody conjugate, or a combination of both. These options are outlined below in order of increasing levels of sensitivity.



Alkaline Phosphatase Detection Options

Method	Product Description	Catalog Number	Unit Size	Stock Concentration	Dilution
A-1	Alkaline Phosphatase Streptavidin	SA-5100	1 ml	1 mg/ml	1:200
A-2	VECTASTAIN® ABC-AP Kit (Standard)	AK-5000	1 kit	N.A.	Follow kit instructions*
A-3	Alkaline Phosphatase Avidin D	A-2100	100 U	100 U/ml	1:500
A-4	Alkaline Phosphatase Anti-Biotin, made in goat	SP-3020	1 ml	1 mg/ml	1:200
B	Anti-Biotin, mouse monoclonal	MB-9100	1 ml	N.A.	1:200
	Plus choose one of the following:				
	Biotinylated Anti-Mouse IgG, made in horse	BA-2000	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Mouse IgG, made in goat	BA-9200	1.5 mg	1.5 mg/ml	1:200
	Plus choose an AP conjugate (in options A-1 through A-4)				
C	Anti-Biotin, made in goat	SP-3000	1 mg	1 mg/ml	1:2000
	Plus choose one of the following:				
	Biotinylated Anti-Goat IgG, made in rabbit	BA-5000	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Goat IgG, made in horse	BA-9500	1.5 mg	1.5 mg/ml	1:200
	Plus choose an AP conjugate (in options A-1 through A-4)				

** Kit instructions are available on our website.*

Peroxidase Detection Options

Method	Product Description	Catalog Number	Unit Size	Stock Concentration	Dilution
A-1	VECTASTAIN® Elite® ABC Kit (Standard)	PK-6100	1 kit	N.A.	Follow kit instructions*
A-2	Peroxidase Streptavidin	SA-5004	1 mg	1 mg/ml	1:200
A-3	Peroxidase Avidin D	A-2004	5 mg	5 mg/ml	1:1000
A-4	Peroxidase Anti-Biotin, made in goat	SP-3010	1 mg	1 mg/ml	1:200
B	Anti-Biotin, mouse monoclonal	MB-9100	1 ml	N.A.	1:200
	Plus the following:				
	ImmPRESS™ Reagent Anti-Mouse Ig, Peroxidase Polymer	MP-7402	50 ml	N.A.	No dilution
C	Anti-Biotin, made in goat	SP-3000	1 mg	1 mg/ml	1:2000
	Plus the following:				
	ImmPRESS™ Reagent Anti-Goat Ig, Peroxidase Polymer	MP-7405	50 ml	N.A.	No dilution
D	Anti-Biotin, mouse monoclonal	MB-9100	1 ml	N.A.	1:200
	Plus choose one of the following:				
	Biotinylated Anti-Mouse IgG, made in horse	BA-2000	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Mouse IgG, made in goat	BA-9200	1.5 mg	1.5 mg/ml	1:200
	Plus choose an HRP conjugate (in options A-1 through A-4)				
E	Anti-Biotin, made in goat	SP-3000	1 mg	1 mg/ml	1:2000
	Plus choose one of the following:				
	Biotinylated Anti-Goat IgG, made in rabbit	BA-5000	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Goat IgG, made in horse	BA-9500	1.5 mg	1.5 mg/ml	1:200
	Plus choose an HRP conjugate (in options A-1 through A-4)				

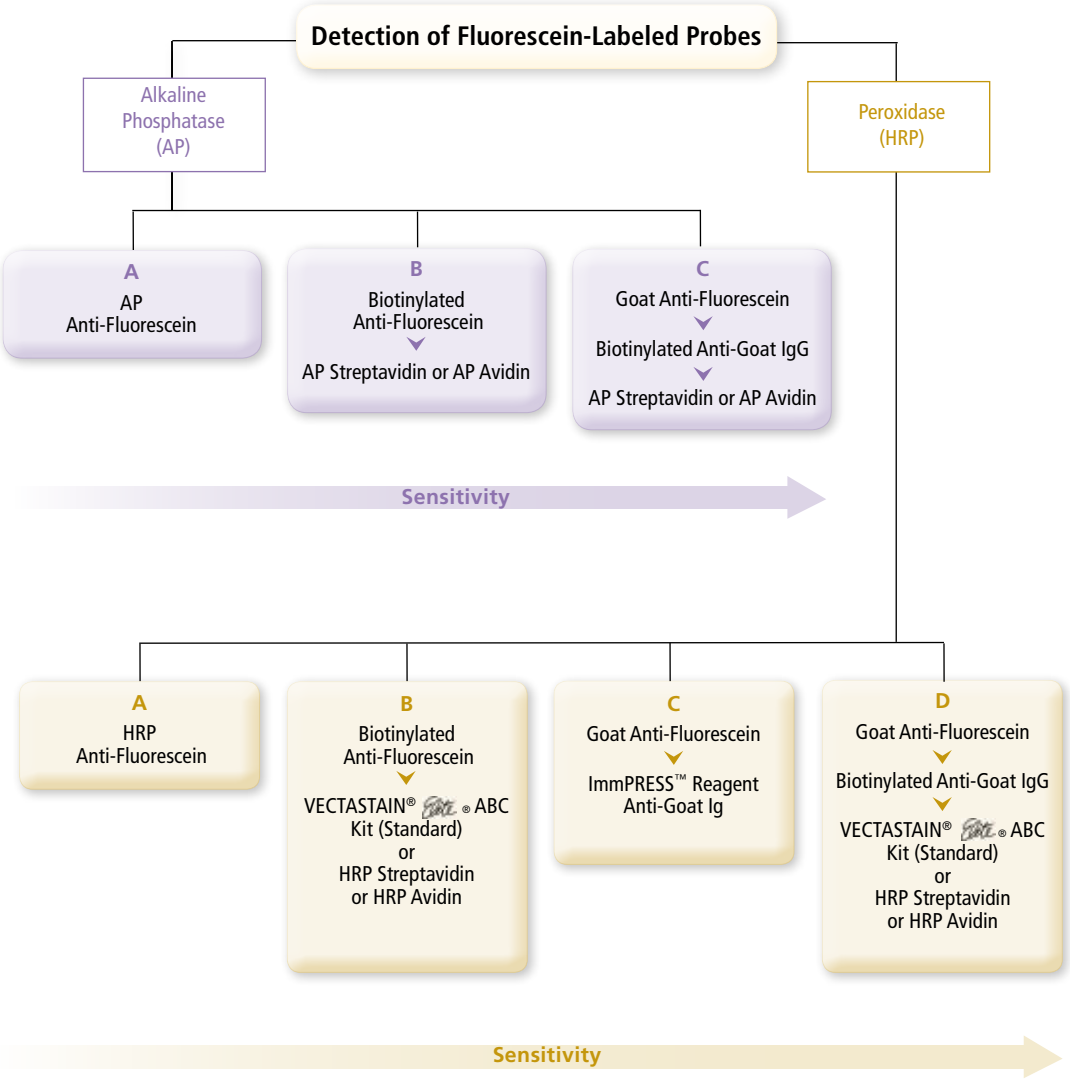
** Kit instructions are available on our website.*

Choose an appropriate alkaline phosphatase or peroxidase substrate on page C16.

Chromogenic Detection Reagents (continued)

Detection of Fluorescein-Labeled Probes

Fluorescein is not found endogenously in biological systems. It can be directly visualized or used as a tag and detected with our high affinity antibody reagents. Several detection options are outlined below in order of increasing levels of sensitivity.



Alkaline Phosphatase Detection Options

Method	Product Description	Catalog Number	Unit Size	Stock Concentration	Dilution
A	Alkaline Phosphatase Anti-Fluorescein, made in goat	MB-2100	150 µg	0.5 mg/ml	1:100
B	Biotinylated Anti-Fluorescein, made in goat	BA-0601	0.5 mg	1 mg/ml	1:100
	Plus choose an AP conjugate				
	Alkaline Phosphatase Streptavidin	SA-5100	1 ml	1 mg/ml	1:200
	VECTASTAIN® ABC-AP Kit (Standard)	AK-5000	1 kit	N.A.	Follow Kit Instructions*
	Alkaline Phosphatase Avidin D	A-2100	100 U	100 U/ml	1:500
C	Anti-Fluorescein, made in goat	SP-0601	1 mg	1 mg/ml	1:100
	Plus choose one of the following:				
	Biotinylated Anti-Goat IgG, made in horse	BA-9500	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Goat IgG, made in rabbit	BA-5000	1.5 mg	1.5 mg/ml	1:200
	Plus choose an AP conjugate				
	Alkaline Phosphatase Streptavidin	SA-5100	1 ml	1 mg/ml	1:200
	VECTASTAIN® ABC-AP Kit (Standard)	AK-5000	1 kit	N.A.	Follow Kit Instructions*
	Alkaline Phosphatase Avidin D	A-2100	100 U	100 U/ml	1:500

** Kit instructions are available on our website.*

Peroxidase Detection Options

Method	Product Description	Catalog Number	Unit Size	Stock Concentration	Dilution
A	Peroxidase Anti-Fluorescein, made in goat	SP-1910	0.5 mg	0.5 mg/ml	1:100
B	Biotinylated Anti-Fluorescein, made in goat	BA-0601	0.5 mg	1 mg/ml	1:100
	Plus choose an HRP conjugate				
	VECTASTAIN® Elite® ABC Kit (Standard)	PK-6100	1 kit	N.A.	Follow Kit Instructions*
	Peroxidase Streptavidin	SA-5004	1 mg	1 mg/ml	1:200
	Peroxidase Avidin D	A-2004	5 mg	5 mg/ml	1:1000
C	Anti-Fluorescein, made in goat	SP-0601	1 mg	1 mg/ml	1:100
	Plus the following:				
	ImmPRESS™ Reagent Anti-Goat Ig, Peroxidase Polymer	MP-7405	50 ml	N.A.	No dilution
D	Anti-Fluorescein, made in goat	SP-0601	1 mg	1 mg/ml	1:100
	Plus choose one of the following:				
	Biotinylated Anti-Goat IgG, made in horse	BA-9500	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Goat IgG, made in rabbit	BA-5000	1.5 mg	1.5 mg/ml	1:200
	Plus choose an HRP conjugate				
	VECTASTAIN® Elite® ABC Kit (Standard)	PK-6100	1 kit	N.A.	Follow Kit Instructions*
	Peroxidase Streptavidin	SA-5004	1 mg	1 mg/ml	1:200
	Peroxidase Avidin D	A-2004	5 mg	5 mg/ml	1:1000

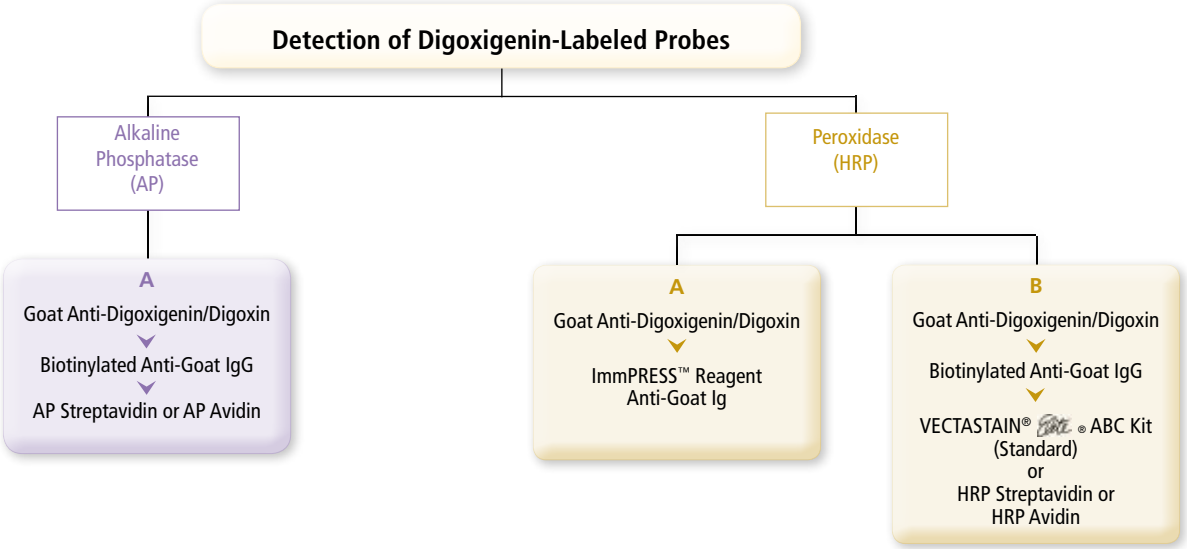
** Kit instructions are available on our website.*

Choose an appropriate alkaline phosphatase or peroxidase substrate on page C16.

Chromogenic Detection Reagents (continued)

Detection of Digoxigenin-Labeled Probes

ISH probes are frequently labeled with digoxigenin (DIG), a steroid found in plants. Digoxigenin-labeled probes can be detected using our high affinity, purified antibody raised against digoxigenin and digoxin.



Alkaline Phosphatase Detection Options

Method	Product Description	Catalog Number	Unit Size	Stock Concentration	Dilution
A	Anti-Digoxigenin/Digoxin, made in goat	MB-7000	1 mg	1 mg/ml	1:100
	Plus choose one of the following:				
	Biotinylated Anti-Goat IgG, made in horse	BA-9500	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Goat IgG, made in rabbit	BA-5000	1.5 mg	1.5 mg/ml	1:200
	Plus choose an AP conjugate				
	Alkaline Phosphatase Streptavidin	SA-5100	1 ml	1 mg/ml	1:200
	VECTASTAIN® ABC-AP Kit (Standard)	AK-5000	1 kit	N.A.	Follow kit instructions*
	Alkaline Phosphatase Avidin D	A-2100	100 U	100 U/ml	1:500

** Kit instructions are available on our website.*

Peroxidase Detection Options

Method	Product Description	Catalog Number	Unit Size	Stock Concentration	Dilution
A	Anti-Digoxigenin/Digoxin, made in goat	MB-7000	1 mg	1 mg/ml	1:100
	Plus the following:				
	ImmPRESS™ Reagent Anti-Goat Ig, Peroxidase Polymer	MP-7405	50 ml	N.A.	No dilution
B	Anti-Digoxigenin/Digoxin, made in goat	MB-7000	1 mg	1 mg/ml	1:100
	Plus choose one of the following:				
	Biotinylated Anti-Goat IgG, made in horse	BA-9500	1.5 mg	1.5 mg/ml	1:200
	Biotinylated Anti-Goat IgG, made in rabbit	BA-5000	1.5 mg	1.5 mg/ml	1:200
	Plus choose an HRP conjugate				
	VECTASTAIN® Elite® ABC Kit (Standard)	PK-6100	1 kit	N.A.	Follow kit instructions*
	Peroxidase Streptavidin	SA-5004	1 mg	1 mg/ml	1:200
	Peroxidase Avidin D	A-2004	5 mg	5 mg/ml	1:1000

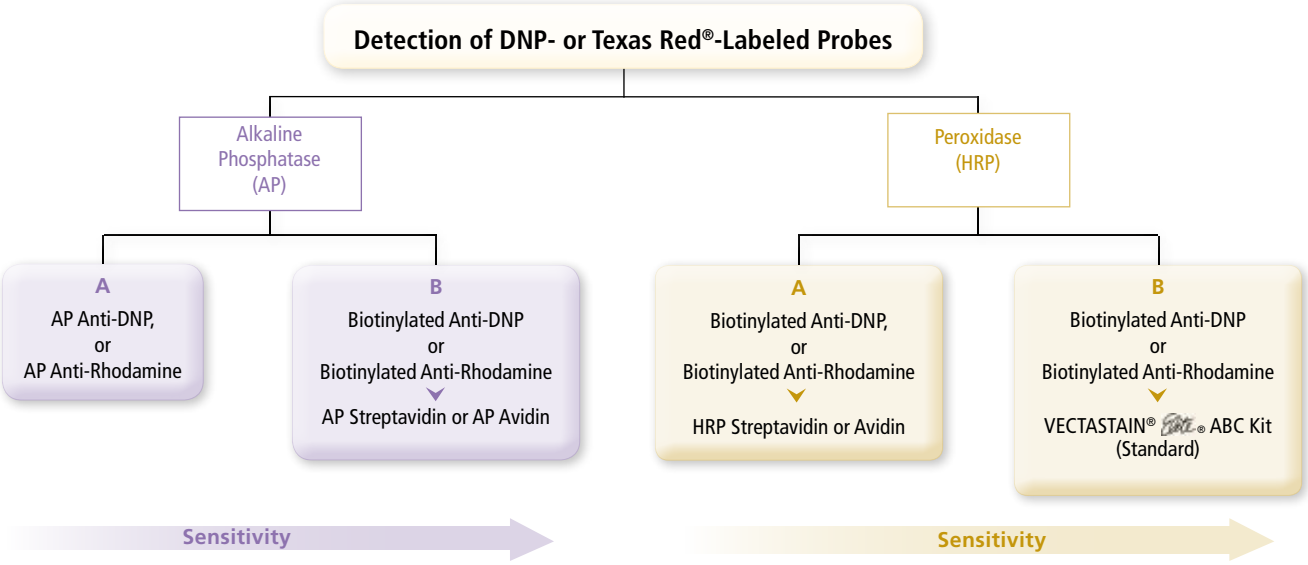
** Kit instructions are available on our website.*

Choose an appropriate alkaline phosphatase or peroxidase substrate on page C16.

Detection of DNP- or Texas Red®-Labeled Probes

Dinitrophenyl (DNP) and Texas Red® are not found endogenously in tissue and therefore are excellent alternatives to biotin. High affinity, purified antibodies are available for detection of DNP.

Texas Red® is a high quantum yield rhodamine derivative that can be directly visualized. It can also be detected using our high affinity, purified anti-rhodamine antibody conjugates. These antibodies bind most rhodamines including Texas Red®.



Alkaline Phosphatase Detection Options

Method	Product Description	Catalog Number	Unit Size	Stock Concentration	Dilution
A	Alkaline Phosphatase Anti-DNP, made in rabbit	MB-3100	150 µg	0.5 mg/ml	1:100
	Alkaline Phosphatase Anti-Rhodamine, made in goat	MB-1920	150 µg	0.5 mg/ml	1:100
B	Biotinylated Anti-DNP, made in rabbit	BA-0603	0.5 mg	1 mg/ml	1:100
	Biotinylated Anti-Rhodamine, made in goat	BA-0605	0.5 mg	1 mg/ml	1:100
	Plus choose an AP conjugate				
	Alkaline Phosphatase Streptavidin	SA-5100	1 ml	1 mg/ml	1:200
	VECTASTAIN® ABC-AP Kit (Standard)	AK-5000	1 kit	N.A.	Follow kit instructions*
	Alkaline Phosphatase Avidin D	A-2100	100 U	100 U/ml	1:500

** Kit instructions are available on our website.*

Peroxidase Detection Options

Method	Product Description	Catalog Number	Unit Size	Stock Concentration	Dilution
A	Biotinylated Anti-DNP, made in rabbit	BA-0603	0.5 mg	1 mg/ml	1:100
	Biotinylated Anti-Rhodamine, made in goat	BA-0605	0.5 mg	1 mg/ml	1:100
	Plus choose an HRP conjugate				
	Peroxidase Streptavidin	SA-5004	1 mg	1 mg/ml	1:200
	Peroxidase Avidin D	A-2004	5 mg	5 mg/ml	1:1000
B	Biotinylated Anti-DNP, made in rabbit	BA-0603	0.5 mg	1 mg/ml	1:100
	Biotinylated Anti-Rhodamine, made in goat	BA-0605	0.5 mg	1 mg/ml	1:100
	Plus the following				
	VECTASTAIN® Elite® ABC Kit (Standard, HRP)	PK-6100	1 kit	N.A.	Follow kit instructions*

** Kit instructions are available on our website.*

Choose an appropriate alkaline phosphatase or peroxidase substrate on page C16.

Chromogenic Detection Reagents (continued)

Enzyme Substrates

Several alkaline phosphatase and peroxidase substrates can be used in ISH detection. The substrate chosen must match the enzyme in the detection system.

All of our substrate kit reagents are supplied in convenient dropper bottles, promoting safer handling of chromogens and eliminating wait times for mixing and dissolving powders or tablets.

Vector Laboratories offers two lines of peroxidase substrates. Our original substrate kits provide sensitivity greater than conventional substrates and are known for their consistency and reliability. The ImmPACT™ Substrates contain specially formulated chromogens and diluents and provide:

- Increased sensitivity of the precipitated substrate
- Better stability of the substrate working solution
- Greater consistency (no variation introduced in the buffer preparation)

Substrates provide varying degrees of sensitivities. For example, ImmPACT™ peroxidase substrates are about 2-4 times more sensitive than the original peroxidase substrate kits, and the alkaline phosphatase substrate BCIP/NBT increases in sensitivity with longer incubation times. See chart for relative substrate sensitivities in Section I, "Enzyme Substrates", page I2.

The heat resistant substrates (DAB Substrate Kit, ImmPACT™ DAB, BCIP/NBT, and Vector® Red) can be used in IHC/ISH double labeling applications. IHC is performed first using one of the heat-resistant substrates followed by ISH detection that requires a heat denaturing step.

Additional descriptions for these substrates can be found in Section I, "Enzyme Substrates".

Substrates

Enzyme System	Substrate Kit	Color	Catalog Number	Unit Size	Total Working Solution	Mounting
AP	BCIP/NBT	Indigo	SK-5400	1 kit	200 ml	Non-aqueous
	Vector® Red	Magenta	SK-5100	1 kit	200 ml	Non-aqueous
	Vector® Blue	Blue	SK-5300	1 kit	200 ml	Non-xylene clearing, Non-aqueous
HRP	DAB	Brown	SK-4100	1 kit	300 ml	Non-aqueous
	DAB with Ni	Gray/Black	SK-4100	1 kit	300 ml	Non-aqueous
	ImmPACT™ DAB	Brown	SK-4105	120 ml	120 ml	Non-aqueous
	Vector® VIP	Purple	SK-4600	1 kit	300 ml	Non-aqueous
	ImmPACT™ VIP	Purple	SK-4605	120 ml	120 ml	Non-aqueous
	Vector® SG	Blue/Gray	SK-4700	1 kit	300 ml	Non-aqueous
	ImmPACT™ SG	Blue/Gray	SK-4705	120 ml	120 ml	Non-aqueous
	Vector® NovaRED™	Red	SK-4800	1 kit	300 ml	Non-aqueous
	ImmPACT™ NovaRED™	Red	SK-4805	120 ml	120 ml	Non-aqueous
	AEC	Red	SK-4200	1 kit	300 ml	Aqueous
	ImmPACT™ AEC	Red	SK-4205	120 ml	120 ml	Aqueous
	ImmPACT™ AMEC Red	Red	SK-4285	120 ml	120 ml	Aqueous
	TMB	Blue	SK-4400	1 kit	300 ml	Non-aqueous

Counterstains

Additional descriptions for counterstains and substrate compatibility can be found in Section A, "Immunohistochemistry", pages A24-A25.

Product Description	Color	Catalog Number	Unit Size	Total Working Solution	Mounting
Vector® Hematoxylin	Blue	H-3401	500 ml	500 ml	Non-aqueous and Aqueous
Vector® Hematoxylin QS	Blue	H-3404	100 ml	100 ml	Non-aqueous and Aqueous
Vector® Methyl Green	Green	H-3402	500 ml	500 ml	Non-aqueous
Vector® Nuclear Fast Red	Red	H-3403	500 ml	500 ml	Non-aqueous and Aqueous

Permanent Mounting Medium

VectaMount™

- Permanent, non-aqueous mounting
- Toluene- and xylene-free
- Low hazard
- Odorless
- Compatible with horseradish peroxidase, alkaline phosphatase, and glucose oxidase substrates
- Dries with an ideal refractive index

VectaMount™ Mounting Medium (H-5000) is an optically clear and odorless formula for permanently preserving histochemical stains or precipitable enzyme substrates in tissue sections or cell preparations. ***VectaMount***™ Mounting Medium contains no toluene or xylene. It has a viscosity which provides for easy application and uniform spreading over the tissue section. Mounted sections are clear with an ideal refractive index suitable for high resolution oil immersion microscopy. ***VectaMount***™ Mounting Medium preserves the color and intensity of preparations stained with enzyme substrates such as DAB, TMB, and BCIP/NBT, as well as our proprietary substrates Vector® NovaRED™, Vector® VIP, Vector® SG, ImmPACT™ DAB, ImmPACT™ NovaRED™, ImmPACT™ VIP, ImmPACT™ SG, Vector® Red, Vector® Blue, and Vector® Black. The crystal formation that frequently occurs with the alkaline phosphatase substrate BCIP/NBT using other permanent mounting media is essentially eliminated.

Aqueous Mounting Medium

VectaMount™ AQ

- Aqueous mounting
- Compatible with many horseradish peroxidase and alkaline phosphatase substrates

VectaMount™ **AQ** Mounting Medium (H-5501) is a hard-setting mounting medium developed for use with enzymatic substrates, such as AEC, ImmPACT™ AEC, and ImmPACT™ AMEC *Red*, whose reaction products are soluble in alcohol or other organic solvents. In applications where aqueous mounting is preferred, ***VectaMount***™ **AQ** is suitable for use with other substrates such as DAB, Vector® SG, BCIP/NBT, Vector® Red, Vector® Blue, ImmPACT™ DAB, and ImmPACT™ SG. ***VectaMount***™ **AQ** is simple to use, requires no mixing, and preserves the color and clarity of the substrates. Stained sections mounted with ***VectaMount***™ **AQ** can be stored in a slide box at room temperature for at least 2 years without fading.

Product	Catalog Number	Unit Size
VectaMount™ Permanent Mounting Medium	H-5000	60 ml
VectaMount™ Aqueous Mounting Medium	H-5501	60 ml

Associated Reagents

Control Fluorescein-Labeled Probe

Fluorescein-Labeled

The Control Fluorescein-Labeled Probe (VP-Y175) is a cocktail of randomly generated oligonucleotide sequences end labeled with fluorescein using the same procedures as applied to the target specific cDNA oligonucleotide probes. It is used as a negative control alongside these specific cDNA probes.

Epstein Barr Virus Probe

Fluorescein-Labeled

The Epstein Barr Virus Probe (VP-Y176) may be used to detect viral RNA in formalin-fixed, paraffin-embedded tissues. The technique is simple to perform, employing steps similar to those used in immunohistochemistry. Tissue sections are dewaxed and rehydrated prior to treatment with proteinase K, followed by incubation with the probe. Bound probe is then detected with an anti-FITC-AP antibody (MB-2100) which is visualized by addition of an enzyme substrate. No specialized equipment is required for *in situ* hybridization and full instructions are supplied with this product.

Epstein Barr Virus Probe ISH Kit

The Epstein Barr Virus Probe ISH Kit (VP-Y177) contains fluorescein-labeled EBV probe, proteinase K, detection probe, (negative) control probe, anti-fluorescein detection system, unlabeled control tissue sections, and glass slides.

Kappa/Lambda Probes

Fluorescein-Labeled

These Kappa/Lambda Probes (VP-Y178) may be used to detect Ig kappa and/or Ig lambda light chain mRNA in formalin-fixed, paraffin-embedded tissues. The technique is simple to perform, employing steps similar to those used in immunohistochemistry. Tissue sections are dewaxed and rehydrated prior to treatment with proteinase K, followed by incubation with the probe. Bound probe is then detected with an anti-FITC-AP antibody (MB-2100) which is visualized by addition of an enzyme substrate. This kit contains sufficient reagents to detect 25 kappa and 25 lambda preparations.

NicKit™ p.s.o. Probe Size Optimization Kit

NicKit™ *p.s.o.* Kit (MB-1905) reduces the size of DNA probes to an optimal length for *in situ* hybridization, avoiding the problem of over- or under-digestion encountered with other methods. The procedure involves a brief U.V. irradiation of probe DNA using a transilluminator or hand-held U.V. lamp followed by enzymatic digestion using a specially prepared endonuclease. The phosphodiester bond at pyrimidine dimers formed by U.V. irradiation is cleaved, resulting in average DNA lengths of 400 bases or less. Probe DNA can be subsequently labeled with 3′ or 5′ EndTag™, PHOTOPROBE® Biotin, or the FastTag® system. The ***NicKit™*** *p.s.o.* reagents can treat up to 300 µg of DNA.

Proteinase K

Proteinase K (VP-Y179) is used for the digestion of tissue sections prior to hybridization to aid in the detection of mRNA for *in situ* hybridization using oligonucleotide probes.

5x *In Situ* Hybridization Blocking Solution

5x *In Situ* Hybridization Blocking Solution (MB-1220) is a blocking reagent used in the fluorescent and enzymatic detection of labeled probes following *in situ* hybridization. The 100 ml concentrate supplies sufficient reagent to prepare 500 ml of working solution.

Product	Catalog Number	Unit Size
Control Fluorescein Labeled Probe	VP-Y175	50 tests
Epstein Barr Virus Probe	VP-Y-176	50 tests
Epstein Barr Virus Probe ISH Kit	VP-Y177	50 tests
Kappa/Lambda Probes	VP-Y178	2x25 tests
NicKit™ p.s.o. Kit	MB-1905	1 kit
Proteinase K	VP-Y179	500 µg
5x *In Situ* Hybridization Blocking Solution	MB-1220	100 ml

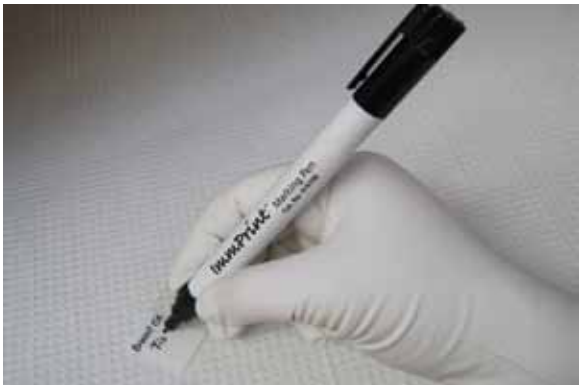
ImmEdge™ Hydrophobic Barrier Pen

The ImmEdge™ Pen (H-4000) is a hydrophobic barrier pen for immunohistochemistry and *in situ* hybridization. It is designed to provide a heat-stable, water-repellent barrier that keeps reagents localized on tissue specimens and prevents mixing of reagents when multiple sections are mounted on the same slide.

The pale blue barrier is insoluble in alcohol and acetone but is completely removed by all commonly used xylene and "xylene substitute" clearing agents. The ImmEdge™ Pen contains no ozone-depleting solvents and is compatible with enzyme- or fluorescence-based detection systems.

ImmPrint™ Histology Pen

The ImmPrint™ Histology Pen (H-6100) is a solvent-resistant permanent marking pen, designed for writing on glass microscope slides, tissue cassettes, and most hard surfaces. The high density, fast-drying black ink is resistant to most organic solvents encountered in histological applications, such as alcohols or clearing agents. Unlike other pens commonly used for histology, the ImmPrint™ pen has a smooth writing tip that resists drying out.



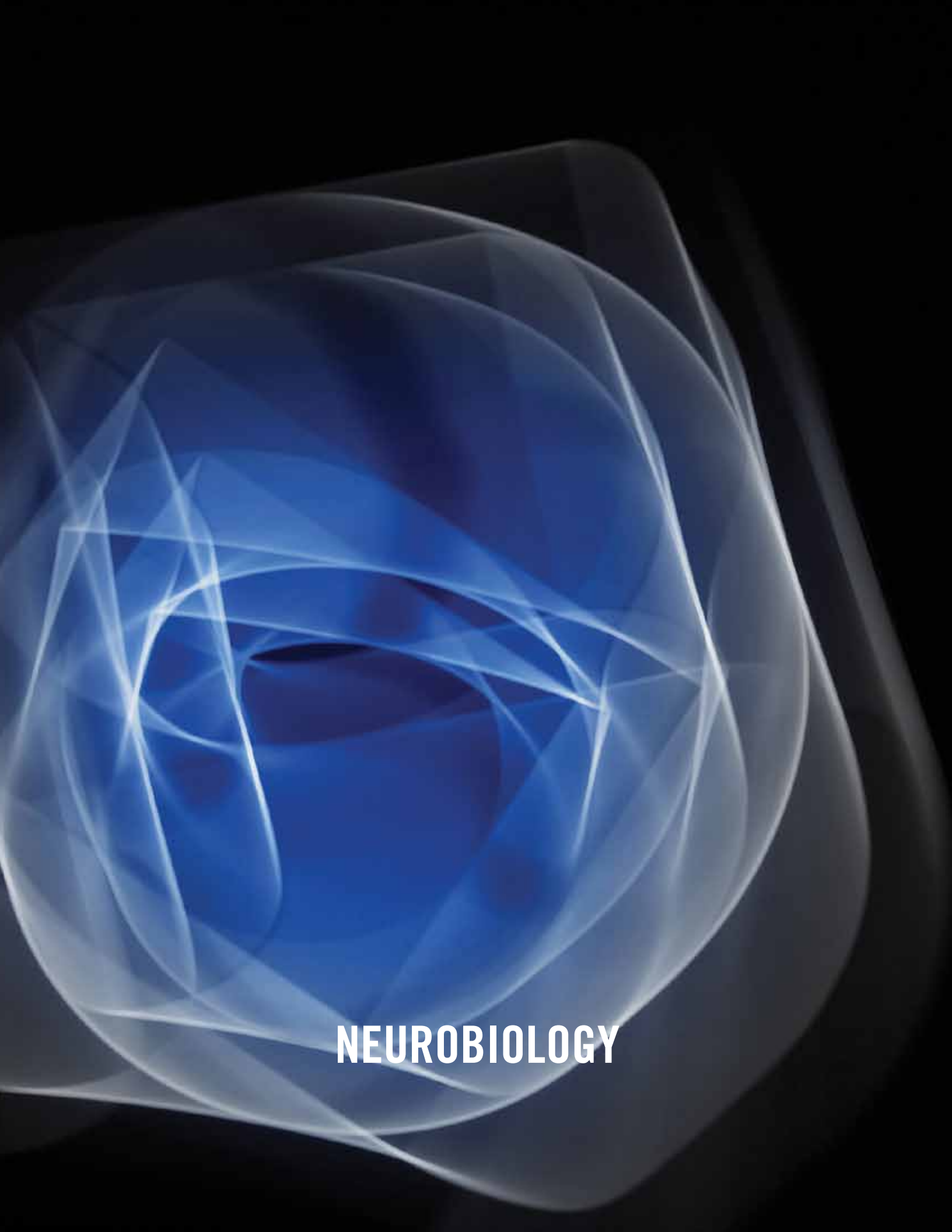
Avidin/Biotin Blocking Kit

Avidin/Biotin Blocking Kit (SP-2001) blocks all endogenous biotin, biotin receptors, and avidin binding sites present in tissues. This kit is designed for use with biotin/avidin detection systems such as the VECTASTAIN® ABC Kits if avidin or biotinylated reagents bind nonspecifically to tissues or proteins. This blocking kit consists of 18 ml of Avidin D and 18 ml of biotin in convenient dropper bottles.

Streptavidin/Biotin Blocking Kit

Streptavidin/Biotin Blocking Kit (SP-2002) blocks all endogenous biotin, biotin receptors, and streptavidin binding sites present in tissues. This kit is designed for use with biotin/streptavidin detection systems such as the VECTASTAIN® Universal Quick kits if streptavidin or biotinylated reagents bind nonspecifically to tissues or proteins. This blocking kit consists of 18 ml of streptavidin and 18 ml of biotin in convenient dropper bottles.

Product	Catalog Number	Unit Size
ImmEdge™ Pen	H-4000	2-pen set
ImmPrint™ Histology Pen	H-6100	5-pen set
Avidin/Biotin Blocking Kit	SP-2001	1 kit
Streptavidin/Biotin Blocking Kit	SP-2002	1 kit


NEUROBIOLOGY

Introduction

Vector Laboratories offers a wide variety of products for neurobiology. Our fluorochrome and enzyme-based detection systems and our proprietary substrates are staples of neurobiological research because of their high sensitivity and low background. We also provide biotinylated neuronal tracers and intracellular labeling reagents such as biotinylated dextran amines and NEUROBIOTIN™ Tracer. Our expertise in lectin isolation and purification led to the development of a range of lectin neuronal tracers and neural glycobiology reagents that are used to study neuroanatomical connections and structure. In addition, we offer several primary antibodies specific for brain and neural antigens.

Neuronal Tracers. Neuronal tracers are used to elucidate anatomical connections of the nervous system. Tracers are transported retrogradely, from axon to the soma, or anterogradely, from the soma to the axon. These tracers are used to determine cells of origin of axons that innervate a certain structure and to identify the target of axonal projections from a particular neuron. Vector Laboratories features reagents for both anterograde and retrograde tracing.

Neuronal Glycobiology. Detection of neurons and support structures using lectins has provided valuable information in the field of neuroscience. For example, small sensory neurons can be broadly classified into two groups based on expression levels of neuropeptides and nerve growth factor receptor. One group expresses a cell surface glycoprotein that binds the lectin *Griffonia simplicifolia* I, Isolectin B_4. As a result, it has become useful to classify neurons as IB_4 positive or IB_4 negative. Other lectins, such as *Lycopersicon esculentum*, *Ricinus communis* agglutinin I, peanut agglutinin, and wheat germ agglutinin have been used to detect various types of glia.

Lectins commonly used in neurobiology are listed in this section. A complete list of all lectins and their descriptions can be found in Section K, "Lectins and Glycobiology Reagents".

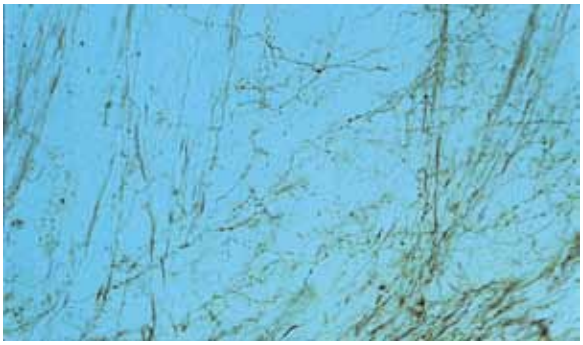
Primary Antibodies. We offer a selection of primary antibodies against brain and neural antigens.

Detection. High sensitivity, low background, consistency, and reliability are hallmarks of detection systems from Vector Laboratories. Various detection and visualization strategies are possible using our reagents. Reagents that are conjugated to fluorochromes can be visualized directly by fluorescence microscopy. Lectins directly conjugated to peroxidase can be detected using a peroxidase substrate. Vector® substrates offer a palette of colors and characteristics that are suitable for different types of analyses (see Section I, "Enzyme Substrates"). Some substrates, such as DAB, Vector® VIP, and Vector® SG result in electron dense products, allowing structural analysis by electron microscopy. In addition, more than one substrate can be introduced into a tissue section for multiple labeling (see our brochure, "Discovery Through Color: A Guide to Multiple Antigen Labeling").

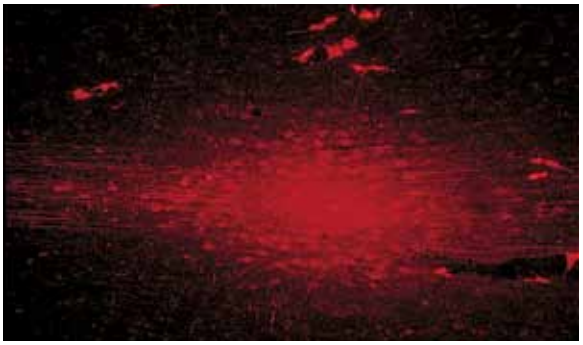
Biotinylated reagents can be detected either with a fluorescence-based avidin or streptavidin detection system or an enzymed-based system such as the VECTASTAIN® Elite® ABC kit followed by a chromogenic substrate.

Unconjugated lectins can be detected using one of several different strategies. When available, use a biotinylated antibody to the lectin followed by an enzyme-based or fluorescence-based avidin or streptavidin detection system. Using an unlabeled antibody to the lectin, a biotinylated antibody to this anti-lectin antibody can be employed followed by a (strept)avidin detection system.

Alternatively, using an unlabeled antibody to the lectin, visualization can be achieved with a non-biotin, one-step ImmPRESS™ (peroxidase) polymer detection reagent followed by a peroxidase substrate.



Rat brain: Biotinylated Dextran Amine – Texas Red® labeled fibers and terminals stained with VECTASTAIN® Elite® ABC (brown, DAB).



Rat frontal cortex: Biotinylated Dextran Amine – Texas Red® at injection site (red fluorescence filter).

Neuronal Tracers

Anterograde/Retrograde Tracing

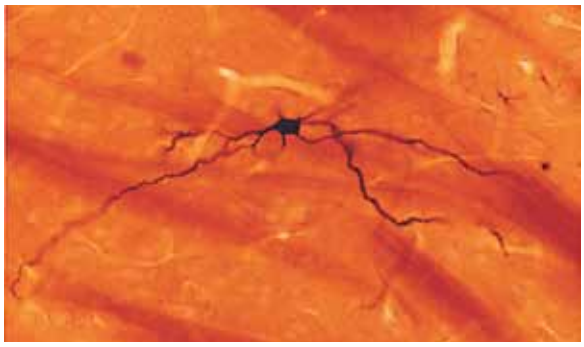
NEUROBIOTIN™ Tracer

NEUROBIOTIN™ Tracer (SP-1120) is an amino derivative of biotin that can be used as an intracellular label for cells, particularly neurons. It is used for visualizing neural architecture and for the identification of gap junction coupling.

Key advantages of NEUROBIOTIN™ Tracer over biocytin and other neuronal labels:

- Better solubility
- More efficiently iontophoresed
- Remains in cell longer
- Non-toxic
- Can be fixed with formalin or glutaraldehyde

NEUROBIOTIN™ Tracer can be used in many types of preparations including *in vivo*, whole mounts, slice preparations, or cultured cells. It can be delivered by many routes such as intracellular electrodes, microinjection, cut-loading, or scrape-loading. NEUROBIOTIN™ Tracer can be detected using avidin or streptavidin systems with either chromogenic (see Section A, "Immunohistochemistry") or fluorescence (see Section B, "Immunofluorescence") visualization methods. See summary chart on page D4.



Intracellular labeling of neurons with NEUROBIOTIN™ Tracer.

Wheat Germ Agglutinin (WGA)

Wheat Germ Agglutinin (WGA) is used for both anterograde and retrograde tracing. Vector Laboratories provides this lectin in several formats. **Peroxidase Wheat Germ Agglutinin (PL-1026)** is specially prepared for neuronal transport studies. This product is supplied at 40 mg/ml of protein in a 2 mg package ready for injection. After tissue preparation, detection can be readily achieved with a peroxidase substrate (see Section I, "Enzyme Substrates"). **Unconjugated Wheat Germ Agglutinin (L-1020)** is available as a salt-free, lyophilized powder. This lectin can be detected with an unconjugated or biotinylated antibody to the lectin. The antibody can be detected with an avidin or streptavidin system with either a chromogenic (see Section A, "Immunohistochemistry") or fluorescence (see Section B, "Immunofluorescence") visualization method. Alternatively, our non-biotin, one-step ImmPRESS™ polymer detection reagent can be used followed by a peroxidase substrate (see Section A, "Immunohistochemistry"). **Biotinylated Wheat Germ Agglutinin (B-1025)** is provided as a solution and can be detected with an avidin or streptavidin system and either a chromogenic (see Section A, "Immunohistochemistry") or fluorescence (see Section B, "Immunofluorescence") visualization method. **Fluorescein Wheat Germ Agglutinin (FL-1021)** and **Rhodamine Wheat Germ Agglutinin (RL-1022)** are provided in solution and can be visualized directly by fluorescence microscopy or with antibodies to fluorescein or rhodamine.

Product	Catalog Number	Unit Size
NEUROBIOTIN™ Tracer	SP-1120	50 mg
Wheat Germ Agglutinin (WGA), peroxidase labeled	PL-1026	2 mg
Wheat Germ Agglutinin (WGA), unconjugated	L-1020	10 mg
		25 mg
Wheat Germ Agglutinin (WGA), biotinylated	B-1025	5 mg
Wheat Germ Agglutinin (WGA), fluorescein-labeled	FL-1021	5 mg
		10 mg
Wheat Germ Agglutinin (WGA), rhodamine-labeled	RL-1022	5 mg
		10 mg
Anti-Wheat Germ Agglutinin Antibodies		
Anti-Wheat Germ agglutinin, unconjugated, made in goat	AS-2024	1 mg
Anti-Wheat Germ agglutinin biotinylated, made in goat	BA-0024	0.5 mg

Neuronal Tracers (continued)

Anterograde Tracing

Biotinylated Dextran Amines

Biotinylated Dextran Amine – Fluorescein (SP-1130)
Biotinylated Dextran Amine – Texas Red® (SP-1140)

Used as anterograde tracers, our Biotinylated Dextran Amines are approximately 10,000 MW and are conjugated with either fluorescein (BDA-F) or Texas Red® (BDA-TR). These tracers can be effectively introduced by iontophoretic or pressure injection methods. The fluorochrome label allows easy location of the injection site using a fluorescence microscope. Details of labeled fibers and fibrillar termini can be observed after detection with an avidin or streptavidin system such as a VECTASTAIN® Elite® ABC kit and a peroxidase substrate (see Section A, "Immunohistochemistry").

Phaseolus Vulgaris Leucoagglutinin (PHA-L)

The lectin *Phaseolus vulgaris* leucoaggutinin (L-1110) is an excellent, specific marker for tracing efferent neuronal projections. After iontophoretic injection of PHA-L, the approximate rate of anterograde transport is about 4-6 mm/day, and survival periods of over 18 days have been observed. Once transported, the PHA-L is visualized with an antibody to the lectin. The antibody can be detected with an avidin or streptavidin system with either a chromogenic (see Section A, "Immunohistochemistry") or fluorescence (see Section B, "Immunofluorescence") visualization method. Alternatively, a non-biotin, one-step ImmPRESS™ polymer detection reagent can be used followed by a peroxidase substrate (see Section A, "Immunohistochemistry"). A protocol is available on our website.

Neuronal Tracing and Associated Reagents

Product	Conjugate	Catalog Number	Direction of Transport	Molecular Weight	Fixable	Means of Detection	Unit Size
NEUROBIOTIN® Tracer		SP-1120	Anterograde, Retrograde	288	Yes	Fluorochrome or enzyme labeled avidin/streptavidin	50 mg
Wheat Germ Agglutinin (WGA)	Unconjugated	L-1020	Anterograde, Retrograde	36,000	Yes	Anti-WGA antibody (unconjugated or biotinylated)	10 mg
							25 mg
	Peroxidase	PL-1026	Anterograde, Retrograde	>76,000	Yes	Peroxidase substrate	2 mg
	Biotin	B-1025	Anterograde, Retrograde	36,000	Yes	Fluorochrome or enzyme labeled avidin/streptavidin	5 mg
	Fluorescein	FL-1021	Anterograde, Retrograde	36,000	Yes	Direct fluorescence	5 mg
							10 mg
	Rhodamine	RL-1022	Anterograde, Retrograde	36,000	Yes	Direct fluorescence	5 mg
							10 mg
Biotinylated Dextran Amine (BDA)	Fluorescein (BDA-F)	SP-1130	Anterograde	10,000	Yes	Direct fluorescence; enzyme labeled avidin/streptavidin	10 mg
	Texas Red® (BDA-TR)	SP-1140	Anterograde	10,000	Yes	Direct fluorescence; enzyme labeled avidin/streptavidin	10 mg
Phaseolus vulgaris leucoagglutinin (PHA-L)	Unconjugated	L-1110	Anterograde	126,000	Yes	Anti-PHA (E+L) antibody (unconjugated or biotinylated)	5 mg
Anti-*Phaseolus vulgaris* Agglutinin Antibodies							
Anti-*Phaseolus vulgaris* Agglutinin (E+L), made in goat,	Unconjugated	AS-2224	For detection of *Phaseolus vulgaris* leucoagglutinin (PHA-L)				1 mg
Anti-*Phaseolus vulgaris* Agglutinin (E+L), made in rabbit	Unconjugated	AS-2300	For detection of *Phaseolus vulgaris* leucoagglutinin (PHA-L)				1 mg
Anti-*Phaseolus vulgaris* Agglutinin (E+L), made in goat	Biotin	BA-0224	For detection of *Phaseolus vulgaris* leucoagglutinin (PHA-L)				0.5 mg

Neuronal Glycobiology Reagents

The lectins listed below are useful in tissue staining applications. Full descriptions of these products can be found in Section K, "Lectins and Glycobiology Reagents", pages K8-K17.

Lectin	Labeling Applications	Page No.
Concanavalin A	Purkinje cell somata and dendrites of rat cerebellum; rat perineuronal glial nets; kainate receptors	K8
Dolichos biflorus Agglutinin	mouse embryonic stem cell marker, mouse olfactory receptor neurons	K9
Griffonia (Bandeiraea) simplicifolia I, Isolectin B_4	non-peptidergic primary afferent neurons; ameboid microglia; rat, mouse and rabbit reactive and ramified microglia; rat dorsal root ganglion and sensory ganglion	K10
Lycopersicon esculentum Lectin	rat ameboid and ramified microglia	K11
Peanut Agglutinin	rat perineuronal glial nets, rat ameboid microglia, rat astrocyte-like processes, cone-specific retinal staining	K12
Ricinus communis Agglutinin I	human reactive and ramified microglia	K14
Soybean Agglutinin	rat perineuronal glial nets; rat and mouse olfactory neurons; sensory neuron soma; and central terminals in rat and cat	K15
Ulex europaeus Agglutinin I	human spinal chord substantia gelatinosa; rabbit, cat and marmoset dorsal root ganglion; primary sensory neurons	K15
Wheat Germ Agglutinin	rat perineuronal glial nets; rat ameboid microglia; rat astrocyte-like processes; substantia gelatinosa; dorsal root ganglion	K16

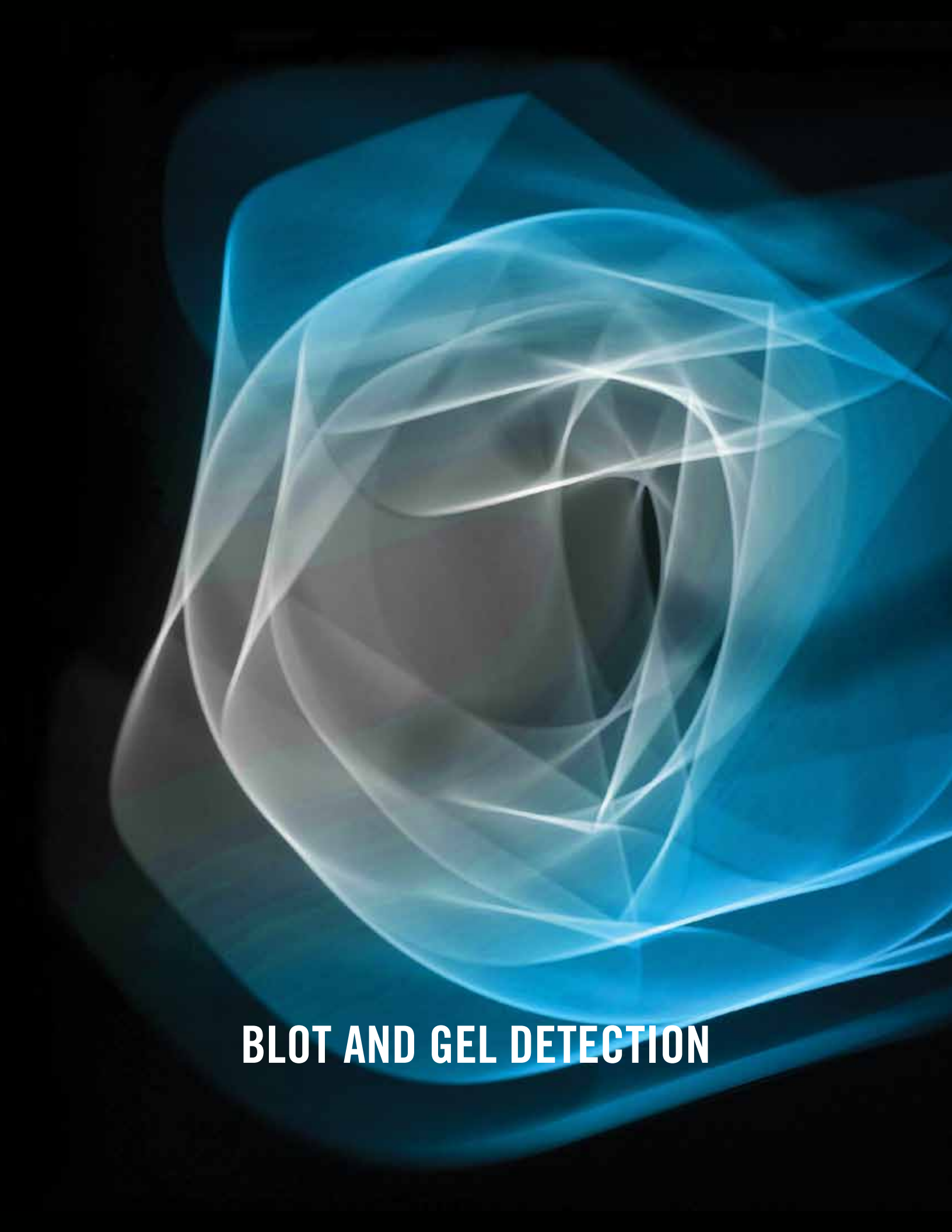
D

Primary Antibodies Against Brain and Neural Antigens

Full descriptions of these products can be found in Section G, "Antibodies", pages G2-G25.

Antibody	Clone	Catalog Number	Applications	Working Dilution	Unit Size
Alpha-Synuclein	KM51	VP-A106	***HE***	1:20 - 1:40	1 ml
Amyloid Precursor Protein	3G12	VP-A109	***HE***	1:25 - 1:50	1 ml
Beta Amyloid	6F/3D	VP-B203	***P***	1:50	1 ml
Calbindin	KR6	VP-C301	***H***	1:100 - 1:200	1 ml
Choline Acetyltransferase	38B12	VP-C383	***HE***	1:25 - 1:50	1 ml
Glial Fibrillary Acidic Protein	GA5	VP-G805	***P***, ***F***	1:100	1 ml
Neuroblastoma Marker	NB84a	VP-N750	***P***, ***F***, ***C***	1:100 - 1:200	1 ml
Neurofilament 200 kD	RT97	VP-N752	***P***, ***F***	1:50	1 ml
	N52.1.7	VP-N753	***H***, ***F***	1:25 - 1:400	1 ml
Neurofilament 68 kD	NR4	VP-N754	***H***, ***F***	1:20 - 1:50	1 ml
Neuron-Specific Enolase	5E2	VP-N755	***P***, ***F***, ***W***	1:50 - 1:100	1 ml
Parvalbumin (Alpha)	2E11	VP-P963	***H***	1:200 - 1:400	1 ml
Peripherin	PJM50	VP-P968	***H***, ***F***, ***W***, ***O***	1:100 - 1:200	1 ml
Protein Gene Product 9.5	10A1	VP-P983	***H***, ***F***, ***W***	1:20 - 1:40	1 ml
S-100 Protein	S1/61/69	VP-S275	***P***, ***F***	1:20 - 1:40	1 ml
	Rabbit Polyclonal	VP-S276	***P***, ***F***	1:200 - 1:400	1 ml
Synaptophysin	27G12	VP-S285	***H***, ***F***, ***W***	1:100 - 1:200	1 ml
	Rabbit Monoclonal, SP11	VP-RM09	***H***, ***W***	1:100	0.5 ml
Tau	Tau-2	VP-T476	***H***	1:50 - 1:100	1 ml
Tyrosine Hydroxylase	1B5	VP-T489	***H***, ***W***	1:20 - 1:40	1 ml

Key: ***P***: Paraffin sections, no pretreatment • ***T***: Paraffin, trypsin pretreatment • ***H***: Paraffin, high temperature antigen unmasking (citrate) • ***HE***: Paraffin, high temperature antigen unmasking (Tris) • ***F***: Frozen sections • ***I***: Immunofluorescence • ***W***: Western blotting • ***C***: Flow cytometry • ***O***: Other



BLOT AND GEL DETECTION

Introduction

Purification, characterization, and identification of proteins or nucleic acids often require analysis of the target in blotting applications. Proteins or nucleic acids are usually separated by size using gel electrophoresis and transferred to a membrane with a blotting technique.

A specific protein can be identified from a mixture using different detection methods. Primary antibodies can be used to recognize unique epitopes or genetically engineered fusion protein tags. Lectins with specificities to certain carbohydrate residues can be used to probe for a particular glycoprotein. These reagents can then be detected and visualized with an enzymatic or fluorescent detection method.

Nucleic acid blotting applications also require high specificity which is derived from the uniqueness of the target sequence and the fidelity of the complementary probe. The target sequence is detected by a complementary probe labeled with tags such as biotin, digoxigenin (DIG), DNP, or fluorescein. The label is then detected with an antibody to the tag and subsequent detection reagents.

Detection sensitivity for both protein and nucleic acid blotting applications depends on the abundance of the target and the quality of the reagents available for detecting the primary antibody or hybridized probe.

Protein Blot Detection Reagents

Vector Laboratories offers a number of reagents for the detection of blotted protein samples. These reagents can recognize epitopes, fusion protein tags, or carbohydrate groups in the targeted protein. The choice of reagents will depend on the nature of the target protein and the chosen visualization method. Sensitivity, reproducibility, and ease of use are important considerations when choosing detection reagents.

One-Step Fluorescence Detection. Fluorochrome conjugated secondary antibodies may be used to detect an unconjugated primary antibody on a blot. These fluorescent detection reagents are used in conjunction with the proper membrane and visualization equipment. This method is fast and simple and allows multiple targets to be visualized simultaneously. Fluorescent reagents are listed and described in detail in Section B, "Immunofluorescence". This section focuses on the more common enzyme-based detection methods.

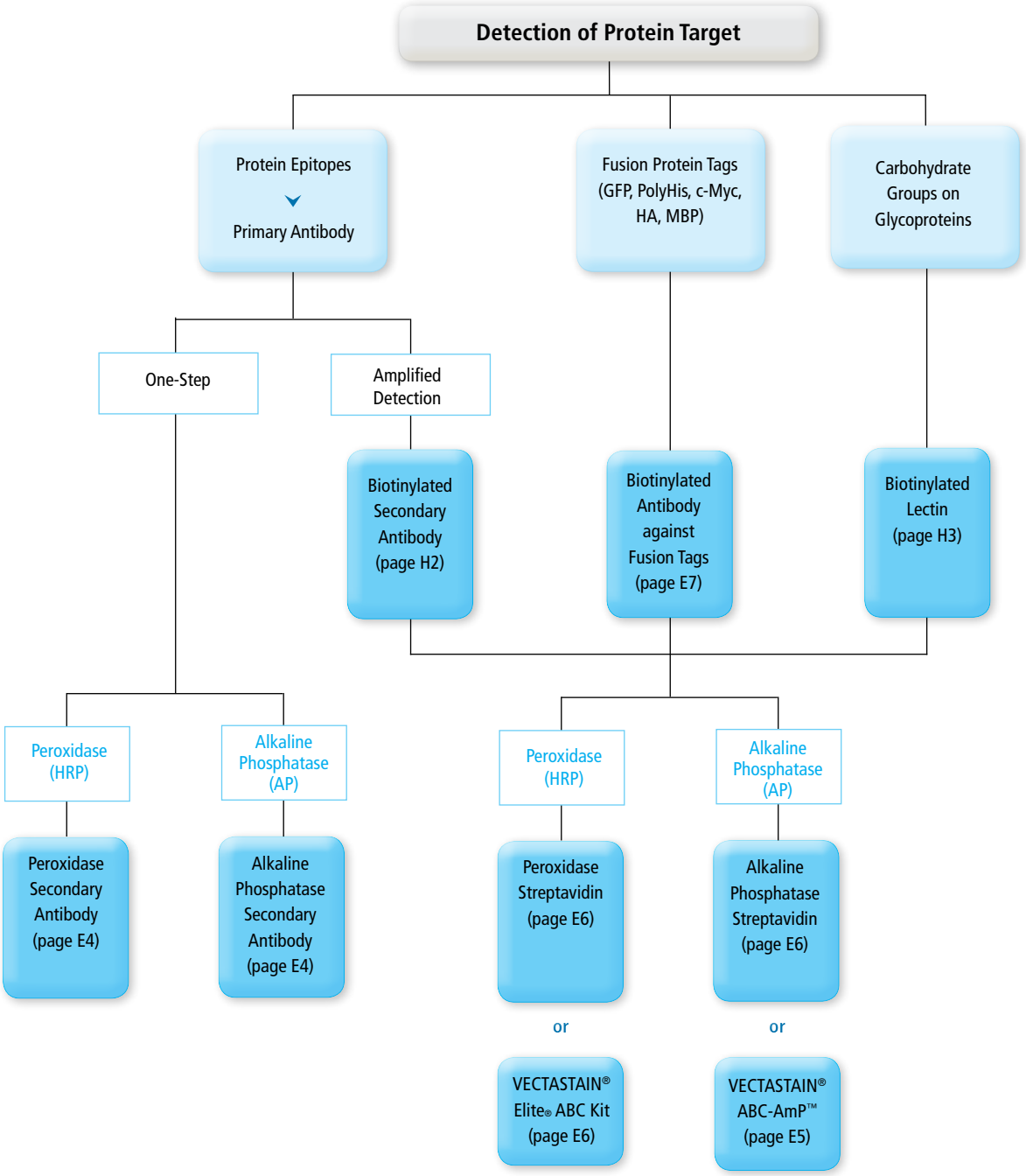
One-Step Enzymatic Detection. One-step enzymatic detection offers greater sensitivity and more versatility due to the types of detection reagents and enzyme substrates available. For one-step detection, alkaline phosphatase (AP) and peroxidase (HRP) conjugated secondary antibodies are available.

Amplified Detection with Biotin/Avidin and Biotin/Streptavidin Systems. Biotin/avidin or biotin/streptavidin detection systems offer the greatest sensitivity by signal amplification and the greatest flexibility in detection methods. The VECTASTAIN® ABC-AmP™ kits, for example, are complete detection systems that offer sensitivity, reliability, and versatility. Streptavidin or avidin based reagents can detect a variety of macromolecules on the blot: biotinylated secondary antibodies (page H2), biotinylated antibodies against fusion protein tags (page E7), biotinylated lectins (page H3), and even biotinylated target proteins blotted directly onto the membrane.

Enzyme Substrates. Substrates that produce either a visible chromogenic precipitate or a chemiluminescent/chemifluorescent signal can be used for visualization. Alkaline phosphatase allows sustained substrate conversion and increased sensitivity with low background. Peroxidase has faster reaction kinetics resulting in a more rapid signal development. Vector Laboratories offers a variety of proprietary peroxidase and alkaline phosphatase substrates that not only satisfies sensitivity requirements and personal preferences, but also allows multiple targets to be visualized simultaneously on the blot. In addition, our DuoLuX™ Chemiluminescent/Chemifluorescent substrates provide highly sensitive chemiluminescent and chemifluorescent visualization.

The flow chart that follows is a guide to several available options to detect protein epitopes, fusion tagged proteins, or glycoproteins. Substrates are included with the VECTASTAIN® ABC-AmP™ kits and are also available separately (see page E10).

Reagent Options for Enzymatic Detection of Proteins on Blots



Protein Blot Detection Reagents (continued)

One-step Detection

Western blot targets are commonly detected with primary antibodies specific to epitopes on the target protein. These antibodies are, in turn, detected with a species-specific secondary antibody. Choose the appropriate secondary antibody based on the species of the primary antibody and desired visualization method.

Fluorescent Secondary Antibodies. Fluorescent detection involves the use of a fluorescent secondary antibody that is directly visualized. Vector Laboratories offers a variety of fluorescently labeled secondary antibodies including DyLight® conjugates (see Section B, "Immunofluorescence", pages B4-B5, for a complete listing). For best signal-to-noise ratio, antibody dilution factors may require optimization.

Enzyme-Conjugated Secondary Antibodies. A wide range of high affinity secondary antibody enzyme conjugates is available for chromogenic or chemiluminescent/chemifluorescent visualization of a target on a blot. The antibodies are purified by affinity chromatography. Cross-reactivities that are likely to interfere with specific labeling are removed by solid phase adsorption techniques. Antibodies are conjugated to either peroxidase or alkaline phosphatase ensuring the maximum degree of labeling with high activity enzyme while not altering the specificity or affinity of the antibody.

The ImmPRESS™ Peroxidase Polymer Detection reagent is a micropolymer of highly active peroxidase directly conjugated to affinity purified antibodies. These reagents achieve outstanding sensitivity with low background. The ImmPRESS™ reagents are provided as prediluted, stabilized solutions that can be further diluted for use on blots. (See also page A12-A13).

Substrates. Appropriate chromogenic or chemiluminescent/chemifluorescent enzyme substrates are listed on page E10.

Enzyme-Conjugated Secondary Antibodies

Antibody	Conjugate	Catalog Number	Unit Size	Concentration
Mouse				
Anti-Mouse IgG (H+L), made in horse	Peroxidase	PI-2000	1 mg	1 mg/ml
	ImmPRESS™ (peroxidase polymer)	MP-7402	50 ml	N/A
	Alkaline Phosphatase	AP-2000	1 ml	1 mg/ml
Rabbit				
Anti-Rabbit IgG (H+L), made in goat	Peroxidase	PI-1000	1 mg	1 mg/ml
	ImmPRESS™ (peroxidase polymer)	MP-7401	50 ml	N/A
	Alkaline Phosphatase	AP-1000	1 ml	1 mg/ml
Human				
Anti-Human IgG (H+L), made in goat	Peroxidase	PI-3000	1 mg	1 mg/ml
	Alkaline Phosphatase	AP-3000	1 ml	1 mg/ml
Rat				
Anti-Rat IgG (H+L), made in goat	ImmPRESS™ (peroxidase polymer)	MP-7404	50 ml	N/A
Goat				
Anti-Goat IgG (H+L), made in horse	Peroxidase	PI-9500	1 mg	1 mg/ml
	ImmPRESS™ (peroxidase polymer)	MP-7405	50 ml	N/A
	Alkaline Phosphatase	AP-9500	1 ml	1 mg/ml
Universal				
Anti-Mouse/Rabbit IgG	ImmPRESS™ (peroxidase polymer)	MP-7500	50 ml	N/A

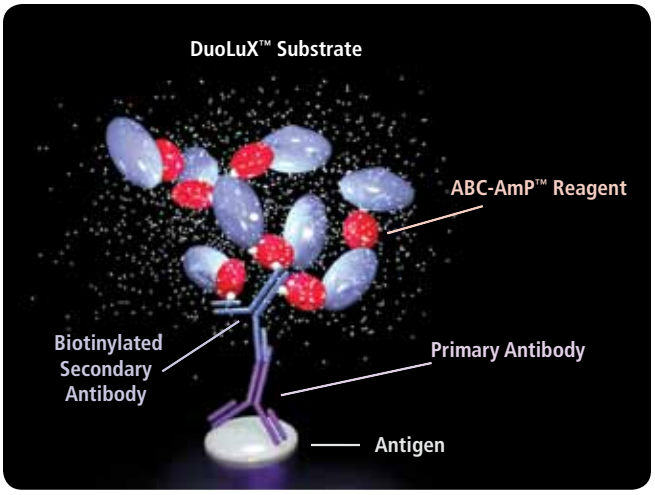
Amplified Detection

Greater sensitivity can be achieved by using an amplified detection procedure. Our biotin/avidin and biotin/streptavidin systems introduce a large number of enzymes to the target site providing signal amplification while maintaining low background.

VECTASTAIN® ABC-AmP™ Detection Kits

The VECTASTAIN® ABC-AmP™ is an amplified ABC alkaline phosphatase reagent for the detection of mouse or rabbit primary antibodies on nitrocellulose or PVDF membranes. The signal can be visualized using either a chemiluminescent/chemifluorescent or a chromogenic substrate. Used with the DuoLuX™ Chemiluminescent/Chemifluorescent Substrate, the VECTASTAIN® ABC- AmP™ system produces a very high and sustained light emission signal, with low background, and permanent fluorescence.

The sensitivity of VECTASTAIN® ABC-AmP™ Reagent combined with the DuoLux™ substrate allows for the detection of as little as 1 pg of target protein.



Protein detection using VECTASTAIN® ABC-AmP™ Reagent and DuoLuX™ Substrate

E

VECTASTAIN® ABC-AmP™ Kits

Product	Catalog Number	Unit Size	Visualization	Kit Components	Approximate Number of 100 cm² blots developed
VECTASTAIN® ABC-AmP™ Kit, Chromogenic Western Blot Detection, For Rabbit IgG	AK-6401	1 Kit	Chromogenic	VECTASTAIN® ABC-AmP™ Reagent 10x Casein Solution Biotinylated Anti-Rabbit IgG BCIP/NBT Substrate	20
VECTASTAIN® ABC-AmP™ Kit, Chromogenic Western Blot Detection, For Mouse IgG	AK-6402	1 Kit	Chromogenic	VECTASTAIN® ABC-AmP™ Reagent 10x Casein Solution Biotinylated Anti-Mouse IgG BCIP/NBT Substrate	20
VECTASTAIN® ABC-AmP™ Kit, Chemiluminescent Western Blot Detection, For Rabbit IgG	AK-6601	1 Kit	Chemiluminescent/ Chemifluorescent	VECTASTAIN® ABC-AmP™ Reagent 10x Casein Solution Biotinylated Anti-Rabbit IgG DuoLuX™ Substrate	20
VECTASTAIN® ABC-AmP™ Kit, Chemiluminescent Western Blot Detection, For Mouse IgG	AK-6602	1 Kit	Chemiluminescent/ Chemifluorescent	VECTASTAIN® ABC-AmP™ Reagent 10x Casein Solution Biotinylated Anti-Mouse IgG DuoLuX™ Substrate	20
VECTASTAIN® ABC-AmP™ Reagent (Standard Kit)	AK-6000	1 Kit	N/A	0.5 ml Reagent A 0.5 ml Reagent B (250 ml working solution)	20

Protein Blot Detection Reagents (continued)

Biotin/Streptavidin-Based Detection Systems

If the desired method is not available in a preconfigured VECTASTAIN® ABC-AmP™ Kit, a detection system can be customized from the following reagents. The following steps will help in the selection of the appropriate system.

If required, other biotinylated reagents (e.g. biotinylated anti-fusion tag antibodies or biotinylated lectins) may be used in place of the biotinylated secondary antibody (see page E7).

1) Based on the species of the primary antibody, choose the biotinylated secondary antibody.

A complete list of biotinylated antibodies can be found in Section H, "Biotin and Avidin/Streptavidin Reagents", page H2.

Species of Primary Antibody	Product	Catalog Number	Unit Size
Goat	Biotinylated Rabbit Anti-Goat IgG	BA-5000	1.5 mg
Hamster	Biotinylated Goat Anti-Hamster IgG	BA-9100	1.5 mg
Human	Biotinylated Goat Anti-Human IgG	BA-3000	1.5 mg
Mouse	Biotinylated Horse Anti-Mouse IgG	BA-2000	1.5 mg
Rabbit	Biotinylated Goat Anti-Rabbit IgG	BA-1000	1.5 mg
Rat	Biotinylated Rabbit Anti-Rat IgG	BA-4000	1.5 mg
Sheep	Biotinylated Rabbit Anti-Sheep IgG	BA-6000	1.5 mg

2) Choose the appropriate detection system.

Enzyme	Product	Catalog Number	Unit Size	Working Solution
Peroxidase	VECTASTAIN® Elite® ABC Kit	PK-6100	1 kit	100 ml
	Horseradish Peroxidase Streptavidin	SA-5004	1 mg	500 ml
Alkaline Phosphatase	VECTASTAIN® ABC-AmP™	AK-6000	1 kit	250 ml
	Alkaline Phosphatase Streptavidin	SA-5100	1 ml	1000 ml

3) Choose the blocking solution.

Product	Catalog Number	Unit Size	Working Solution	More Info (page)
10x Casein Solution	SP-5020	250 ml	2.5 L	E11
Animal-Free Blocker™	SP-5030	250 ml	1.25 L	E11
BSA	SP-5050	500 mg	250 ml of 0.2% soln	E11
Carbo-Free™ Blocking Solution[a]	SP-5040	125 ml	1.25 L	E11

[a] *This blocking reagent is especially recommended when probing blots using lectins.*

4) If necessary, choose a Streptavidin/Biotin or Avidin/Biotin Blocking Kit.

Product	Catalog Number	Unit Size	More Info (page)
Streptavidin/Biotin Blocking Kit	SP-2002	1 kit (18 ml Streptavidin and 18 ml Biotin)	E11
Avidin/Biotin Blocking Kit	SP-2001	1 kit (18 ml Avidin and 18 ml Biotin)	E11

5) Choose a substrate appropriate for the enzyme (listed on page E10).

Detection of Fusion Protein Tags

Fusion protein reagents are part of a methodology for purification, detection, and investigation of expressed proteins. The common fusion tags [green fluorescent protein (GFP), poly histidine (polyHis), c-Myc, human influenza virus hemagglutinin (HA), maltose binding protein (MBP)] can be easily detected with our antibodies against the specific tag.

Our biotinylated, affinity-purified polyclonal antibodies (produced in goat) are optimized to detect fusion proteins in western blot detection. They can be used in combination with a VECTASTAIN® ABC-AmP™ detection system or other streptavidin/avidin-based reagents.

For maximum sensitivity, GFP can also be detected using ImmPRESS™ (peroxidase) Anti-GFP antibody (made in goat). This reagent is prepared using our proprietary peroxidase micropolymer technology.

Recombinant GFP Standard (MB-0752) contains purified recombinant *Aquorea victorea* GFP (28 kD) overexpressed in *E. coli*. It is designed for quantitation of GFP fusion protein in the test sample, and can be used as a positive control on western blots in conjuction with ImmPRESS™ Anti-GFP (MB-0712) or Biotinylated Anti-GFP (BA-0702).

To choose accessory reagents for blot detection follow the steps on the previous page starting with step 2 to configure your own detection system.

Labeled Antibodies Against Fusion Protein Tags

Product	Catalog Number	Unit Size	Stock Concentration	Dilution
Biotinylated Anti-MBP, made in goat	BA-0701	0.25 mg	1 mg/ml	1:1000
Biotinylated Anti-cMyc, made in goat	BA-0703	0.25 mg	1 mg/ml	1:1000
Biotinylated Anti-HA, made in goat	BA-0704	0.25 mg	1 mg/ml	1:1000
Biotinylated Anti-polyHistidine, made in goat	BA-0705	0.25 mg	1 mg/ml	1:1000
Biotinylated Anti-GFP, made in goat	BA-0702	0.25 mg	1 mg/ml	1:1000
ImmPRESS™ (peroxidase) Anti-GFP, made in goat	MB-0712	100 µl	5 mg/ml	1:1000
Recombinant GFP Standard	MB-0752	100 µg	1 mg/ml	N/A

Detection of Glycoproteins

Detection of carbohydrate residues on blotted glycoproteins can be accomplished by using biotinylated lectins. These reagents can be detected with a VECTASTAIN® ABC-AmP™ detection system or other biotin/streptavidin- or biotin/avidin-based reagents.

Lectin descriptions and product listings can be found in Section K, "Lectins and Glycobiology Reagents".

When probing with a biotinylated lectin, we recommend blocking the blot with the Carbo-Free™ Blocking Solution (SP-5040) as other blocking agents may contain glycoproteins that may introduce background.

To choose accessory reagents for blot detection, follow the steps on the previous page starting with step 2 to configure a customized detection system.

Nucleic Acid Blot Detection Reagents

Vector Laboratories offers a number of reagents that can be used to detect blotted nucleic acid samples. The label on the nucleic acid probe determines the choice of reagents. For labeling of nucleic acid probes see Section F, "Labeling Reagents" pages F2-F7.

Biotin - Biotin is one of the most commonly used labels for nucleic acid probes. Because of the extraordinary affinity of avidin and streptavidin for biotin and the many biotin-avidin/streptavidin systems available, this label is ideal for a variety of applications. The UltraSNAP™ Detection Kit is designed for the detection of biotinylated nucleic acid probes in Southern blots (see page E9).

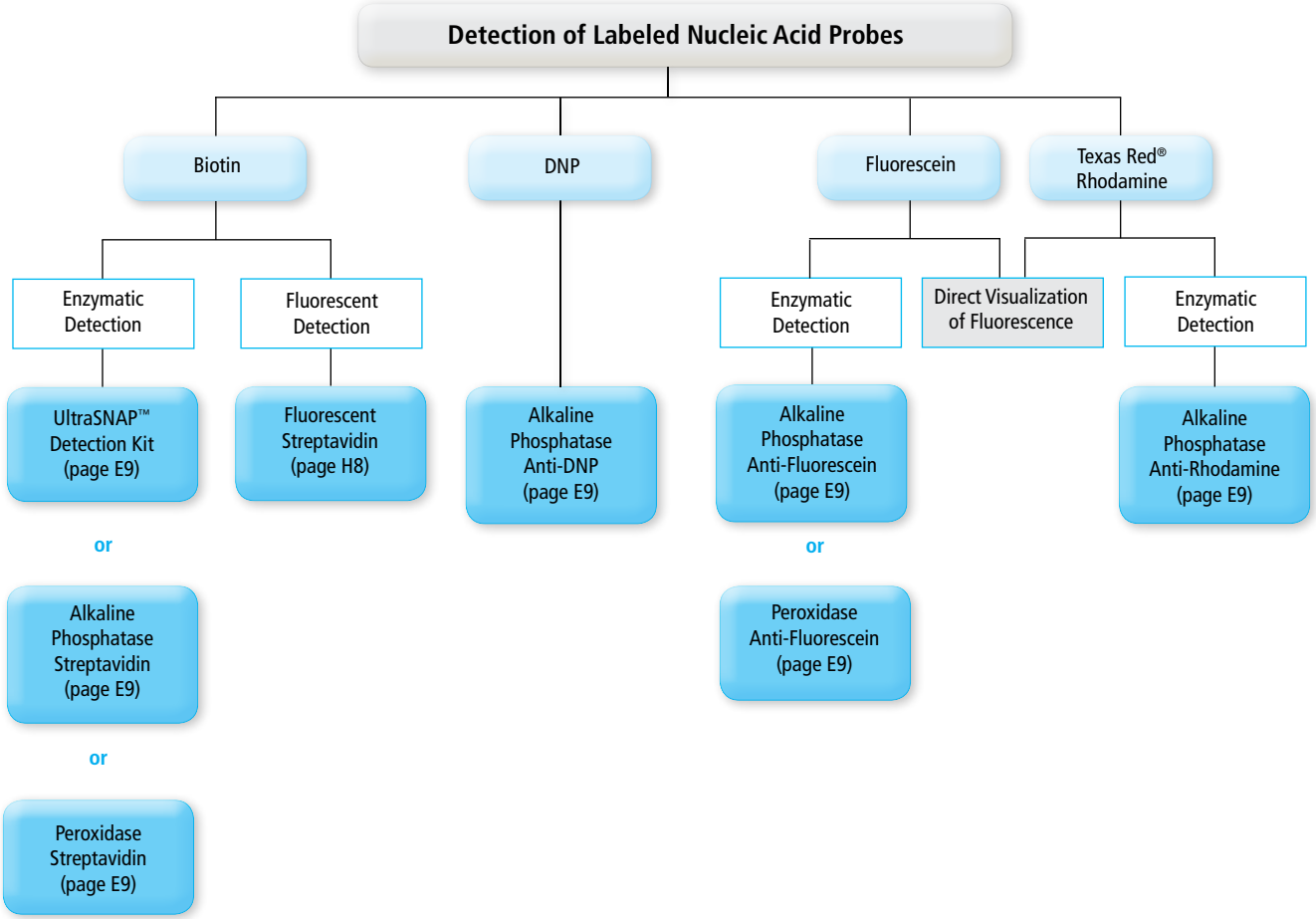
DNP (Dinitrophenyl) - DNP is an excellent alternative to biotin. A high affinity, purified anti-DNP antibody labeled with alkaline phosphatase or biotin is available for detection or amplification of the target.

Fluorescein - Fluorescein can be directly visualized (ex 495 nm; em 515 nm) but is generally detected using a biotin or enzyme conjugated, affinity-purified, anti-fluorescein antibody.

Texas Red® - Texas Red®, a rhodamine fluorochrome, has a high quantum yield (ex 595 nm; em 615 nm), but generally is not visualized directly. The label is usually detected using a high affinity, alkaline phosphatase or biotin-conjugated antibody to rhodamine.

For available detection options see the chart below.

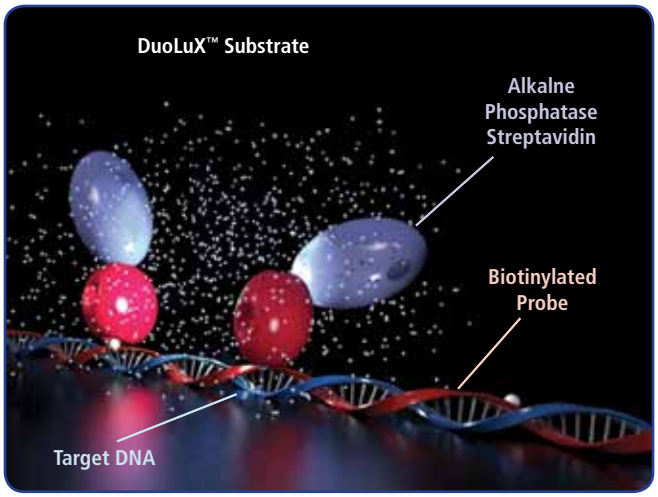
Reagent options for nucleic acid probe detection or blots

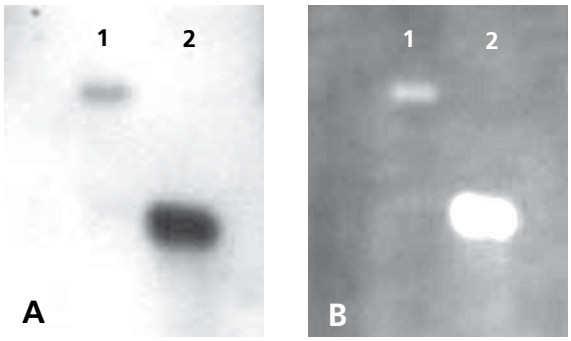


UltraSNAP™ Detection System for Southern Blots

The UltraSNAP™ Detection Kit is designed to detect biotinylated nucleic acids on nylon or nitrocellulose. Biotin can be incorporated into a nucleic acid probe using PHOTOPROBE® Biotin, FastTag®, 3′ or 5′ EndTag™ Labeling Systems or other established enzymatic methods (see Section F, "Labeling Reagents", pages F2-F7).

The UltraSNAP™ kit consists of Alkaline Phosphatase Streptavidin, the DuoLuX™ Chemiluminescent/Chemifluorescent Substrate as well as specially optimized PolyBlock™ Blocking Reagent and washing solutions formulated to produce high sensitivity with low background.





Chemiluminescent [A] and fluorescent [B] detection of a single-copy gene in a yeast genomic digest (lane 1) and a control plasmid DNA (lane 2) using the UltraSNAP™ Kit. Both images were acquired from the same blot.

Streptavidin coupled to alkaline phosphatase binds to the biotinylated probe on the blot. The probe is visualized by the conversion of the DuoLuX™ Chemiluminescent/Chemifluorescent Substrate to a luminescent and fluorescent product by alkaline phosphatase. The sensitivity is enhanced with the use of the PolyBlock™ Blocking Reagent and wash buffers included in the kit which are specifically designed to minimize background.

The kit can be used to develop approximately twenty 100 cm^2 blots.

Substrates for development can be found on page E10.

Nucleic Acid Blot Detection Reagents

Label to be Detected	Detection System/Reagent	Catalog Number	Components/Unit Size
Biotin	UltraSNAP™ Detection Kit	MB-6500	600 µl Alkaline Phosphatase Streptavidin 120 ml 10x Polyblock™ Blocking Reagent 120 ml 20x Wash A 120 ml 10x Wash B 100 ml DuoLuX™ Substrate
	Alkaline Phosphatase Streptavidin	SA-5100	1 ml
	Peroxidase Streptavidin	SA-5004	1 mg
DNP	Alkaline Phosphatase Anti-DNP, made in rabbit	MB-3100	150 µg
Fluorescein	Alkaline Phosphatase Anti-Fluorescein, made in goat	MB-2100	150 µg
	Peroxidase Anti-Fluorescein, made in goat	SP-1910	0.5 mg
Texas Red®/Rhodamines	Alkaline Phosphatase Anti-Rhodamine*, made in goat	MB-1920	150 µg

** Detects most rhodamines including Texas Red®*

Enzyme Substrates

Targets that are detected with an enzyme conjugate can be visualized with an appropriate enzyme substrate that produces a chemiluminescent/chemifluorescent or chromogenic reaction product. Complete substrate descriptions can be found in Section I, "Enzyme Substrates".

The **DuoLuX™ Chemiluminescent/Chemifluorescent Substrate** is a unique formula based on acridan chemistry that produces both a strong chemiluminescent and fluorescent signal. The half-life of luminescent emission exceeds that of many other luminescent substrates. Reacted DuoLuX™ substrate luminesces in the blue range with a peak emission at 453 nm. This emitted light can be captured by film or with an imager. The fluorescence excitation maximum is at 405 nm, but other wavelengths (254 nm and 365 nm) can also be used. Maximum fluorescent emission occurs at 453 nm.

DuoLuX™ Substrate can be used for both peroxidase and alkaline phosphatase. The DuoLuX™ Chemiluminescent/Chemifluorescent Substrate for peroxidase (SK-6604) is supplied in two bottles, consisting of 100 ml of the DuoLuX™ Substrate (Reagent 1) and 100 ml of a peroxidase converter solution (Reagent 2). Reagents 1 and 2 are mixed in equal volumes just prior to use, providing 200 ml of peroxidase substrate working solution. The DuoLuX™ Chemiluminescent/Chemifluorescent Substrate for alkaline phosphatase (SK-6605) is supplied in ready-to-use form, consisting of 100 ml of ready-to-use substrate solution.

Chromogenic substrates can also be used to visualize targets detected with enzyme-based systems. The substrates deposit a colored precipitate directly on the membrane, eliminating the additional steps of exposing and developing film, or image capture. The variety of chromogenic substrates also allows multiple targets to be visualized simultaneously on the blot.

Enzyme Substrates

Enzyme	Substrate	Catalog Number	Unit Size	Visualization/Color	Working Solution
Peroxidase	DuoLuX™ for HRP	SK-6604	200 ml	Chemiluminescent/Chemifluorescent	200 ml
	DAB	SK-4100	1 Kit	Brown	300 ml
	DAB +Ni	SK-4100	1 Kit	Gray-Black	300 ml
	ImmPACT™ DAB	SK-4105	120 ml	Brown	120 ml
	Vector® VIP	SK-4600	1 Kit	Purple	300 ml
	Vector® SG	SK-4700	1 Kit	Blue-Gray	300 ml
	Vector® NovaRED™	SK-4800	1 Kit	Red	300 ml
	AEC	SK-4200	1 Kit	Red	300 ml
	TMB	SK-4400	1 Kit	Blue	300 ml
Alkaline Phosphatase	DuoLuX™ for AP	SK-6605	100 ml	Chemiluminescent/Chemifluorescent	100 ml
	Vector® Red	SK-5100	1 Kit	Magenta	200 ml
	Vector® Blue	SK-5300	1 Kit	Blue	200 ml
	Vector® Black	SK-5200	1 Kit	Black	200 ml
	BCIP/NBT	SK-5400	1 Kit	Indigo	200 ml

Blocking Reagents

10x Casein Solution

10x Casein Solution (SP-5020) is a general blocking agent for nucleic acid and protein blotting, histochemical and other applications. It is supplied as 250 mls of a 10x concentrate.

Animal-Free Blocker™

Animal-Free Blocker™ (SP-5030) is a plant-derived blocking agent and diluent for immunohistochemical, nucleic acid or protein blotting, and for 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. It is supplied as 250 ml of a 5x concentrate.

Carbo-Free™ Blocking Solution

Carbo-Free™ Blocking Solution (SP-5040) is a protein-based agent intended for use as a general blocking or diluent solution for blotting, ELISA, and immunohistochemical applications. Unlike serum, nonfat dry milk, casein, or other common protein-containing blocking agents, this product is essentially free of glycoproteins. This solution is ideal for applications using lectins in which glycoprotein contamination could generate background staining or false positive results. It is supplied as 125 ml of a 10x concentrate.

Bovine Serum Albumin (BSA)

Immunohistochemical Grade

This ultrapure grade of bovine serum albumin (BSA; SP-5050) can be used as a diluent or a blocking agent. It is free of impurities present in other grades of BSA which can introduce artifacts or increase background staining in immunohistochemical staining, ELISAs, or blot development.

Avidin/Biotin Blocking Kit

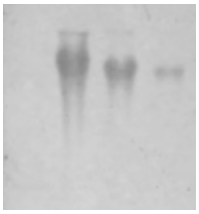
Avidin/Biotin Blocking Kit (SP-2001) blocks all endogenous biotin, biotin receptors, and avidin binding sites. This kit is designed for use with biotin/avidin detection systems such as the VECTASTAIN® ABC Kits if avidin or biotinylated reagents bind nonspecifically to tissues or proteins. This blocking kit consists of 18 ml of Avidin D and 18 ml of biotin in convenient dropper bottles.

Streptavidin/Biotin Blocking Kit

Streptavidin/Biotin Blocking Kit (SP-2002) blocks all endogenous biotin, biotin receptors, and streptavidin binding sites. This kit is designed for use with biotin/streptavidin detection systems such as the VECTASTAIN® Universal Quick kits if streptavidin or biotinylated reagents bind nonspecifically to tissues or proteins. This blocking kit consists of 18 ml of streptavidin and 18 ml of biotin in convenient dropper bottles.

HYBEX™ Hybridization Solution

HYBEX™ Hybridization Solution (MB-1230) is a ready-to-use hybridization formula for general hybridization of nucleic acid probes in membrane-based assays. HYBEX™ Hybridization Solution contains no formamide and can be used with both nylon and nitrocellulose membranes. It is supplied as 200 ml of working solution.



Detection of streptavidin gene in DNA of S. avidinii. The blots with 10, 5 or 1 µg of S. avidinii genomic DNA digests were hybridized with a biotin-labeled probe specific to streptavidin gene (75 ng/ml in HYBEX™ Hybridization Solution) and subsequently detected with alkaline phosphatase-streptavidin and BCIP/NBT.

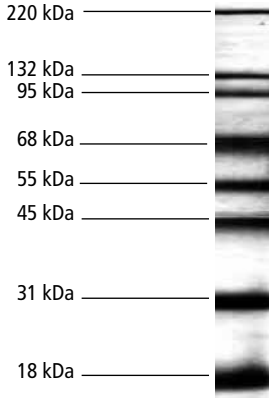
Blocking Reagents

Product	Catalog Number	Stock	Unit Size
10x Casein Solution	SP-5020	10x	250 ml
Animal-Free Blocker™	SP-5030	5x	250 ml
Carbo-Free™ Blocking Solution	SP-5040	10x	125 ml
Bovine Serum Albumin (BSA)	SP-5050	-	500 mg
Avidin/Biotin Blocking Kit	SP-2001	18 ml Avidin D 18 ml Biotin	1 Kit
Streptavidin/Biotin Blocking Kit	SP-2002	18 ml Streptavidin 18 ml Biotin	1 Kit
HYBEX™ Hybridization Solution	MB-1230	1x	200 ml

Molecular Weight Markers

Vector® Biotinylated Protein Molecular Weight Markers

Vector® Biotinylated Protein Molecular Weight Markers (SP-1400) provide ideal standards for western blots. This product consists of eight distinct biotinylated protein bands with a molecular weight range from 19 kDa to 222 kDa. It is designed to be run in an SDS-PAGE under denaturing conditions. This mixture of biotinylated proteins can be run alongside the sample under study and, following transfer, developed with any avidin or streptavidin-based detection system.



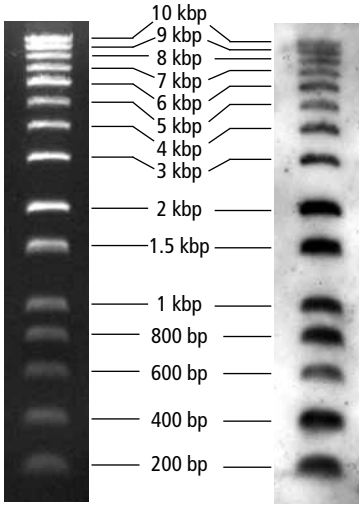
Biotinylated protein molecular weight marker separated by 7.5% denaturing PAGE, transferred to nitrocellulose, and detected with VECTASTAIN® ABC-AmP™, using BCIP/NBT substrate.

Vector® DNA Molecular Weight Markers

This product is an electrophoresis standard consisting of 15 bands between 0.2 and 10 kilobase pairs. Bands are regularly spaced so that sizes are easy to identify. Markers are supplied ready-to-use containing loading dye so that electrophoresis can be monitored.

The Unlabeled DNA Molecular Weight Marker (MB-1301) is used when the marker is to be visualized in a gel by ethidium bromide or other DNA stains.

The Biotinylated DNA Molecular Weight Marker (MB-1302) is used when nucleic acids are to be transferred to a membrane and later detected using an avidin or streptavidin-based system.



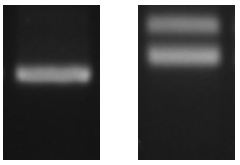
Ready-to-use marker (5 µl or 500 ng) was loaded on a 0.5% agarose gel. The gel was run in 1x TBE at 10 V/cm, stained with ethidium bromide (left) or blotted onto nylon membrane using standard methods. Biotinylated DNA bands (right) were detected using the UltraSNAP™ Kit (Alkaline Phosphatase Streptavidin followed by DuoLuX™ Chemiluminescent Substrate).

Product	Catalog Number	Unit Size	Number of Applications
Biotinylated Protein Molecular Weight Markers	SP-1400	250 µl	50
DNA Molecular Weight Markers, Unlabeled	MB-1301	25 µg	50
DNA Molecular Weight Markers, Biotinylated	MB-1302	25 µg	50

Sequence Specific DNA Ligands

Resolve-It™ Kit

Sequence specific DNA ligands bind to GC- or AT-rich sites on DNA and retard the electrophoretic migration of DNA in a sequence specific manner. This ability is critical for applications such as differential display in which multiple DNA species of similar size need to be separated before subsequent excision and analysis. The Resolve-It™ Kit contains two ligands. AT-Yellow™, a bisbenzimide-PEG conjugate, similar to the discontinued HA-Yellow, binds to AT-rich regions; GC-Red™, a phenyl neutral red-PEG conjugate, similar to HA-Red binds GC-rich regions. Either ligand is added to the agarose during gel preparation, and, during electrophoresis, ligand interaction retards the mobility of DNA depending on the amount of ligand bound. The quantity of each ligand supplied is sufficient to prepare about 200 ml of agarose gel.



A B

Two 600 bp DNAs of different sequence form a single band using typical electrophoretic conditions (A). The same DNAs are separated into two distinct bands in a gel containing Resolve-It™ AT-Yellow™ (B).

Product	Catalog Number	Unit Size
Resolve-It™ Kit	MB-1401	1 kit

LABELING REAGENTS

Nucleic Acid Labeling

Choosing a Labeling Method

When labeling nucleic acids, two considerations determine which labeling system will work best for a given application:

- size and type of the nucleic acid to be labeled
- choice of label required for the application

Size and Type

Longer strands of DNA (>100 bp), circular DNA (i.e. plasmid DNA), RNA, or PNA (peptide nucleic acid) are efficiently and reliably labeled with the PHOTOPROBE® labeling reagents or the FastTag® Nucleic Acid Labeling System. With these chemical labeling methods, Vector Laboratories offers an alternative to traditional enzymatic labeling methods such as random priming or nick translation which are difficult to control and don't label the original nucleic acid sample. While random priming or nick translation result in a labeled copy produced from the original template, PHOTOPROBE® labeling reagents and the FastTag® systems ensure labeling of the original nucleic acid. Multiple site labeling over the entire length of the nucleic acid with PHOTOPROBE® labeling reagents and FastTag® systems results in greater accessibility of the affinity tag or increased detection sensitivity.

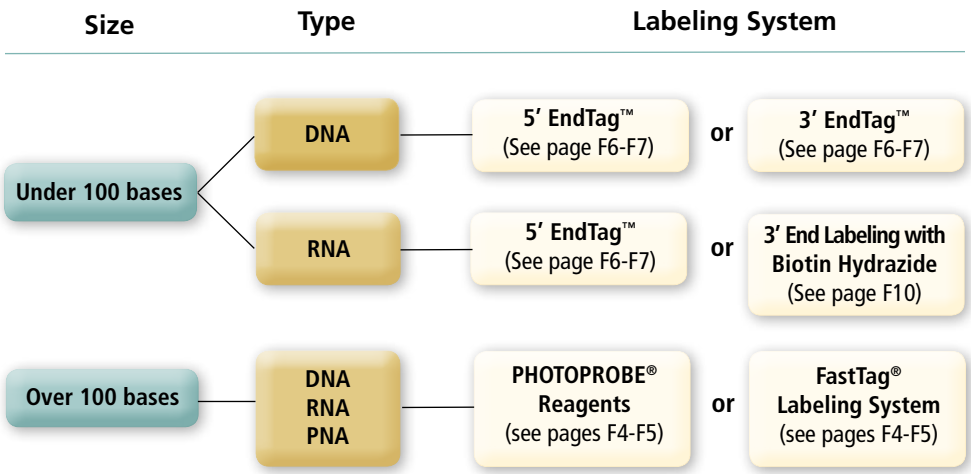
The integrity of the nucleic acid is preserved in this non-destructive reaction making it useful for applications where it is necessary to use the intact, original sample. The PHOTOPROBE® or FastTag® chemical labeling method is especially convenient for labeling samples that will be used to observe cellular localization of nucleic acid (e.g. plasmid DNA in gene delivery or siRNA) or for quantitative comparison.

Shorter strands of nucleic acids such as oligonucleotides, PCR primers, or capture probes used to identify nucleic acid binding proteins are specifically and efficiently labeled at either the 5′ or the 3′ end using the 5′ EndTag™ or the 3′ EndTag™ Nucleic Acid Labeling Systems, respectively. The 5′ EndTag™ or the 3′ EndTag™ Kits can be used to attach a single fluorochrome or affinity tag at the appropriate end of nucleic acids. The 5′ EndTag™ Labeling Kit uses both DNA and RNA as a substrate, whereas the 3′ EndTag™ Kit will selectively label only DNA.

Choice of Label

The second important factor in determining the most appropriate labeling system is the choice of label or affinity tag required for the application. The PHOTOPROBE® Biotin reagents incorporate biotin into nucleic acid in one simple step. The versatility of the FastTag®, 5′ EndTag™ or 3′ EndTag™ Labeling Kits, or PHOTOPROBE® Amine allows a variety of tags to be incorporated including fucose for reversible binding.

Choose a Labeling System Based on Nucleic Acid Type and Size:



Some commonly used labels that are available from Vector Laboratories are:

Biotin – The extraordinary affinity of avidin and streptavidin for biotin and the many biotin-avidin/streptavidin systems available make this label ideal for a variety of applications including *in situ* hybridization, blotting, and affinity binding.

DNP (Dinitrophenyl) – DNP is not found endogenously in tissue so it is an excellent alternative to biotin. High affinity, purified antibodies are available for detection or amplification of the signal.

Fluorescein – Fluorescein is not found endogenously in biological systems. This fluorescent label can be visualized directly (ex 495 nm; em 515 nm) or used as a hapten and detected with our biotinylated or enzyme-conjugated antibody to fluorescein.

Texas Red® - Texas Red® is also not found endogenously in tissues. This fluorescent label is a high quantum yield rhodamine that can be directly visualized (ex 595 nm; em 615 nm). The label can also be detected, and the signal amplified, using our biotinylated or alkaline phosphatase conjugated antibody to rhodamine.

Fucose – This unique label is ideal for reversible binding of labeled nucleic acid to VECTREX® AAL, a matrix containing the fucose-specific lectin *Aleuria aurantia*. Fucose-labeled nucleic acids can be bound and eluted under mild conditions. Alkaline phosphatase conjugated *Aleuria aurantia* lectin can be used in dot blot applications to assess labeling efficiency with the fucose label. (For more information, please see page K7).

Choose a Labeling System Based on Application and Label:

Application	Labeling Method	Label
Oligonucleotide labeling	3′ or 5′ EndTag™	Biotin, DNP, Fluorescein, Texas Red®, Fucose
In situ hybridization	3′ or 5′ EndTag™	Biotin, DNP, Fluorescein, Texas Red®
	FastTag®	Biotin, DNP, Fluorescein, Texas Red®
	PHOTOPROBE®	Biotin, Amine
Blot hybridization	3′ or 5′ EndTag™	Biotin, DNP, Fluorescein, Texas Red®
	FastTag®	Biotin, DNP, Fluorescein, Texas Red®
	PHOTOPROBE®	Biotin, Amine
Matrix binding (e.g. subtractive hybridization)	3′ or 5′ EndTag™	Biotin, Fucose
	FastTag®	Biotin, Fucose
	PHOTOPROBE®	Biotin, Amine
Cellular localization	3′ or 5′ EndTag™	Biotin, DNP, Fluorescein, Texas Red®
	FastTag®	Biotin, DNP, Fluorescein, Texas Red®
	PHOTOPROBE®	Biotin
Comparative genomic hybridization	FastTag®	Biotin, DNP, Fluorescein, Texas Red®
	PHOTOPROBE®	Biotin

Nucleic Acid Labeling (continued)

PHOTOPROBE®/FastTag® System

PHOTOPROBE® Reagents and the FastTag® Nucleic Acid Labeling Systems are the methods of choice for incorporating a label at multiple sites along the entire length of the nucleic acid. The labeling reaction does not destroy the original nucleic acid or create a copy. The integrity of the original sample is preserved. Single or double stranded DNA, circular DNA, RNA, siRNA, or PNA (peptide nucleic acid) can be labeled with the same reagents.

Labeling with either system is based on aryl azide chemistry in which either reagent, when exposed to heat or light, becomes activated and is incorporated into the nucleic acid. The labeling reaction is not base specific and can be carried out using either a mercury vapor bulb, a halogen lamp, a heating block, or a thermal cycler.

PHOTOPROBE® Biotin is the best choice for simple and rapid labeling of nucleic acids with biotin. Biotin is incorporated directly in a short heat or light activation step.

PHOTOPROBE® (Long Arm) Biotin is incorporated in a single step but this reagent has an extra long linker arm and should be used if increased distance between the sample and the tag is needed.

PHOTOPROBE® Biotin or PHOTOPROBE® (Long Arm) Biotin Kit contain enough reagents to label up to 250 µg of nucleic acid (or to carry out up to 50 labeling reactions).

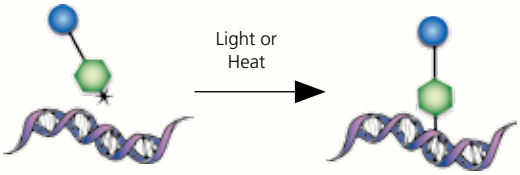
PHOTOPROBE® Amine uses the same aryl azide chemistry to incorporate primary amines into nucleic acids. These amino groups can subsequently be used to attach amine-reactive haptens, affinity tags, or fluorochromes, or to immobilize nucleic acids to a solid matrix. The PHOTOPROBE® Amine Kit includes enough reagents to label up to 360 µg of nucleic acid (or to carry out up to 72 labeling reactions).

FastTag® Labeling Kit enables nucleic acid labeling with a choice of different tags (haptens, fluorochromes, affinity ligands, or other markers). FastTag® labeling consists of three easy steps:

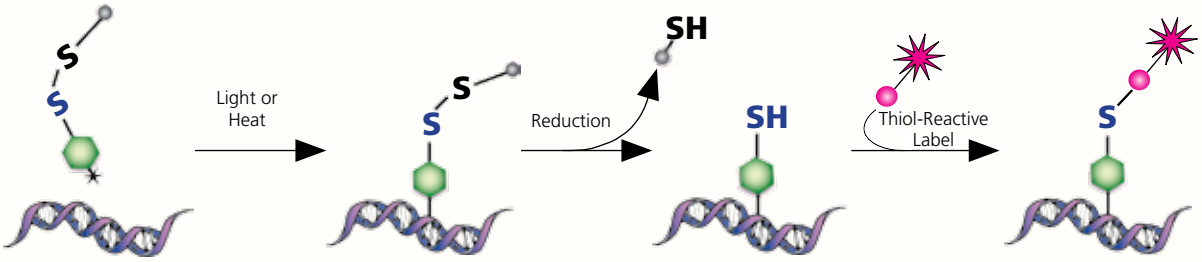
1. The disulfide-containing FastTag® universal linker is incorporated into the nucleic acid upon exposure to heat or light.
2. Reduction of the disulfide bond yields a thiol group.
3. A variety of labels containing thiol-reactive groups (maleimides, iodoacetamides, etc.) can then be covalently bound to the nucleic acid via the introduced FastTag® thiol.

The FastTag® Labeling Kit contains enough reagents to label up to 250 µg of nucleic acid (or to carry out up to 50 labeling reactions). A thiol-reactive label is not included in the kit but a label can be selected from the variety of maleimide tags listed in the table on the adjacent page.

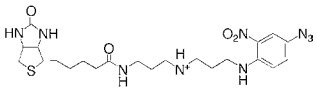
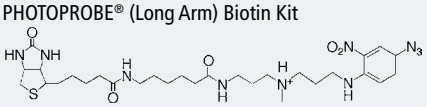
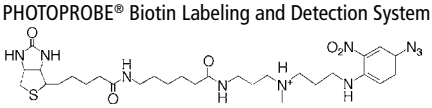
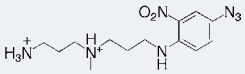
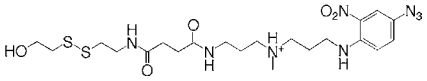
PHOTOPROBE® Biotin Labeling Reaction



FastTag® Labeling Reaction



FastTag® and PHOTOPROBE® Reagents

Product	Catalog Number	Unit Size	Components	Amount of Nucleic Acid Labeled
PHOTOPROBE® Biotin Kit 	SP-1000	1 Kit	PHOTOPROBE® Biotin Reagent (0.5 mg) Tris Buffer *sec*-Butanol Precipitant Biotinylated DNA Standard	250 µg (up to 50 reactions)
PHOTOPROBE® (Long Arm) Biotin Kit 	SP-1020	1 Kit	PHOTOPROBE® (LA) Biotin Reagent (0.5 mg) Tris Buffer *sec*-Butanol Precipitant Biotinylated DNA Standard	250 µg (up to 50 reactions)
PHOTOPROBE® Biotin Labeling and Detection System 	SPK-1906	1 Kit	PHOTOPROBE® (LA) Biotin Kit 10x Casein Solution (250 ml) Alkaline Phosphatase Streptavidin (1 ml) BCIP/NBT Substrate Kit	250 µg (50 blots)
PHOTOPROBE® Amine Kit 	SP-1070	1 Kit	PHOTOPROBE® Amine Reagent (0.5 mg) Borate Buffer *sec*-Butanol Precipitant	360 µg (up to 72 reactions)
FastTag® Labeling Kit 	MB-8000	1 Kit	FastTag® Reagent (0.5 mg) Tris Buffer Citrate Buffer Reducing Reagent *sec*-Butanol Precipitant	250 µg (up to 50 reactions)

F

Thiol-reactive Labels

Product	Catalog Number	Unit Size	Components
Biotin Maleimide	SP-1501	12 mg	Labeling Reagent, plus Labeled DNA Standard
DNP Maleimide	SP-1503	1 mg	Labeling Reagent, plus Labeled DNA Standard
Fluorescein Maleimide	SP-1502	12 mg	Labeling Reagent, plus Labeled DNA Standard
Fucose Maleimide	SP-1504	1 mg	Labeling Reagent, plus Labeled DNA Standard
Texas Red® Maleimide	SP-1505	3.6 mg	Labeling Reagent, plus Labeled DNA Standard

Nucleic Acid Labeling (continued)

5′ EndTag™ and 3′ EndTag™ Labeling Systems

End labeling is a favored method for applications where an internal label might interfere with hybridization or sequence-specific protein binding.

5′ or 3′ EndTag™ labeled nucleic acids can be used for applications such as DNA hybridization, PCR, *in situ* hybridization, the binding of capture probes to affinity matrices, or electrophoretic mobility shift assays (EMSA). Short oligonucleotides are labeled more efficiently with these systems than with other methods. In addition, end labeling of oligonucleotides is an economical alternative to having labels inserted during synthesis.

Both the 5′ EndTag™ and the 3′ EndTag™ Nucleic Acid Labeling Systems enable the covalent attachment of a variety of fluorescent dyes, haptens, or affinity tags to the respective ends of the nucleic acids using thiol-specific chemistry. Labels containing thiol-reactive groups (maleimides, iodoacetamides, etc.) can easily be incorporated.

Labeling time is about 1 hour with very little hands-on time.

5′ EndTag™ Labeling Kit

The 5′ EndTag™ System labels 5′ ends of DNA, RNA, or unmodified oligonucleotides. 5′ EndTag™ is ideal for labeling PCR primers because a label is attached only at the 5′ end, leaving the 3′ end available for polymerization.

Labeling with the 5′ EndTag™ Kit is achieved in two steps:

1. T4 polynucleotide kinase transfers a thiophosphate from ATPγS to the 5′-OH group of the nucleic acid. (5′ phosphorylated ends are converted to 5′-OH groups with the included alkaline phosphatase).
2. Thiolated sample is coupled to a thiol-reactive label.

The 5′ EndTag™ Kit is designed to perform 10 labeling reactions of up to 0.6 nmols of 5′ ends (e.g. about 5 µg of a 25 base oligo) per reaction. Thiol-reactive label is not included in the kit and should be selected from the table on page F7.

3′ EndTag™ Labeling Kit

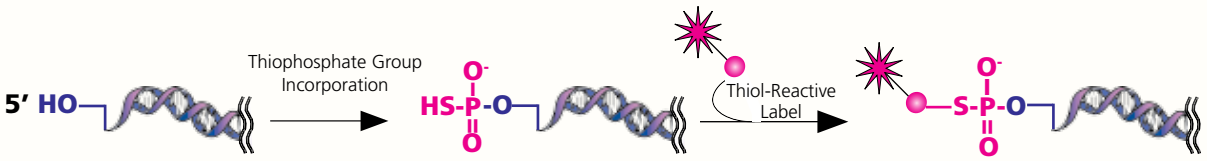
The 3′ EndTag™ system enables simple and uniform labeling of blunt, overhanging, or recessed 3′ ends of DNA. 3′ end labeling is the preferred method if the terminal phosphate at the 5′ end must be preserved.

Labeling using the 3′ EndTag™ Labeling Kit is achieved in two steps:

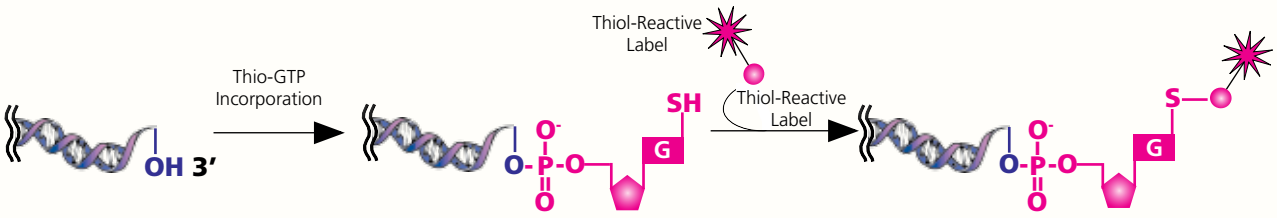
1. Terminal deoxynucleotidyl transferase (TdT) incorporates the thiol-containing nucleotide (SH-GTP) onto the 3′ end.
2. Thiolated DNA is coupled with a thiol-reactive label.

The 3′ EndTag™ Labeling Kit is designed to perform 20 labeling reactions of up to 0.5 nmols of 3′ ends (e.g. about 4.2 µg of a 25 base oligonucleotide) per reaction. Thiol-reactive label is not included in the kit and should be selected from the table on page F7.

5′ EndTag™ Labeling Reaction



3′ EndTag™ Labeling Reaction



EndTag™ Labeling Kits

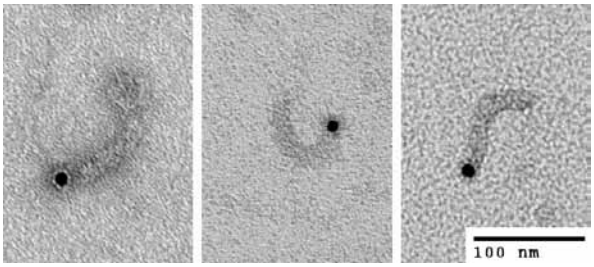
Product	Catalog Number	Unit Size	Components	Number of Reactions
5′ EndTag™ Kit	MB-9001	1 Kit	T4 Polynucleotide Kinase 10x Reaction Buffer ATPγS Precipitant Alkaline Phosphatase	10 (up to 0.6 nmol/reaction)
3′ EndTag™ Kit	MB-9002	1 Kit	Terminal Transferase (TdT) SH-GTP 10x TdT Buffer Precipitant	20 (up to 0.5 nmol/reaction)

Thiol-reactive Labels

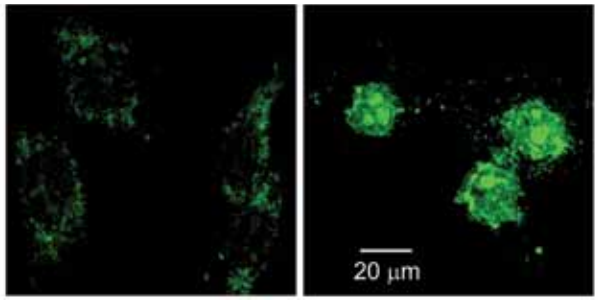
Product	Catalog Number	Unit Size	Components
Biotin Maleimide	SP-1501	12 mg	Labeling Reagent, plus Labeled DNA Standard
DNP Maleimide	SP-1503	1 mg	Labeling Reagent, plus Labeled DNA Standard
Fluorescein Maleimide	SP-1502	12 mg	Labeling Reagent, plus Labeled DNA Standard
Fucose Maleimide	SP-1504	1 mg	Labeling Reagent, plus Labeled DNA Standard
Texas Red® Maleimide	SP-1505	3.6 mg	Labeling Reagent, plus Labeled DNA Standard

RNA 3′ End Labeling with Biotin Hydrazide

RNA can be specifically labeled with biotin on the 3′ end using Biotin (Long Arm) Hydrazide. The two step procedure involves mild periodate oxidation and introduces two biotins into the RNA only at the 3′ end of uncapped RNA. The 5′ cap of mRNA can also be labeled. For additional information, see Biotin (Long Arm) Hydrazide (SP-1100) product description (page F10).



Transmission electron micrographs of molecules of influenza A viral ribonucleoprotein particles (vRNPs) labeled at the 5′ end of the vRNA with biotin using the 5′ EndTag™ Kit, and further labeled with streptavidin gold. Courtesy of Drs. Winco WH Wu and Nelly Panté, University of British Columbia, Vancouver BC, Canada.



- energy
- cytosol

+ energy
+ cytosol

Nuclear import assay in digitonin-permeabilized HeLa cells of biotinylated vRNPs. vRNPs were labeled first at the 5′ end of the vRNA with biotin using the 5′ EndTag™ Kit, and then with Vector® Fluorescein Streptavidin. This allowed for direct fluorescence visualization of the vRNPs with a confocal fluorescence microscope. The negative control consists of vRNPs added to the cells in the absence of energy and exogenous cytosol. In the presence of energy and cytosol, the fluorescein-labeled vRNPs successfully enter the nucleus, with a high degree of nucleolar staining. Courtesy of Drs. Winco WH Wu and Nelly Panté, University of British Columbia, Vancouver BC, Canada.

Protein Labeling

Protein labeling and modification is a powerful tool for the isolation and analysis of proteins. The ProtOn™ Protein Labeling Kits simplify the attachment of either biotin or fluorescein to antibodies or other proteins. Vector Laboratories offers a variety of reagents and kits for protein labeling allowing for greater flexibility of experimental design.

Labeling Reagents

Two functional groups on proteins that are often used in protein modification are primary amino groups, present in lysine residues and chain termini, and thiol groups in cysteine residues. The objective for protein modification determines the functional group that is chosen for labeling. Generally, a protein will have many primary amino groups accessible on its surface. Modification of these surface primary amino groups usually does not affect binding or enzymatic activity of the protein. This allows several labels to be covalently attached and subsequently detected and visualized. Thiol groups are usually less abundant and may be involved in structual elements of the protein like disulfide bridges. Modification of thiol groups, on the other hand, may yield insight into protein structure and function.

Vector Laboratories offers reagents for the attachment of labels to either primary amino groups or thiol groups along with their corresponding detection reagents.

Glycoproteins may also be labeled with biotin hydrazide through their carbohydrate groups (see page F10).

Primary Amino Group Modification:

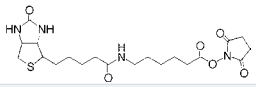
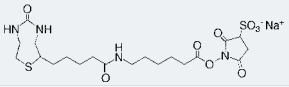
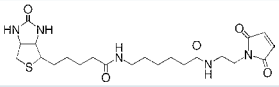
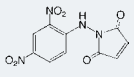
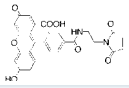
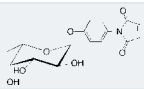
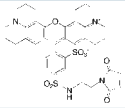
Biotin (Long Arm) NHS is an N-hydroxysuccinimide derivative of biotin with an aminohexanoate spacer arm to provide for maximum accessibility of biotin by avidin and streptavidin conjugates. The reagent will readily couple biotin under mild alkaline conditions to primary amino groups of proteins. Biotin (Long Arm) NHS is ideally suited to label antibodies, hormones, or other polypeptide molecules containing lysine residues. The linkage between biotin and proteins produced with this reagent is stable. Fifty mg of Biotin (Long Arm) NHS is generally sufficient to label 500 mg of protein.

Biotin (Long Arm) NHS, water soluble, is a sulfonated derivative of our Biotin (Long Arm) NHS. Like Biotin (Long Arm) NHS, this reagent is useful for attaching biotin to proteins or other molecules containing a primary amino group. The spacer arm allows full accessibility of attached biotins by avidin or streptavidin conjugates. Unlike Biotin (Long Arm) NHS, this derivative is completely water soluble and does not require DMF or DMSO to dissolve the activated biotin.

Thiol Group Modification:

Biotin (Long Arm) Maleimide reacts readily under mild acid or neutral pH conditions in aqueous solution with free sulfhydryl groups, such as those of cysteine residues or sulfhydryls introduced chemically into proteins or other macromolecules. The spacer arm in this derivative ensures that the biotin is fully accessible to avidin or streptavidin detection systems.

Protein Labeling Reagents

Functional Group Labeled	Product Description	Catalog Number	Unit Size
Primary Amino Group	Biotin (Long Arm) NHS 	SP-1200	50 mg
	Biotin (Long Arm) NHS, water soluble 	SP-1210	50 mg
Thiol Group	Biotin (Long Arm) Maleimide 	SP-1501	12 mg
	DNP Maleimide 	SP-1503	1 mg
	Fluorescein Maleimide 	SP-1502	12 mg
	Fucose Maleimide 	SP-1504	1 mg
	Texas Red® Maleimide 	SP-1505	3.6 mg

Thiol Group Modification (continued)

DNP Maleimide also reacts with free sulfhydryl groups introducing dinitrophenyl (DNP) groups. DNP is an excellent alternative to biotin because it is not found endogenously in the cells. (For detection reagents see page G32.)

Fluorescein Maleimide is used to label free thiol groups with fluorescein. This fluorescent label can be directly visualized or used as a specific tag that can be detected with anti-fluorescein antibodies (see page G33).

Fucose Maleimide is an ideal tag for proteins that need to be reversibly bound to a matrix (for more information on this system, see page J7).

Texas Red® Maleimide can be used to label free thiol groups with this high quantum yield rhodamine derivative. This tag can either be directly visualized or detected using anti-rhodamine antibodies (see page G33).

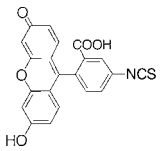
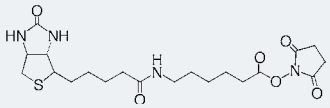
ProtOn™ Protein Labeling Kits

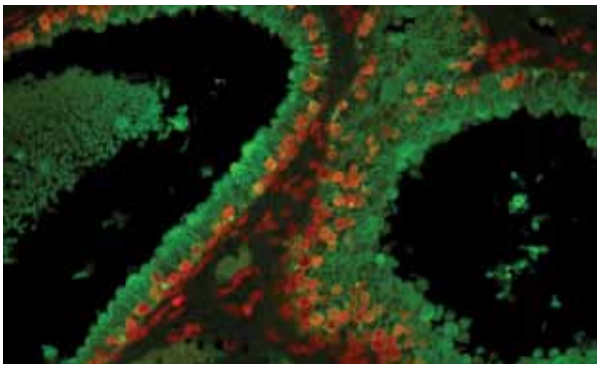
The ProtOn™ Protein Labeling Kits are designed for the simple and fast labeling of proteins with biotin or fluorescein. The kits are ideally suited to label macromolecules containing primary amino groups, such as antibodies, hormones, or other proteins.

The ProtOn™ labeling reagents react with primary amines (i.e. lysine residues or terminal amino groups) on the protein forming a stable covalent bond.

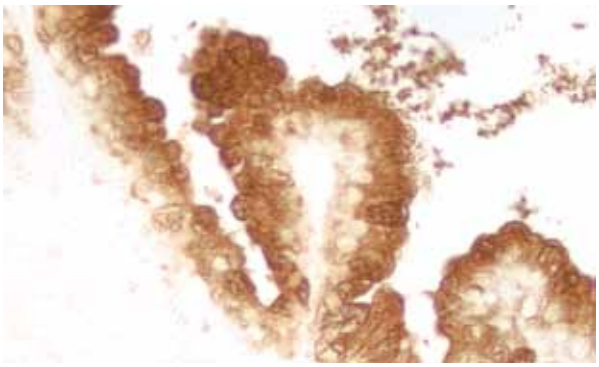
Each kit contains all the components needed for at least five labeling reactions (up to 1 mg of protein per reaction).

ProtOn™ Protein Labeling Kits

Product	Catalog Number	Unit Size	Components	Number of Reactions (amount of labeled proteins)
ProtOn™ Fluorescein Labeling Kit 	PLK-1201	1 Kit	Labeling Reagent (Fluorescein isothiocyanate) Reagent Solvent Reaction Buffer Stop Reagent Gel Filtration Slurry 5 x 1 ml Spin Columns 5 x 2 ml Collection Tubes	5 (up to 1 mg/reaction)
ProtOn™ Biotin Labeling Kit 	PLK-1202	1 Kit	Labeling Reagent (Biotin [Long Arm] NHS) Reagent Solvent Reaction Buffer Stop Reagent Gel Filtration Slurry 5 x 1 ml Spin Columns 5 x 2 ml Collection Tubes Agarose Avidin D Slurry	5 (up to 1 mg/reaction)



Paraffin-embedded human prostate tissue stained directly with rabbit anti-prostate specific antigen labeled with the ProtOn™ Fluorescein Labeling Kit and mounted in VECTASHIELD® Hard✦Set™ Mounting Medium with PI.



Paraffin embedded human prostate tissue stained using rabbit anti-prostate specific antigen labeled with the ProtOn™ Biotin Labeling Kit and detected with VECTASTAIN® Elite® ABC Reagent and Vector® DAB substrate.

Carbohydrate Labeling

Biotin (Long Arm) Hydrazide

Biotin (Long Arm) Hydrazide (SP-1100) reacts with aldehyde groups and thus can be used to label carbohydrate groups present in a wide variety of macromolecules. The spacer arm in this product allows optimal accessibility of avidin or streptavidin conjugates to biotin.

Polysaccharides, glycoproteins, or glycolipids. Biotin can be incorporated into viruses, bacteria, or animal and plant cells via their surface carbohydrate groups.

Incorporation of Biotin (Long Arm) Hydrazide occurs onto sugars containing unsubstituted vicinal hydroxyl groups that are usually present at the non-reducing termini of the oligosaccharide.

Coupling of biotin is accomplished in two steps:

(1) A brief treatment of the saccharide with periodate (not included) produces aldehyde groups on the sugars containing unsubstituted vicinal hydroxyl groups.

(2) Biotin (Long Arm) Hydrazide reacts with the aldehyde groups, producing a stable linkage between the saccharide and biotin.

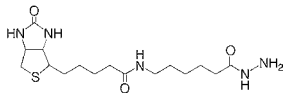
RNA 3′ End Labeling. RNA can be specifically labeled with biotin at the 3′ end. The two-step procedure involves:

(1) Mild oxidation of the ribose located at the 3′ end of RNA, the only ribose susceptible to periodate oxidation. The bond between the 2′ and 3′ hydroxyl groups on the ribose is cleaved, generating two aldehyde groups.

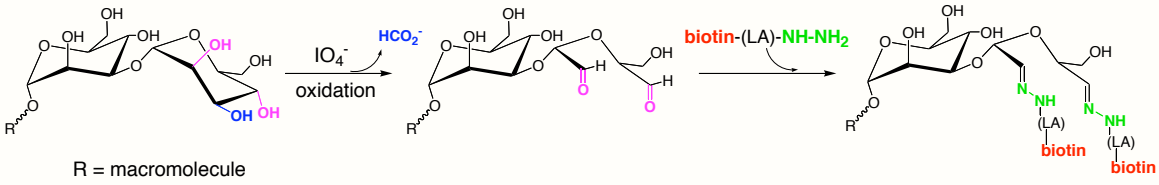
(2) Reaction of both aldehyde groups with Biotin (Long Arm) Hydrazide, introducing two biotins at the 3′ end.

Proteins and other molecules. This reagent can also be used to couple biotin to carboxyl groups on proteins and other molecules using a carbodiimide coupling method.

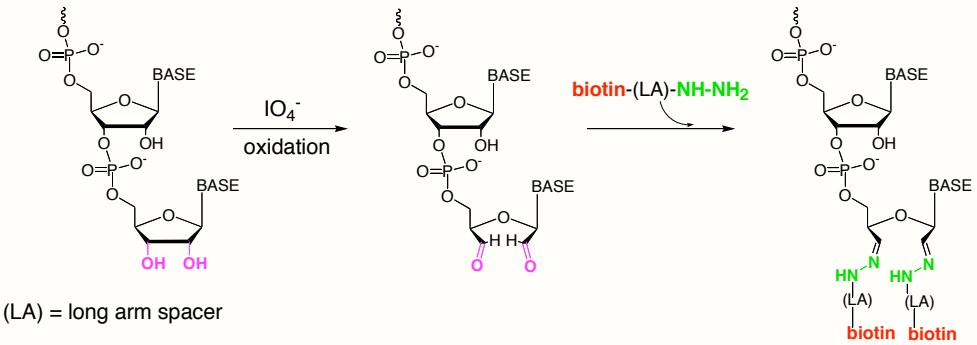
Other materials. Initial activation of a material such as plastics with glutaraldehyde allows subsequent incorporation of Biotin (Long Arm) Hydrazide onto the surface.

Product	Catalog Number	Unit Size
Biotin (Long Arm) Hydrazide 	SP-1100	50 mg

Saccharide, Glycoprotein, or Glycolipid Labeling



RNA 3′ End Labeling



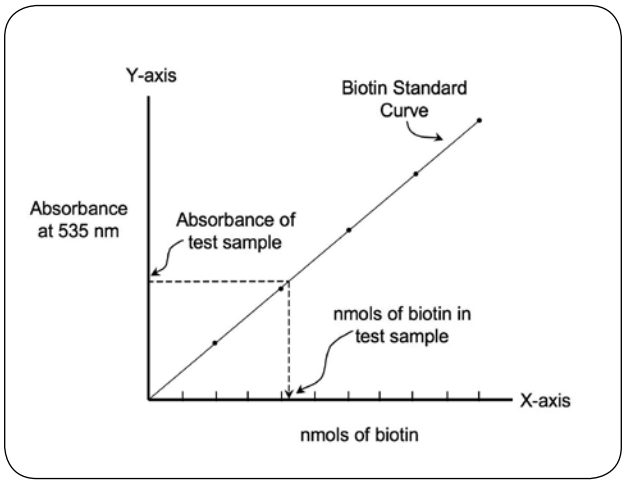
Biotin Quantitation Kit

Quant*Tag™ Biotin Kit

The Quant*Tag™ Biotin Kit (BDK-2000) is designed to determine the amount of free biotin in solution or the number of biotins attached to nucleic acids, proteins, or other macromolecules. This kit can be used to determine accurately the labeling efficiency of biotin-labeled molecules.

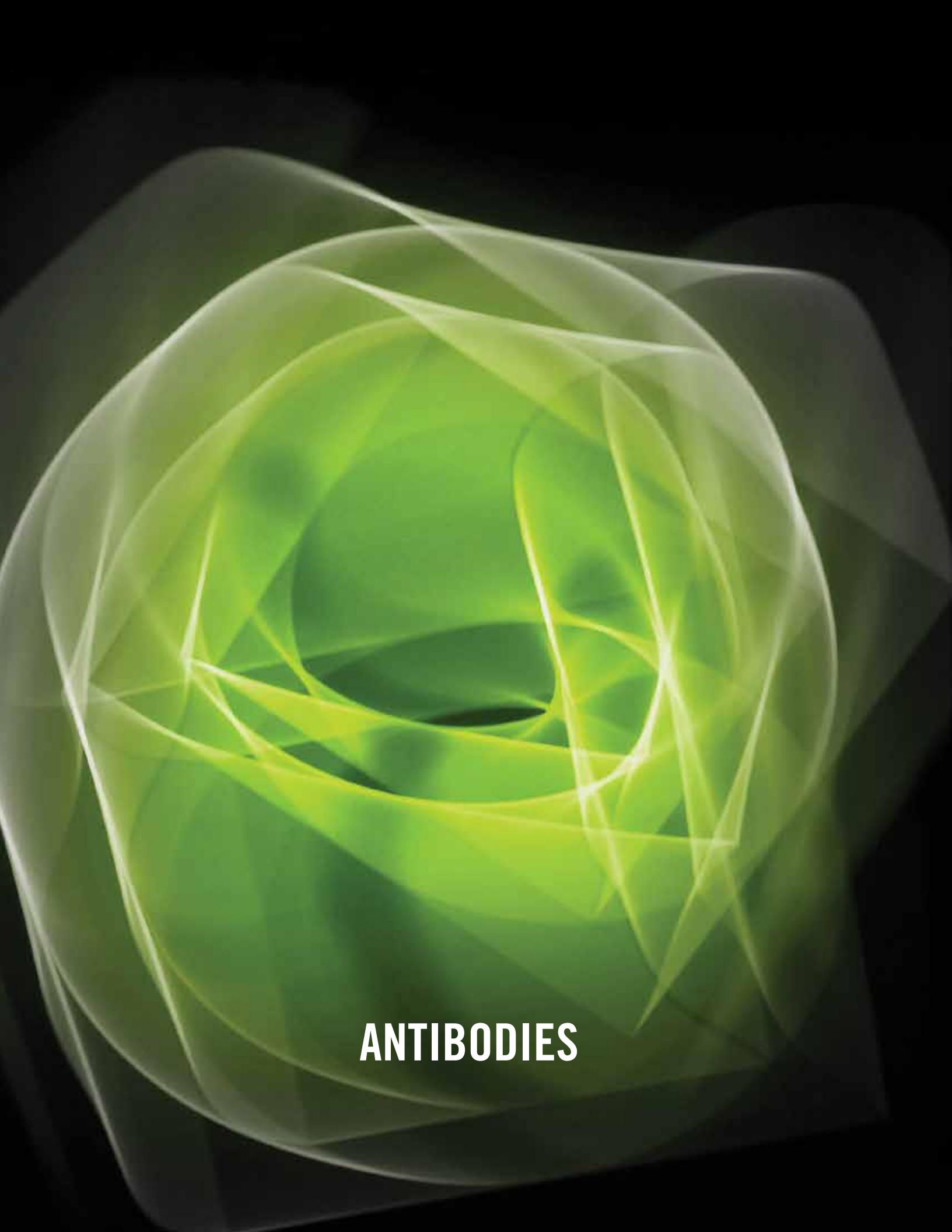
Unlike conventional biotin quantitation methods like the HABA assay, no predigestion of protein or nucleic acids is required, saving time and increasing accuracy. The Quant*Tag™ Biotin Kit, more sensitive than the HABA assay, is able to detect less than 1 nmol of biotin. Quant*Tag™ Kit reagents chemically react with free or bound biotin producing a colored product that can be quantified using a spectrophotometer. The absorbance is measured in the visible spectrum allowing the use of plastic cuvettes or microtitre plates.

The Quant*Tag™ Biotin Kit is quick and easy to use, and the assay can be completed in 30 minutes. A biotin standard is included. The kit contains sufficient reagents to perform from 25 to 250 tests depending on assay size.



The sample to be tested is reacted with the kit reagents along with standards containing known amounts of biotin. The absorbance readings of the known samples are plotted producing a standard curve. The absorbance of the test sample is located on the standard curve indicating the amount of biotin present.

Product	Catalog Number	Unit Size
Quant*Tag™ Biotin Kit	BDK-2000	1 kit



ANTIBODIES

Primary Antibodies

Almost all of these antibodies are mouse monoclonal antibodies and are suitable for immunohistochemistry on formalin-fixed, paraffin-embedded human tissue sections. The antibodies are listed in alphabetical order by the antigen they recognize. A brief description of antigen expression and antibody use is provided for each product.

Additional information such as individual product specification sheets and protocols for use can be obtained via our website. We will be expanding our range of primary antibodies and continually updating our website to include these new products and photomicrographs of stained tissue sections.

Current prices (US, UK or Canada) are also listed on our website and are subject to change without notice.

Unless otherwise indicated please note that all of these antibodies are for research use only.

Working dilutions are given for immunohistochemistry applications. Please refer to product data sheet for dilution factors for other applications.

Application Key:

- ***P***: Paraffin sections with no pretreatment
- ***T***: Trypsin pretreatment technique for paraffin sections
- ***H***: High temperature antigen unmasking technique for paraffin sections using Antigen Unmasking Solution (citrate), Cat. No. H-3300 (see page A30).
- ***HE***: High temperature antigen unmasking technique for paraffin sections using Antigen Unmasking Solution (Tris), Cat. No. H-3301 (see page A30).
- ***F***: Frozen sections
- ***I***: Immunofluorescence
- ***W***: Western blotting
- ***C***: Flow cytometry
- ***O***: Other applications

G

Alpha-Actinin

Clone RBC2/1B6 VP-A102 • 1 ml

F, ***W*** • Working Dilution: 1:40 - 1:80

Alpha-actinin is expressed in skeletal muscle. This antibody recognizes alpha-actinin in type I but not type IIa or IIb human muscle fibers. Clone RBC2/1B6 was raised against erythrocyte membrane ghosts.

Alpha B Crystallin

Clone G2JF VP-A103 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:80 - 1:160

Alpha B crystallin is expressed in tissues such as muscle, Schwann cells, and glial cells. It is also expressed in renal tubular, thyroid, colonic, and stratified squamous epithelium. Alpha B crystallin is found in ubiquitinated intermediate filament inclusion bodies, such as Lewy bodies, Rosenthal fibers, and Mallory bodies. It is rarely found in neurofibrillary tangles. Alpha B crystallin is expressed in various carcinomas. Clone G2JF was raised against amino acids 1 – 10 of alpha B crystallin.

Alpha Fetoprotein

Clone C3 VP-A104 • 1 ml

P, ***F*** • Working Dilution: 1:50

Alpha fetoprotein (AFP) is an oncofetal antigen found in body fluids, which if detected in high concentrations has clinical implications. AFP is expressed in fetal liver but is not present under normal circumstances in healthy adult tissues. It is expressed in a proportion of germ cell tumors, with high frequency in yolk sac tumors. Clone C3 was raised against alpha fetoprotein affinity purified from hepatoma patient serum.

Alpha-Sarcoglycan (Adhalin)

Clone Ad1/20A6 VP-A105 • 1 ml

F, ***W*** • Working Dilution: 1:100 - 1:200

Alpha-sarcoglycan is present in normal muscle but has a differential expression in several types of muscular dystrophy. Clone Ad1/20A6 was raised against amino acids 217 to 289 of rabbit adhalin.

Alpha-Synuclein

Clone KM51 VP-A106 • 1 ml

HE • Working Dilution: 1:20 - 1:40

Alpha-synuclein is present in brain tissue and studies implicate a role in Lewy body formation and Parkinson's disease. Clone KM51 is specific for alpha-synuclein and is unreactive with beta-synuclein.

Amyloid Precursor Protein

Clone 3G12 VP-A109 • 1 ml

HE • Working Dilution: 1:25 - 1:50

Amyloid precursor protein (APP) is a transmembrane protein, and some isoforms are constituents of neurofibrillary tangles and senile plaques as seen in Alzheimer's disease. This antibody does not react with APP-like proteins but reacts with deposited APP isoforms in late-stage neurofibrillary tangle-bearing neurons, neuritic processes surrounding senile plaques, and neuropil threads in grey matter of Alzheimer's disease brain as well as some soluble APP isoforms. Clone 3G12 was raised against the extracellular region of APP between the Kunitz protease inhibitor domain and the beta-amyloid region.

Anaplastic Lymphoma Kinase (p80)

Clone 5A4 VP-A110 • 1 ml

H • Working Dilution: 1:100

The p80 protein is expressed in some large cell lymphomas. Clone 5A4 was raised against a recombinant protein representing a region spanning the tyrosine kinase catalytic domain and part of the C-terminus of the NPM-ALK transcript.

Androgen Receptor

Clone 2F12 VP-A111 • 1 ml

H, ***F*** • Working Dilution: 1:25

Clone AR27 VP-A112 • 1 ml

HE, ***F*** • Working Dilution: 1:50 - 1:100

Androgen receptor is found in a variety of tissues including prostate, skin, and oral mucosa. Androgen receptor has been reported in a diverse range of human tumors. Both clones 2F12 and AR27 were raised against the N-terminus of the human androgen receptor.

Apolipoprotein D

Clone 36C6 VP-A114 • 1 ml

H, ***W*** • Working Dilution: 1:40 - 1:80

Apolipoprotein D is expressed in a range of normal tissues including axillary apocrine glands, adrenal cortex, and corpus luteum. Peripheral nerves, pituitary, testis, cerebellum, and renal tubules are also positive. Apolipoprotein D expression has been described in malignant prostatic glands but is absent in the normal glands. It is also highly expressed in cyst fluid from females with gross cystic disease of the breast and may be a feature of some breast carcinomas. Clone 36C6 was raised against full-length apolipoprotein D.

bcl-2 Oncoprotein

Clone bcl-2/100/D5 VP-B201 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:80

The bcl-2 oncoprotein is an inhibitor of apoptosis and is present in normal and neoplastic lymphoid tissues. Proliferating lymphoid cells and reactive germinal centers do not express bcl-2. Clone bcl-2/100/D5 was raised against a synthetic peptide sequence.

Bcl-6

Clone P1F6 VP-B202 • 1 ml

HE, ***F*** • Working Dilution: 1:20 - 1:40

Bcl-6 is found in normal germinal center B cells and related lymphomas. Clone P1F6 was raised against the N-terminal region of Bcl-6.

Beta Amyloid

Clone 6F/3D VP-B203 • 1 ml

P • Working Dilution: 1:50

Beta amyloid is the major protein component of amyloid cores, neuritic cores, and neurofibrillary tangles, such as those seen in Alzheimer's disease. Beta amyloid deposits are also detected in Lewy body dementia, Down's syndrome, amyloidosis (Dutch type), and in the Guam Parkinson-Dementia complex. Clone 6F/3D was raised against amino acids 8 – 17 of the amyloid protein.

Beta-Catenin

Clone 17C2 VP-B204 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:100 - 1:200

Beta-catenin is widely expressed. Aberrant expression is observed in human tumorigenesis, and especially in colorectal cancer. Clone 17C2 was raised against the C-terminus of beta catenin.

Beta-Dystroglycan

Clone 43DAG1/8D5 VP-B205 • 1 ml

F, ***W*** • Working Dilution: 1:50 - 1:200

Beta-dystroglycan is found in normal muscle but has a differential expression in certain instances of muscular dystrophy. Clone 43DAG1/8D5 was raised against a C-terminal peptide of beta-dystroglycan.

Beta Sarcoglycan

Clone SARC/5B1 VP-B206 • 1 ml

F • Working Dilution: 1:50 - 1:200

Beta-sarcoglycan is found in normal muscle but has a differential expression in certain instances of muscular dystrophy. Clone SARC/5B1 was raised against a fusion protein RBSG-NT of the human beta-sarcoglycan sequence.

Primary Antibodies (continued)

Blood Coagulation Factor XIIIa

Clone E980.1 VP-B207 • 1 ml

H • Working Dilution: 1:40 - 1:80

Factor XIIIa is is found in several cell types in numerous tissues. Increased expression has been correlated with cell differentiation and tumor aggressiveness. Clone E980.1 was raised against a portion of the C-terminus of the blood coagulation factor XIIIa protein.

Bone Morphogenetic Protein 4

Clone 3H2 VP-B208 • 1 ml

P, *W* • Working Dilution: 1:10 - 1:20

Bone morphogenetic protein 4 (BMP4) stimulates bone formation in adult mammals and is also expressed in embryonic tissues such as nervous system, musculature and skeleton. It also seems to be involved with early tooth morphogenesis, and is expressed in prostate adenocarcinoma and benign prostatic hyperplasia. Clone 3H2 was raised against recombinant mouse BMP4.

Bromodeoxyuridine (BrdU)

Clone 85-2C8 VP-B209 • 1 ml

T, *F* • Working Dilution: 1:200 - 1:400

5-bromodeoxyuridine (BrdU) is an analog of thymidine. When incubated with tissue, proliferating cells incorporate BrdU into DNA during S phase and this provides an accurate means of determining cell proliferation. This antibody detects incorporated BrdU in cells or tissues.

Calbindin

Clone KR6 VP-C301 • 1 ml

H • Working Dilution: 1:100 - 1:200

Calbindin is found in brain, kidney, gut, and pancreatic islets. Altered expression of calbindin has been reported in progressive supranuclear palsy, striatal degeneration, and Huntington's disease. Clone KR6 was raised against the majority of the calbindin molecule.

Calcitonin

Rabbit Polyclonal VP-C302 • 0.5 ml

P, *F* • Working Dilution: 1:100 - 1:200

Rabbit Monoclonal

Clone SP17 VP-RM14 • 0.5 ml

H • Working Dilution: 1:100

Calcitonin is found in C cells of normal and hyperplastic thyroid. Clone SP17 and the polyclonal antibody were raised against full-length calcitonin.

Calpain

Clone Calp3c/12A2 VP-C304 • 2.5 ml

W • Working Dilution: 1:100

Clone Calp3d/2C4 VP-C305 • 2.5 ml

W • Working Dilution: 1:25 - 1:50

Calpain 3 is a muscle-specific, calcium-activated neutral protease. Defects in the gene encoding for calpain 3 appear to be the origin of a form of limb-girdle muscular dystrophy. Clone Calp3c/12A2 was raised against amino acids 355-370 and Calp3d/2C4 was raised against amino acids 1-19 of calpain 3.

Calretinin

Clone 5A5 VP-C306 • 1 ml

H • Working Dilution: 1:100

Rabbit Monoclonal

Clone SP13 VP-RM11 • 0.5 ml

H • Working Dilution: 1:100

Calretinin is a calcium-binding protein found in the nervous system and thymus. Calretinin is highly expressed in malignant mesothelioma cells. Both clones SP13 and 5A5 were raised against the full-length mouse calretinin.

Carcinoembryonic Antigen (CD66e)

Clone 12-140-10 VP-C307 • 1 ml

T, *F* • Working Dilution: 1:100 - 1:200

Carcinoembryonic antigen (CEA) is an oncofetal protein found in normal tissues, tumors of the digestive tract, and certain adenocarcinomas. Clone 12-140-10 was raised against CEA isolated from liver metastases of colorectal carcinomas.

Caspase-3 (CPP32)

Clone JHM62 VP-C308 • 1 ml

H, *W* • Working Dilution: 1:25 - 1:50

Caspase-3 is regarded as a control mediator of apoptosis in mammalian cells. Caspase-3 is found in some epithelia, kidney, thymus, bone, tonsil, lymph node, and bone marrow cells. Clone JHM62 was raised against an 182 amino acid region of CPP32.

Cathepsin D

Clone C5 VP-C310 • 1 ml

P, *F* • Working Dilution: 1:100 - 1:200

Cathepsin D is expressed in a variety of tissues. A high level of Cathepsin D in breast cancer may indicate a functional estrogen receptor complex suggesting a likely response to endocrine therapy. Clone C5 was raised against full-length Cathepsin D.

CD1a

Clone JPM30 VP-C311 • 1 ml
H, ***F*** • Working Dilution: 1:20 - 1:40
Clone MTB1 VP-C312 • 1 ml
H, ***F*** • Working Dilution: 1:15 - 1:30

CD1a is expressed on dendritic cells, cortical thymocytes, Langerhans cells, and certain interdigitating cells. Both clones MTB1 and JPM30 were raised against the external domain of CD1a. Clone JPM30 may show some cross-reactivity with CD1d.

CD2 (LFA-2)

Clone AB75 VP-C313 • 1 ml
HE • Working Dilution: 1:40 - 1:80

CD2 is a glycoprotein that mediates adhesion of activated T cells and thymocytes with antigen presenting cells and other target cells. Clone AB75 was raised against the external domain of CD2.

CD3

Clone UCHT1 VP-C314 • 1 ml
F, ***C*** • Working Dilution: 1:100 - 1:200
Clone PS1 VP-C316 • 1 ml
H, ***W***, ***O*** • Working Dilution: 1:100 - 1:200
Clone LN10 VP-C429 • 1 ml
H • Working Dilution: 1:200
Rabbit Monoclonal
Clone SP7 VP-RM01 • 0.5 ml
H • Working Dilution: 1:150 - 1:300

CD3 is regarded as a pan T cell marker and is expressed on early thymocytes as well as mature T cells. Both clones LN10 and PS1 are specific for the non-glycosylated epsilon chain of the human CD3 molecule. Clone UCHT1 was raised against human infant thymocytes and lymphocytes from a patient with Sezary disease. Clones LN10, PS1, and UCHT1 recognize T cells in thymus, bone marrow, peripheral lymphoid tissue, and blood and are all pan T cell markers.

CD4

Clone 1F6 VP-C318 • 1 ml
HE, ***W*** • Working Dilution: 1:20 - 1:40
Clone 4B12 VP-C319 • 1 ml
H, ***HE***, ***F***, ***W*** • Working Dilution: 1:20 - 1:40

CD4 is a transmembrane glycoprotein expressed predominantly on a defined T cell subset (helper/inducer cells). Most cutaneous T cell lymphomas have been reported to have CD4 positive cells. Both clones 1F6 and 4B12 were raised against the external domain of CD4.

CD4 and CD8 (duo pack)

Clones 1F6 and 4B11 VP-C320 • 2x0.5 ml
CD4 ***HE***, ***W*** • Working Dilution : 1:20 - 1:40
CD8 ***HE***, ***F***, ***W*** • Working Dilution : 1:20 - 1:40

CD5

Clone 4C7 VP-C322 • 1 ml
H, ***W*** • Working Dilution: 1:25 - 1:50
Rabbit Monoclonal
Clone SP19 VP-RM16 • 0.5 ml
H • Working Dilution: 1:50

CD5 is found on most thymocytes and peripheral blood lymphocytes. It is highly expressed on T cells and on a B cell subset in the mantle zone of lymph nodes. CD5 antigen is also expressed by many T and B cell leukemias and lymphomas. Clone 4C7 was raised against the external domain while SP19 was raised against the intracellular region of CD5.

CD7

Clone LP15 VP-C426 • 1 ml
H • Working Dilution: 1:50 - 1:100

CD7 is the earliest T cell specific antigen expressed on lymphocytes. CD7 is found on most T cells, NK cells, and thymocytes. Clone LP15 was raised against the external domain of CD7.

CD8

Clone 4B11 VP-C324 • 1 ml
HE, ***F***, ***W*** • Working Dilution: 1:20 - 1:40
Clone 1A5 VP-C325 • 1 ml
H, ***F***, ***W*** • Working Dilution: 1:20 - 1:40
Rabbit Monoclonal
Clone SP16 VP-RM13 • 0.5 ml
H • Working Dilution: 1:100

CD8 is expressed on a T cell subset of normal cytotoxic/suppressor cells. It is also found on NK cells, some thymocytes, peripheral blood null cells, and bone marrow cells. Clones 4B11, 1A5 and SP16 were raised against synthetic peptides from the cytoplasmic domain of CD8.

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Primary Antibodies (continued)

CD9 (Motility-Related Protein-1)

Clone 72F6 VP-C326 • 1 ml

HE, ***F*** • Working Dilution: 1:20 - 1:40

CD9 is expressed on developing B lymphocytes, platelets, monocytes, eosinophils, basophils, stimulated T lymphocytes, and neurons and glial cells of the peripheral nervous system. In melanoma and breast cancer, CD9 antigen expression has been reported to occur predominantly on primary, non-metastatic tumors. Clone 72F6 was raised against the major extracellular loop of CD9.

CD10

Clone 56C6 VP-C328 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:80

CD10, also called neprilysin, is widely expressed on normal and neoplastic cells. CD10 is also expressed in some lymphoid malignancies, such as lymphoblastic, Burkitt's, and follicular lymphomas, and chronic myelocytic leukemia. Clone 56C6 was raised against the external domain of CD10.

CD11b (Mac-1)

Clone 44 VP-C329 • 1 ml

F • Working Dilution: 1:100

CD11b is expressed on granulocytes, monocytes, and macrophages. Clone 44 was raised against synovial cells/ monocytes.

CD13

Clone 38C12 VP-C330 • 1 ml

H • Working Dilution: 1:100

CD13 antigen is expressed on granulocytes, monocytes and their precursors, most acute myeloid leukemias, and a smaller proportion of acute lymphoid leukemias. It is also found on a variety of non-hematopoietic cells. CD13 was raised against the C-terminal region of the extracellular domain of CD13.

CD14

Clone 7 VP-C331 • 1 ml

H, ***W*** • Working Dilution: 1:50 - 1:100

CD14 is expressed predominantly on myelomonocytic cells such as monocytes, macrophages, and Langerhans cells. Clone 7 was raised against the external domain of CD14.

CD15

Clone BY87 VP-C332 • 1 ml

HE, ***F***, ***T*** • Working Dilution: 1:20

CD15 is found on most circulating granulocytes and some monocytes, but is absent from normal lymphocytes. It has been reported to be present on Reed-Sternberg cells of Hodgkin's disease, T cell lymphomas, and some leukemias. Clone BY87 was raised against peripheral blood cells from a patient with B cell lymphoma.

CD16

Clone 2H7 VP-C333 • 1 ml

H • Working Dilution: 1:20 - 1:40

CD16 is expressed on NK cells, granulocytes, activated macrophages, and a subset of T cells. Clone 2H7 was raised against the external domain of CD16.

CD20

Clone L26 VP-C335 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:200 - 1:400

CD20 is found on normal and malignant B cells. Clone L26 was raised against human tonsil B cells.

CD21

Clone 2G9 VP-C336 • 1 ml

H, ***F*** • Working Dilution: 1:10 - 1:20

CD21 is present on mature B cells. CD21 antigen is found in B cell chronic lymphocytic leukemias and in a subset of T cell acute lymphocytic leukemias but is absent on T lymphocytes, monocytes and granulocytes. It has been reported that CD21 is on follicular dendritic cells of follicular and centrocytic lymphomas. Clone 2G9 was raised against the external domain of CD21.

CD22 (BL-CAM)

Clone FPC1 VP-C338 • 1 ml

HE • Working Dilution: 1:20 - 1:40

CD22 is present both on the membrane and in the cytoplasm of B lymphocytes. Surface expression, however, is variable and may be lost upon differentiation. CD22 has been reported to be present in certain leukemias. It is absent from peripheral blood T cells, T cell leukemias, granulocytes, and monocytes. Clone FPC1 was raised against the external domain of CD22.

CD23

Clone 1B12 VP-C339 • 1 ml
H, ***F***, ***C*** • Working Dilution: 1:20 - 1:40
Rabbit Monoclonal
Clone SP23 VP-RM18 • 0.5 ml
H • Working Dilution: 1:100

CD23 functions as a low affinity IgE receptor on B cells. It is found on B lymphocytes, some peripheral blood cells, and on EBV transformed B lymphoblastoid cell lines. Expression of CD23 antigen has been reported on monocytes and dendritic cells. Clone 1B12 was raised against the external domain of CD23. Clone SP23 was raised against amino acids 48 – 248.

CD25 (Interleukin-2 Receptor)

Clone 4C9 VP-C340 • 1 ml
H • Working Dilution: 1:100 - 1:200

CD25 antigen is the alpha subunit of interleukin-2 receptor. It is expressed on a subpopulation of activated T cells. CD25 is also found on HTLV-1 transformed T and B cells, EBV transformed B cells, myeloid precursors, and oligodendrocytes. IL-2 receptor expression is associated with inflammatory and malignant conditions, lymphoid neoplasia, auto-immune diseases, and allograft rejection. Clone 4C9 was raised against the external domain of CD25.

CD29 (ß1 integrin subunit)

Clone 7F10 VP-C341 • 1 ml
H • Working Dilution: 1:20 - 1:40

CD29 antigen is expressed on most cells including all leukocytes, although only at low levels on granulocytes. CD29 expression is indicative of certain T cell subsets. It is also reported to be expressed on hepatocytes from diseased liver and in peripheral blood lymphocytes in patients with Graves' disease. Clone 7F10 was raised against the N-terminus of the extracellular domain.

CD30 Antibodies

Clone 15B3 VP-C342 • 1 ml
H, ***F*** • Working Dilution: 1:20 - 1:40
Clone 1G12 VP-C343 • 1 ml
H, ***F*** • Working Dilution: 1:20 - 1:40

CD30 is expressed on normal and neoplastic cells including multinucleated Reed-Sternberg cells, mononuclear Hodgkin's cells, most anaplastic large cell lymphomas, other lymphomas, and virally transformed cells. Clone 1G12 recognizes the internal domain while 15B3 recognizes the external domain of CD30.

CD31 (PECAM-1)

Clone 1A10 VP-C344 • 1 ml
H • Working Dilution: 1:50 - 1:100

CD31 is found on platelets, monocytes, granulocytes, B cells, and at the endothelial intracellular junction. Clone 1A10 recognizes the extracellular domain of CD31.

CD33

Clone PWS44 VP-C430 • 1 ml
HE • Working Dilution: 1:100

CD33 is expressed on both the myeloid and monocyte lineages, although it is absent on granulocytes. CD33 expression has been demonstrated in various leukemias and myeloproliferative disorders arising from these cells. Clone PWS was raised against the C2 domain of CD33.

CD34 (Endothelial Cell Marker)

Clone QBEnd/10 VP-C345 • 1 ml
T, ***F*** • Working Dilution: 1:50

CD34 is expressed on human lymphoid and myeloid hemopoietic progenitor cells as well as vascular endothelium. Clone QBEnd/10 was raised against a vesicular suspension prepared from a perfusate of human term placenta.

CD35

Clone RLB25 VP-C431 • 1 ml
HE, ***F*** • Working Dilution: 1:40 - 1:80

CD35 antigen is found on erythrocytes, B cells, a subset of T cells, monocytes, macrophages cultured in vitro, neutrophils, eosinophils, glomerular podocytes, and follicular dendritic cells. Decreased levels of CD35 antigen have been reported on B cells in patients with HIV infection. Clone RLB25 was raised against the first four complement control protein domains of CD35.

CD38

Clone SPC32 VP-C348 • 1 ml
H, ***F*** • Working Dilution: 1:50 - 1:100

CD38 is found on immature B and T cells, thymocytes, pre-B cells, germinal center B cells, mitogen-activated T cells, some plasma cells, monocytes, NK cells, some bone marrow cells, and brain cells. CD38 antigen has also been reported in neurofibrillary tangles, the pathological indicator of Alzheimer's disease. Clone SPC32 was raised against the external domain of CD38.

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Primary Antibodies (continued)

CD40

Clone 11E9 VP-C349 • 1 ml

HE, *W* • Working Dilution: 1:20 - 1:40

CD40 is present on mature B cells, most B cell leukemias and lymphomas, interdigitating reticulum cells, follicular dendritic cells, and Reed-Sternberg cells. CD40 antigen is expressed on some epithelial cells of certain carcinomas and in malignant melanomas. Clone 11E9 was raised against the external domain of CD40.

CD42b (GP1b)

Clone MM2/174 VP-C350 • 1 ml

H, *F* • Working Dilution: 1:100

CD42b is found on platelets and also on megakaryocytes in bone marrow and in megakaryoblastic leukemias. The absence of CD42b antigen on platelets is a possible indicator of Bernard-Soulier disease. Clone MM2/174 was raised against human platelet plasma membrane.

CD43

Clone MT1 VP-C352 • 1 ml

P, *F*, *W* • Working Dilution: 1:20 - 1:40

CD43 is expressed on normal and neoplastic T cells and cells of myeloid lineage. A small proportion of B cell chronic leukemias and centrocytic lymphomas are also reported to express CD43 antigen. Clone MT1 was raised against human lymphocytes.

CD44 (H-CAM)

Clone DF1485 VP-C353 • 1 ml

H, *F* • Working Dilution: 1:40 - 1:80

CD44 is expressed on T cells, B cells, monocytes, granulocytes, erythrocytes, platelets, epithelial cells, glial cells, fibroblasts and myocytes as well as some carcinomas. Alterations in expression on some tumor cell lines suggest a role in metastasis. Clone DF1485 was raised against full-length CD44.

CD45 (Leukocyte Common Antigen)

Clone RP2/18 and RP2/22 VP-C354 • 1 ml

P, *F* • Working Dilution: 1:100

CD45 is present on most human leukocytes such as lymphocytes, monocytes and eosinophils but absent from erythrocytes and platelets. Both clones were raised against purified CD45 glycoproteins.

CD45RO

Clone UCHL1 VP-C356 • 1 ml

P, *F* • Working Dilution: 1:100 - 1:200

CD45RO is an isoform of CD45 and is one of four isoforms of the CD45R subfamily that constitute the restricted form of the leukocyte common antigen. CD45RO is expressed on some peripheral blood T lymphocytes, CD4 positive lymphocytes, thymocytes, and on the majority of T cell malignancies. Monocytes, granulocytes, and tissue macrophages also express CD45RO. Clone UCHL1 was raised against an IL-2 dependent T cell line (CA1).

CD54 (ICAM-1)

Clone 23G12 VP-C357 • 1 ml

H • Working Dilution: 1:25 - 1:50

CD54 or intercellular adhesion molecule (ICAM-1) is expressed on monocytes and endothelial cells. CD54 is induced on B and T cells, thymocytes, fibroblasts, keratinocytes, and epithelial cells. Clone 23G12 was raised against a portion of the external domain of CD54.

CD56 (NCAM)

Clone CD564 VP-C358 • 1 ml

H • Working Dilution: 1:50 - 1:100

Clone 1B6 VP-C360 • 1 ml

H, *W* • Working Dilution: 1:50 - 1:100

CD56, also known as neural cell adhesion molecule, is found on neurons, astrocytes, Schwann cells, NK cells, and a subset of activated T lymphocytes. Both clones CD564 and 1B6 were raised against the external domain of CD56.

CD57

Clone NK-1 VP-C361 • 1 ml

P, *F* • Working Dilution: 1:50

CD57, also known as HNK-1, is expressed on a subset of mononuclear cells with NK activity and on defined neuroectodermal cells. It is often co-expressed with CD8 on suppressor/cytotoxic T cells that are involved with graft rejection. Clone NK-1 was raised against human peripheral blood mononuclear cells.

CD61 (GPIIIa)

Clone 2f2 VP-C362 • 1 ml

H, *F* • Working Dilution: 1:50 - 1:100

CD61 is expressed on platelets, monocytes, endothelial cells, smooth muscle cells, B cells, macrophages, mast cells, and fibroblasts. Individuals with Glanzmann's thrombasthenia are reported to express little or no CD61 antigen. CD61 antigen is also reported to be expressed in most cases of megakaryocytic leukemia. Clone 2f2 was raised against a portion of the external domain of CD61.

CD66a (CEACAM1)

Clone 29H2 VP-C363 • 1 ml

H • Working Dilution: 1:50 - 1:100

CD66a is a member of the carcinoembryonic antigen (CEA) family and is expressed by hematopoietic cells. It is also expressed on apical membranes of some epithelia, on endothelium of certain organs, and on the cells of myeloid lineage, granulocytes and myelocytes. CD66a is downregulated in some cancers. Clone 29H2 was raised against a truncated CD66 molecule.

CD68

Clone KP1 VP-C364 • 1 ml

H, ***F*** • Working Dilution: 1:200 - 1:400

CD68 is associated with cytoplasmic granules and the membranes of macrophages. It is also expressed in monocytes, neutrophils, basophils, and large lymphocytes in addition to non-hematopoietic tissues such as liver and renal tubules. Clone KP1 was raised against lysosomal granules from human lung macrophages.

CD79a

Clone 11D10 VP-C366 • 1 ml

H, ***F***, ***C*** • Working Dilution: 1:50 - 1:100

Clone 11E3 VP-C367 • 1 ml

H, ***F*** • Working Dilution: 1:100 - 1:200

Rabbit Monoclonal

Clone SP18 VP-RM15 • 0.5 ml

H • Working Dilution: 1:100

CD79a is part of the complex that constitutes the B cell antigen receptor. CD79a is found early in B cell maturation, and it has been reported on normal and the majority of acute leukemias of precursor B cell type, B cell lines, B cell lymphomas, and in some myelomas. It is not present on myeloid or T cells. Both clones 11D10 and 11E3 were raised against the C-terminus while SP18 was raised against the N-terminus of CD79a.

CD83

Clone 1H4b VP-C368 • 1 ml

H • Working Dilution: 1:20 - 1:40

CD83 is a useful target antigen to identify mature and activated dendritic cells. CD83 is found on Langerhans cells in the skin, peripheral blood dendritic cells, and interdigitating reticulum cells within the T cell areas of lymphoid organs. It is also expressed on Reed-Sternberg cells such as those in Hodgkin's disease. Clone 1H4b was raised against the external N-terminus of CD83.

CD99 (MIC2)

Clone HO36-1.1 VP-C369 • 1 ml

H, ***F*** • Working Dilution: 1:50

CD99 is expressed on most human tissues including cortical thymocytes, pancreatic islets cells, Leydig and Sertoli cells, virtually all hematopoietic cell types (except granulocytes), peripheral blood lymphocytes, granulose cells of the ovary, endothelial cells, and basal/parabasal squamous epithelial cells. CD99 expression has been reported in a wide range of tumors. Clone HO36-1.1 was raised against the E-rosette forming cells isolated from human peripheral blood lymphocytes.

CD105 (Endoglin)

Clone 4G11 VP-C371 • 1 ml

H • Working Dilution: 1:50 - 1:100

CD105 is expressed on endothelial cells of capillaries, arterioles, and venules in a variety of tissues, and at low levels on acute lymphoblastic and myelocytic leukemia cells. CD105 may function as a TGF-beta receptor. Clone 4G11 was raised against the extracellular domain of CD105.

CD146 (MCAM)

Clone N1238 VP-C373 • 1 ml

H, ***W*** • Working Dilution: 1:25 - 1:50

Originally, the CD146 molecule was defined as a marker of tumor progression and metastasis formation in human melanoma. CD146 can be induced on some T cells. More recently, it has been reported to be expressed on endothelial cells, smooth muscle and cerebellar cortex. Clone N1238 was raised against the extracellular domain of CD146.

CD163

Clone 10D6 VP-C374 • 1 ml

H • Working Dilution: 1:50 - 1:100

CD163 protein is restricted in its expression to the monocytic/macrophage lineage. It is found on all circulating monocytes and most tissue macrophages except those in the mantle zone and germinal centers of lymphoid follicles, Langerhans cells, and interdigitating reticulum cells. Clone 10D6 was raised against domain 1 – 4 of the N-terminal region of CD163.

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Primary Antibodies (continued)

CD166 (ALCAM)

Clone MOG/07 VP-C375 • 1 ml

H • Working Dilution: 1:40 - 1:80

CD166 is also referred to as activated leukocyte cell adhesion molecule (ALCAM). In addition to activated leukocytes, it is expressed on activated monocytes, epithelial cells, fibroblasts, neurons, melanoma cells, and in sweat and sebaceous glands. CD166 is upregulated in metastasizing melanoma cell lines. Clone MOG/07 was raised against a 200 amino acid portion of CD166.

CDX2

Clone AMT28 VP-C376 • 1 ml

HE • Working Dilution: 1:50 - 1:100

CDX2 is an intestine-specific transcription factor expressed early in intestinal development and is found predominantly on the surface of villi and crypts. Differential expression has been reported in some intestinal and colorectal cancers. Clone AMT28 was raised against the N-terminus of CDX2.

c-erbB-2 Oncoprotein (HER2/neu)

(External Domain)

Clone 10A7 VP-C378 • 1 ml

P, ***W*** • Working Dilution: 1:40 - 1:80

Rabbit Monoclonal

Clone SP3 VP-RM07 • 0.5 ml

H • Working Dilution: 1:100

(Internal Domain)

Clone CB11 VP-C380 • 1 ml

P, ***F***, ***C*** • Working Dilution: 1:40

c-erbB-2 oncoprotein is present in a wide variety of cell types in a range of normal human fetal and adult tissues, including breast, stomach, and ovary. It has also been documented as being present in a percentage of breast and other adenocarcinomas and transitional cell carcinomas. Clones 10A7 and SP3 are directed against the external domain while CB11 recognizes the internal domain of c-erbB-2 oncoprotein.

c-erbB-3 Oncoprotein

Clone RTJ1 VP-C381 • 1 ml

H, ***F***, ***O*** • Working Dilution: 1:20 - 1:40

c-erbB-3 oncoprotein is expressed in chronic pancreatitis, exocrine pancreatic cancer and in tumors of the gastro-intestinal tract. This antibody shows no cross-reactivity with similar structured proteins such as c-erbB-2 and EGFR. Clone RTJ1 was raised against the cytoplasmic domain of c-erbB-3.

c-fos Oncoprotein

Clone CF2 VP-F701 • 1 ml

F • Working Dilution: 1:40

Expression of the c-fos gene is low in most adult tissues, however, high levels of expression have been detected in normal skin. Clone CF2 was raised against a peptide from c-fos.

Checkpoint Kinase 1

Clone DCS-310.1 VP-C382 • 1 ml

H • Working Dilution: 1:50 - 1:100

Checkpoint Kinase 1 regulates cell cycle progression in response to agents that block DNA replication. Cell cycle kinases act upstream of p53 in DNA damage response, and mutations of these kinases are implicated in certain cancer development. Clone DCS-310.1 was raised against a CHK-1 fusion protein.

Choline Acetyltransferase

Clone 38B12 VP-C383 • 1 ml

HE • Working Dilution: 1:25 - 1:50

Choline Acetyltransferase (ChAT) is found in cholinergic neurons in the CNS. Specific loss of cholinergic neurons has been reported in the basal forebrain in Lewy body dementia and Alzheimer's disease. It does not react with axons in the insular cortex or the internal capsule, non-cholinergic structures, endothelial cells, or microglia. Clone 38B12 was raised against the C-terminus of ChAT.

Chromogranin A

Clone 5H7 VP-C425 • 1 ml

H • Working Dilution: 1:200

Rabbit Monoclonal

Clone SP12 VP-RM10 • 0.5 ml

H • Working Dilution: 1:100

Chromogranin A protein is widely expressed in neuronal tissues and secretory granules of endocrine cells such as parathyroid gland, adrenal medulla, anterior pituitary, pancreatic islets, and C cells of the thyroid. Chromogranin A expression has been reported in neuroendocrine tumors such as pituitary adenomas, islet cell tumors, phaeochromocytomas, medullary thyroid carcinomas, Merkel cell tumors, and carcinoids. Clone 5H7 was raised against the central region while SP12 was raised against the full-length chromogranin A.

c-kit Oncoprotein (CD117)

Clone T595 VP-C385 • 1 ml

H, ***HE*** • Working Dilution: 1:20 - 1:40

c-kit is involved with blood, gamete, and melanocyte development. It is found in normal breast epithelium, melanocytes, mast cells, glial cells and glioblastomas, melanomas, in certain carcinomas and certain leukemias. Clone T595 was raised against the three N-terminal C2-like extracellular domains of the c-kit oncoprotein.

Clusterin (Apolipoprotein J)

Clone 7D1 VP-C386 • 1 ml

H, ***F*** • Working Dilution: 1:30 - 1:60

Clusterin has several names including apolipoprotein J. It is found in certain conditions such as amyloid disorders, Alzheimer's disease, breast cancer, and anaplastic large cell lymphoma. Clone 7D1 was raised against the alpha chain of clusterin.

Collagen Type II

Rabbit Polyclonal VP-C387 • 1 ml

T • Working Dilution: 1:15 - 1:30

Collagen type II is found mostly throughout cartilage matrix and also in very small amounts in the eye. This antibody does not cross-react with collagen types I, III, IV, V, VI, serum proteins, or non-collagenous extracellular associated proteins. This antibody was raised against collagen type II from adult human knee cartilage.

Collagen Type IV

Clone PHM-12 VP-C388 • 1 ml

H+***T***, ***F*** • Working Dilution: 1:100 - 1:200

In kidney, collagen type IV is expressed in glomerular and tubular basement membranes and also mesangial cells and the matrix within glomeruli, the basal lamina of capillaries, as well as basement membrane structures in many organs. Clone PHM-12 was raised against human glomeruli.

Complement Component C9

Clone 10A6 VP-C389 • 1 ml

H • Working Dilution: 1:50 - 1:100

Complement component C9 binds to the C5b-8 complex as the final protein of the membrane attack complex. In this manner, it acts similarly to perforin. This antibody is particularly useful in identifying regions of recent ischemic myocardial damage postmortem. Clone 10A6 was raised against the C-terminus of complement component C9.

Cyclin A

Clone 6E6 VP-C391 • 1 ml

H, ***W***, ***C*** • Working Dilution: 1:50 - 1:100

Cyclin A is detectable in S phase, increasing during cell cycle progression to G2 phase and may prove useful as a marker of proliferation. Cyclin A is expressed in normal human epidermis and various proliferative skin diseases including psoriasis, seborrhoeic keratosis and squamous cell carcinoma. Clone 6E6 was raised against an N-terminal fragment of cyclin A.

Cyclin B1

Clone 7A9 VP-C392 • 1 ml

H, ***W***, ***C*** • Working Dilution: 1:20 - 1:40

Cyclin B expression is restricted to a specific short period of the cell cycle with cyclin B1 expression detected earlier and peaking in concentration before cyclin B2 expression. Cyclin B positive cells, indicated by cytoplasmic staining, in proliferating tissue are reported to represent a subset of Ki67 positive cells. Clone 7A9 was raised against full-length cyclin B1.

Cyclin D1

Clone DCS-6 VP-C393 • 1 ml

HE/***T***, ***F***, ***W*** • Working Dilution: 1:20

Clone P2D11F11 VP-C394 • 1 ml

HE/***T***, ***W*** • Working Dilution: 1:25 - 1:50

Rabbit Monoclonal

Clone SP4 VP-RM03 • 0.5 ml

HE • Working Dilution: 1:50 - 1:100

Maximum expression of cyclin D1 occurs at a critical point in mid to late G1 phase of the cell cycle. Cyclin D1 expression has been shown to be amplified in tumors such as breast cancer and mantle cell lymphoma. Clones DCS-6 and P2D11F11 were both raised against full-length cyclin D1 and SP4 was raised against the C-terminus of cyclin D1.

Cyclin D3

Clone DCS-22 VP-C395 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:20 - 1:40

Cyclin D3 expression is induced later than cyclin D1 in G1 phase of the cell cycle. Cyclin D3 appears to have activity similar to the other D-type cyclins when complexed with cyclin dependent kinases. Clone DCS-22 was raised against full-length cyclin D3.

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Primary Antibodies (continued)

Cyclin E

Clone 13A3 VP-C396 • 1 ml

H, *F*, *W* • Working Dilution: 1:40 - 1:80

Cyclin E activity is mediated via its activation of cyclin dependent kinase 2 and is modulated by certain tumor suppressor proteins. Cyclin E overexpression shortens the length of G1 phase, accelerating progression of the cell cycle into S phase. Clone 13A3 was raised against full-length cyclin E.

Cyclooxygenase-2 (Cox-2)

Rabbit Monoclonal

Clone SP21 VP-RM02 • 0.5 ml

H • Working Dilution: 1:25 - 1:50

Cyclooxygenase-2 is found in normal skin and epithelial cells of various diseases and cancers. In Crohn's disease and ulcerative colitis, cyclooxygenase-2 is strongly expressed in the upper crypts, surface epithelial cells, and in the mononuclear cells of the lamina propria. It is expressed in both epithelial and interstitial cells of adenomatous polyps and several adenocarcinomas as well as in a high proportion of esophageal squamous cell carcinomas. Clone SP21 was raised against the C-terminus of rat Cox-2.

Cytokeratin 1

Clone 34ßB4 VP-C398 • 0.5 ml

H, *F* • Working Dilution: 1:20 - 1:40

Cytokeratin 1 is found in complex squamous epithelium such as foot sole epidermis, ectocervix, and anal canal epithelium. It is not present in normal or abnormal epithelia of hepatocellular origin or ductal origin like breast or pancreas. Clone 34ßB4 was raised against extract from human stratum corneum from the sole of the foot.

Cytokeratin 4

Clone 6B10 VP-C399 • 0.5 ml

H, *F*, *W* • Working Dilution: 1:100 - 1:200

Cytokeratin 4 is found in non-keratinizing squamous epithelia such as those in the esophagus, tongue, vagina, laryngeal area, and superficial cells of the cornea. Cytokeratin 4 is expressed in the suprabasal cells of the urinary bladder transitional epithelium, in cells of sweat glands, prostatic ducts, and in some bronchial epithelial cells. Cytokeratin 4 is reported to be present in some squamous cell carcinomas. Clone 6B10 was raised against an epithelial cell preparation from human esophagus.

Cytokeratin 5

Clone XM26 VP-C400 • 1 ml

H, *F*, *W* • Working Dilution: 1:100 - 1:200

Cytokeratin 5 is normally expressed in the basal epithelia of many tissues and non-keratinizing stratified squamous epithelia. Cytokeratin 5 is expressed in most epithelial and biphasic mesotheliomas as well as basal cell epitheliomas and some squamous cell carcinomas. Clone XM26 was raised against the C-terminus of cytokeratin 5.

Cytokeratin 6

Clone LHK6B VP-C401 • 1 ml

F, *O* • Working Dilution: 1:20 - 1:40

Cytokeratin 6 is found in hair follicles, suprabasal cells of internal stratified epithelia, and in epidermis in both normal and hyperproliferative situations. Cytokeratin 6 has been described in a large percentage of head and neck squamous cell carcinomas. Clone LHK6B was raised against a C-terminus peptide of cytokeratin 6.

Cytokeratin 7

Clone OV-TL 12/30 VP-C403 • 1 ml

H/*T*, *F*, *W* • Working Dilution: 1:50

Cytokeratin 7 is found in many, but not all, ductal and glandular epithelia, including lung and breast. Cytokeratin 7 has been reported in adenocarcinomas of the lung, breast, endometrium, ovary, and thyroid as well as in carcinomas of the bladder and chromophobe renal cell carcinoma. Clone OV-TL 12/30 was raised against the OTN 11 ovarian carcinoma cell line.

Cytokeratin 8

Clone TS1 VP-C404 • 1 ml

H, *F* • Working Dilution: 1:100 - 1:200

Cytokeratin 8 is expressed in the embryo and persists in adult tissues and is a major component of all simple epithelia but not of stratified squamous epithelia. Cytokeratin 8 is expressed in adenocarcinomas of individuals and is also found in their sera. Clone TS1 was raised against purified tumor-derived cytokeratins.

Cytokeratin 8/18

Clone 5D3 (liquid) VP-C407 • 1 ml

Clone 5D3 VP-C428 • 1 ml

T, *F*, *C* • Working Dilution: 1:100

Cytokeratin 8 and cytokeratin 18 are expressed in the embryo and persist in adult tissues. Both cytokeratins 8 and 18 are major components of all simple epithelia but not of stratified squamous epithelia. Clone 5D3 was raised against cytokeratins from the breast carcinoma cell line MCF-7.

Cytokeratin 10

Clone LHP1 VP-C408 • 1 ml

T, *F* • Working Dilution: 1:50

Cytokeratin 10 is found in suprabasal layers of keratinizing stratified epithelia, such as foot sole epidermis and anal canal epithelium, as well as the suprabasal layers of some non-keratinizing stratified epithelia and squamous carcinomas. Clone LHP1 was raised against a cytoskeletal extract prepared from the epidermal component of trypsin-split normal adult skin.

Cytokeratin 13

Clone KS-1A3 VP-C409 • 0.5 ml

H, *F*, *W* • Working Dilution: 1:100 - 1:200

Cytokeratin 13 is a major component of squamous, non-keratinized epithelium, transitional epithelium, pseudostratified epithelium, and myoepithelium. It is reported to be expressed in carcinomas of the trachea, apocrine and eccrine sweat glands, salivary glands, reserve cells of endocervical glands, bladder, ectocervix, tongue, esophagus, anal canal, and the basal layer of keratinized epidermis. Clone KS-1A3 was raised against cultured A431 cells from a human epidermoid carcinoma of the vulva.

Cytokeratin 14

Clone LL002 VP-C410 • 1 ml

H, *F* • Working Dilution: 1:20

Cytokeratin 14 is present in the basal layer of stratified epithelia. Cytokeratin 14 has been reported to be expressed in neoplasms of squamous cell origin. Clone LL002 was raised against a 15 amino acid peptide from the C-terminal region of cytokeratin 14.

Cytokeratin 15

Clone LHK15 VP-C411 • 1 ml

H, *F* • Working Dilution: 1:40 - 1:80

Cytokeratin 15 is expressed only in basal keratinocytes of stratified squamous epithelium and fetal epidermis and nail. All trichoepitheliomas, derived from hair follicle stem cells, and some basal cell carcinomas are reported to express cytokeratin 15. Clone LHK15 was raised against full-length cytokeratin 15.

Cytokeratin 16

Clone LL025 VP-C412 • 1 ml

H, *F* • Working Dilution: 1:20 - 1:40

Cytokeratin 16 is expressed where keratinocytes are undergoing rapid turnover in the suprabasal region. It is found in hair follicles, tongue epiglottis epithelium, and the sole of the foot. Cytokeratin 16 is reported to be found in various pathological states, including wound healing, psoriasis and certain carcinomas. Clone LL025 was raised against a peptide from the C-terminal region of cytokeratin 16.

Cytokeratin 17

Clone E3 VP-C413 • 1 ml

H, *F*, *W* • Working Dilution: 1:20 - 1:40

In normal tissues cytokeratin 17 is expressed in basal cells of pseudostratified epithelium in the trachea, larynx, and bronchi, myoepithelial cells in salivary glands, and sweat glands. In neoplastic tissue, cytokeratin 17 is expressed in squamous cell carcinomas of the lung, cervix, and oral cavity. Clone E3 was raised against a rat enterocyte keratin preparation.

Cytokeratin 18

Clone DC-10 VP-C414 • 1 ml

H, *F* • Working Dilution: 1:20 - 1:40

Cytokeratin 18 is normally co-expressed with cytokeratin 8 and is found in most simple ductal and glandular epithelia. Cytokeratin 18 is reported not to be expressed in stratified squamous epithelium on most squamous cell carcinomas. Clone DC-10 was raised against a human breast carcinoma PMC-42 cell line.

Cytokeratin 19

Clone b170 VP-C415 • 1 ml

T, *F* • Working Dilution: 1:100 - 1:150

Cytokeratin 19 is expressed in a large number of epithelial cell types, including many ductal and glandular epithelia. It is detected in non-keratinizing squamous epithelia and hair follicles, with strong staining of the basal layer observed. Clone b170 was raised against a human MCF-7 cell line cytoskeletal preparation.

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Primary Antibodies (continued)

Cytokeratin 20

Clone KS20.8 VP-C416 • 1 ml

H/*T*, *W* • Working Dilution: 1:25 - 1:50

Cytokeratin 20 expression is almost entirely confined to the gastric and intestinal epithelium, urothelium, and Merkel cells of the skin. In tumors it is reported, there is a marked difference in the expression of cytokeratin 20 within different carcinomas. Clone KS20.8 was raised against a cytoskeletal preparation isolated from microdissected villi of human duodenal mucosa.

Cytokeratin 1/5/10/14

Clone 34βE12 VP-C417 • 1 ml

H, *F*, *W* • Working Dilution: 1:100 - 1:200

This antibody recognizes keratins in the high molecular weight range and labels squamous, ductal, and other complex epithelia and carcinomas arising from these tissues. It has also been reported to react with benign small acinar lesions of the prostate. It generally does not react with simple epithelia and reacts variably with adenocarcinomas. Clone 34βE12 was raised against keratin extracted from human stratum corneum.

Cytokeratin 5/6/18

Clone LP34 VP-C418 • 1 ml

T, *F* • Working Dilution: 1:100

This antibody demonstrates a broad pattern of reactivity with epithelial tissues, from simple glandular epithelia to stratified squamous epithelia. Clone LP34 was raised against the detergent-insoluble fraction of psoriatic human epidermis.

Cytokeratin-Multi AE1/AE3

Clone AE1 and AE3 VP-C419 • 1 ml

H, *F* • Working Dilution: 1:20 - 1:100

This mixture of antibodies exhibits broad recognition with both acidic and basic cytokeratins. This mixture specifically recognizes 56.5, 50, 50', 48, and 40 kDa acidic cytokeratins and 65 to 67, 64, 59, 58, 56 and 52 kDa basic cytokeratins.

Cytokeratin-Multi 4/5/6/8/10/13/18

Clone C-11 VP-C420 • 1 ml

H, *F* • Working Dilution: 1:10

This is a broad spectrum antibody that recognizes a variety of normal, reactive, and neoplastic epithelia. It labels simple epithelium and basal and suprabasal layers of cornifying and non-cornifying squamous epithelium. Clone C-11 was raised against a human A431 cell cytoskeleton preparation.

Cytomegalovirus (pp65 antigen)

Clone 2 and 6 VP-C422 • 1 ml

H, *I*, *W* • Working Dilution: 1:200

Cytomegalovirus (CMV) is an opportunistic pathogen that infects lung, kidney, gut, and other organs in immunologically immature individuals or in immunosuppressed patients. This pool of 2 antibodies will detect the pp65 antigen in tissues and cytospins.

Delta-Sarcoglycan

Clone Sarc3/12C1 VP-D501 • 1 ml

F, *W* • Working Dilution: 1:25 - 1:50

Delta-sarcoglycan is found in normal muscle but has a differential expression in certain instances of muscular dystrophy. Clone Sarc3/12C1 was raised against amino acids 1 – 19 of delta-sarcoglycan.

Desmin

Clone DE-R-11 VP-D502 • 1 ml

F, *T*, *W* • Working Dilution: 1:50 - 1:100

Desmin is expressed in skeletal, cardiac, and smooth muscle cells. Clone DE-R-11 was raised against full-length porcine desmin.

Dysferlin

Clone Ham1/7B6 VP-D503 • 1 ml

P, *F* • Working Dilution: 1:20 - 1:40

Clone HAM3/17B2 VP-D504 • 1 ml

H, *F*, *W* • Working Dilution: 1:20 - 1:40

Dysferlin is found in normal muscle but expression is reduced or lost in individuals with limb-girdle muscular dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM). Clone HAM3/17B2 was raised against a peptide from exons 11 and 12 of dysferlin. Clone Ham1/7B6 was raised against a peptide from exon 53.

Dystrophin

Rod Domain
Clone Dy4/6D3 VP-D508 • 2.5 ml
F, *W* • Working Dilution: Neat - 1:20

C-terminus
Clone Dy8/6C5 VP-D505 • 2.5 ml
F, *W* • Working Dilution: Neat - 1:20

N-terminus
Clone Dy10/12B2 VP-D507 • 2.5 ml
F, *W* • Working Dilution: Neat - 1:20

Dystrophin is expressed in muscle tissue. Abnormal expression is seen in Duchenne muscular dystrophy (DMD). Clone Dy8/6C5 was raised against the C-terminal 17 amino acids while clone Dy10/12B2 was raised against a peptide from the N-terminal region of dystrophin. Clone Dy4/6D3 was raised against a dystrophin fusion protein.

E-Cadherin

Clone 36B5 VP-E601 • 1 ml
H • Working Dilution: 1:25 - 1:50

E-cadherin is expressed in epithelial cells and is reduced in some cancers including prostate and breast cancer. Clone 36B5 was raised against the N-terminal external region of E-Cadherin.

Emerin

Clone 4G5 VP-E602 • 1 ml
H, *F*, *W* • Working Dilution: 1:20 - 1:40

Emerin is found ubiquitously in tissues. Emerin is found in the highest concentrations in skeletal and cardiac muscle. Deficiency in its expression is a key factor in Emery-Dreifuss muscular dystrophy. Clone 4G5 was raised against the N-terminal region of emerin.

Enterovirus

Clone 5-D8/1 VP-E603 • 1 ml
I, *W*, *O* • Working Dilution: 1:10

Enterovirus primarily infects the alimentary tract and probably infects other organs through the blood vascular systems. This antibody recognizes most enterovirus and poliovirus strains and some echovirus strains in tissue culture preparations. Clone 5-D8/1 was raised against Coxsackie virus B5.

Epidermal Growth Factor Receptor

Clone EGFR-113 VP-E605 • 1 ml
H, *F* • Working Dilution: 1:10 - 1:20

Increased expression of epidermal growth factor receptor (EGFR) is found in several squamous cell carcinomas. Clone EGFR-113 is raised to the extracellular domain of EGFR.

Epithelial Membrane Antigen

Clone GP1.4 VP-E606 • 1 ml
P, *F* • Working Dilution: 1:200 - 1:400

Epithelial membrane antigen is widely expressed in epithelia including ductal and glandular epithelia and most squamous epithelia. EMA is also expressed in a subset of Hodgkin's lymphomas. Clone GP1.4 was raised against human milk fat globule membrane.

Epithelial Specific Antigen

Clone VU-1D9 VP-E607 • 1 ml
T, *F*, *W*, *O* • Working Dilution: 1:50 - 1:100

Epithelial specific antigen (ESA) is expressed in the majority of human epithelial cells and is rarely expressed in mesothelial cells. Clone VU-1D9 was raised against a small cell lung carcinoma cell line (HG9).

Epstein-Barr Virus

(Early Antigen Diffuse)
Clone G3-E31 VP-E608 • 1 ml
W, *O* • Working Dilution: 1:200 - 1:400

Epstein-Barr virus early antigens are non-structural proteins that do not require viral DNA replication for synthesis. The early antigen diffuse is expressed during the early lytic phase of virus replication, most notably in keratinocytes of hairy cell leukoplakia.

Epstein-Barr Virus

(Latent Membrane Protein)
Clones CS1, CS2,
CS3 and CS4 VP-E609 • 1 ml
T, *F* • Working Dilution: 1:100 - 1:200

This pool of four monoclonal antibodies detects the latent membrane protein (LMP-1) derived from the BNLF1 gene of Epstein-Barr virus (EBV).

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Primary Antibodies (continued)

Epstein-Barr Virus

(Nuclear Antigen 2)

Clone PE2 VP-E610 • 1 ml

F, *W* • Working Dilution: 1:25 - 1:50

Epstein-Barr virus nuclear antigen 2 (EBNA2) is crucial for growth transformation of B lymphocytes and is known to modulate the activity of several viral and cellular promoters during infection. This antibody will generally not label true EBV-positive lymphomas but will label EBV induced atypical lymphoproliferative disease.

Estrogen Receptor

Clone 6F11 VP-E613 • 1 ml

Clone 6F11 VP-E614 • 2ml

H, *F*, *W*, *C* • Working Dilution: 1:40 - 1:80

Clone EMR02 (ER beta) VP-E615 • 1 ml

H, *W* • Working Dilution: 1:25 - 1:50

Clone 1D5 VP-E617 • 1 ml

H • Working Dilution: 1:200 - 1:400

Rabbit Monoclonal

Clone SP1 VP-RM05 • 0.5 ml

H • Working Dilution: 1:40

Estrogen receptor (ER) is expressed in a variety of different tissues but is found most prominently in the female reproductive tract. A large amount of research has focused on the differential expression of ER in human breast cancer. Clone 6F11 was raised against a prokaryotic recombinant protein corresponding to the full-length alpha form of the human ER molecule. Clone EMR02 is directed to the wild type C-terminus of human ER beta. Clone 1D5 reacts specifically with ER alpha and shows no reaction with ER beta. Clone SP1 is a rabbit monoclonal antibody generated from a synthetic peptide derived from the C-terminus of human ER alpha molecule.

Estrogen and Progesterone Receptor (duo pack)

Clones 6F11 and 16 VP-E611 •2x0.5 ml

H, *F*, *W* • Working Dilution (Clone 6F11): 1:40 - 1:80

H, *F*, *W* • Working Dilution (Clone 16): 1:100 - 1:200

Estrogen and Progesterone receptors (ER/PR) are expressed in a variety of different tissues but are found most prominently in the female reproductive tract. A large amount of research has focused on the differential expression of ER/PR in human breast cancer. Clone 6F11 was raised against a prokaryotic recombinant protein corresponding to the full-length alpha form of the human ER molecule. Clone 16 is specific for a region of the N-terminus of the A form of PR.

Fas (CD95)

Clone GM30 VP-F702 • 1 ml

H, *F* • Working Dilution: 1:40 - 1:80

Fas is an apoptosis-related glycoprotein expressed on the surface of various cell types including activated T and B lymphocytes and T lymphoblastoid cell lines. This clone recognizes a site on the internal domain near the C-terminal end of the human Fas molecule.

Fascin

Clone IM20 VP-F703 • 1 ml

H, *W* • Working Dilution: 1:200 - 1:400

Fascin is found in dendritic cells and medullary dendritic cells of the thymus and Reed-Sternberg cells in certain lymphomas. This clone binds to a site on the C-terminal region of the fascin molecule.

Feline Calicivirus (Capsid protein)

Clone 1G9 VP-F704 • 0.5 ml

W, *O* • Working Dilution: 1:500 - 1:1000

Feline calicivirus (FCV) is a pathogen of cats that produces a variety of ailments. This clone recognizes a capsid protein of 62 kDa in western blots.

Fibronectin

Clone 568 VP-F705 • 1 ml $385

P, *F* • Working Dilution: 1:100 - 1:200

Fibronectin is found primarily in basement membranes. This clone is specific for the cell attachment domain of human fibronectin.

Filaggrin

Clone 15C10 VP-F706 • 1 ml

H • Working Dilution: 1:50 - 1:100

Filaggrin exhibits aberrant expression in some human keratinizing disorders. This clone reacts with a portion of the N-terminus of the human filaggrin molecule.

Galectin-1

Clone 25C1 VP-G801 • 1 ml

H, *W* • Working Dilution: 1:100 - 1:200

Galectin-1 is expressed in various malignant tumors such as thyroid and colon carcinomas. This clone was generated against the full length of the mature human galectin-1 molecule.

Galectin-3

Clone 9C4 VP-G802 • 1 ml

H, *W* • Working Dilution: 1:100 - 1:200

Galectin-3 expression is downregulated in certain cancers such as colon and breast cancer. It is detected in anaplastic large cell lymphomas. This clone was generated against the full length of the human galectin-3 molecule.

Gamma-Sarcoglycan

Clone 35DAG/21B5 VP-G803 • 1 ml

F • Working Dilution: 1:100

Gamma-sarcoglycan is found in normal muscle but has a differential expression in certain instances of muscular dystrophy. This clone was raised against a synthetic peptide containing amino acids 167-178 of the rabbit gamma-sarcoglycan sequence.

Glial Fibrillary Acidic Protein

Clone GA5 VP-G805 • 1 ml

P, *F* • Working Dilution: 1:100

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein found in glial cells such as astrocytes and ependymal cells. GFAP is also seen in Schwann cells, enteric glial cells, and satellite cells of the sensory ganglia in the peripheral nervous system. This clone was generated using porcine spinal cord.

Glucagon

Rabbit Polyclonal VP-G806 • 0.5 ml

P, *F* • Working Dilution: 1:25 - 1:50

Glucagon is found primarily in islet cells of the pancreas. This polyclonal antibody was generated using porcine glucagon.

Glucocorticoid Receptor

Clone 4H2 VP-G807 • 1 ml

H, *W* • Working Dilution: 1:10 - 1:20

Glucocorticoid receptor is expressed in many cell types including neoplastic cells of chronic lymphocytic leukemia. This clone is specific for the N-terminal modulating region of the human glucocorticoid receptor.

Glutathione S-Transferase, pi

Clone LW29 VP-G810 • 1 ml

P, *W* • Working Dilution: 1:200 - 1:400

Four glutathione s-transferase isoenzymes exist and are expressed in numerous tissues such as brain, liver, stomach, breast, kidney, and skin at varying levels. Expression varies in certain cancers. This clone is specific for the pi isoform.

Gonadotropin-Releasing Hormone Receptor

Clone A9E4 VP-G811 • 1 ml

H • Working Dilution: 1:10 - 1:20

Gonadotropin-releasing hormone receptor is expressed primarily in the anterior pituitary. This clone was raised using a synthetic peptide representing amino acids 1-29 of the human gonadotropin-releasing hormone receptor extracellular region.

Granzyme B

Clone 11F1 VP-G812 • 1 ml

H • Working Dilution: 1:40 - 1:80

Granzyme B is found in lytic granules of cytotoxic T lymphocytes and NK cells. This clone was generated against the N-terminus of the mature granzyme B molecule.

Gross Cystic Disease Fluid Protein-15

Clone 23A3 VP-G813 • 1 ml

H • Working Dilution: 1:20 - 1:40

Gross cystic disease fluid protein-15 is found in apocrine cystic secretions in gross cystic disease of the breast. It is also present in apocrine epithelia in major organs and salivary glands. This clone was generated against the excreted domain of the gross cystic disease fluid protein (15 kDa) molecule.

Growth Associated Phosphoprotein 43 (GAP43)

Clone 1G7 VP-G814 • 1 ml

P • Working Dilution: 1:20 - 1:40

GAP43 is expressed in neuronal growth cones and some presynaptic terminals. This clone was generated using the complete GAP43 molecule.

Heat Shock Protein 27

Clone 2B4 VP-H901 • 1 ml

P, *F*, *W* • Working Dilution: 1:20 - 1:40

Heat shock proteins are induced in organisms when exposed to stress such as heat and chemicals. This clone was generated using the full length human heat shock protein 27 molecule.

Heat Shock Protein 70

Clone 8B11 VP-H902 • 1 ml

H • Working Dilution: 1:20 - 1:40

Heat shock proteins are induced in organisms when exposed to stress such as heat and chemicals. Heat shock protein 70 has been reported in certain cancers. This clone recognizes human heat shock protein (Hsp) 70 and heat shock cognate (Hsc) protein 70.

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Primary Antibodies (continued)

Helicobacter Pylori

Rabbit Polyclonal VP-H918 • 1 ml

P • Working Dilution: 1:20 - 1:40

Helicobacter pylori is a known causal agent of chronic gastritis and peptic ulcer disease in humans, and strains can be grouped into two broad families, type I and type II, based on their expression of the hopQ allele. This rabbit polyclonal antibody is reactive with heat stable, somatic antigens of the whole *Helicobacter pylori* organism.

Hepatitis C Virus (NS3)

Clone MMM33 VP-H905 • 1 ml

H, *F* • Working Dilution: 1:25 - 1:50

Hepatitis C virus (HCV) is the primary cause of blood-born and community acquired non-A and non-B hepatitis. This clone was raised against a recombinant NS3 protein and will help detect HCV antigen in infected hepatocytes. It exhibits minimal cross-reactivity with normal or diseased liver sections.

c-MET (Hepatocyte Growth Factor Receptor)

Clone 8F11 VP-H906 • 1 ml

H, *F* • Working Dilution: 1:20 - 1:30

c-MET is expressed on hepatocytes, microglial cells in white matter, astrocytes, kidney tubular cells, keratinocytes, and endothelial cells. This clone binds to the external domain of the beta chain of the c-MET molecule.

Hepatocyte Specific Antigen

Clone OCH1E5 VP-H907 • 1 ml

P • Working Dilution: 1:50

This antibody recognizes an uncharacterized antigen found in normal adult and fetal hepatocytes and hepatocellular carcinomas. Tissue staining appears as a distinct granular cytoplasmic pattern.

Human Follicle Stimulating Hormone (beta 2)

Clone INN-hFSH-60 VP-H910 • 1 ml

T, *F* • Working Dilution: 1:25 - 1:50

Follicle stimulating hormone (FSH) is derived from gonado-trophic cells of the pituitary. This clone recognizes the beta 2 epitope of the beta subunit of human FSH.

Human Gastric Mucin

Clone 45M1 VP-H911 • 1 ml

H, *F* • Working Dilution: 1:50

This antibody recognizes mucin of stomach epithelium, and also fetal, pre-malignant, and malignant colonic epithelium, but not normal colon. It was derived from mucin isolated from fluid taken from an ovarian cyst.

Human Growth Hormone

Rabbit Polyclonal VP-H912 • 0.25 ml

P, *F* • Working Dilution: 1:50 - 1:100

Growth hormone is made by somatotroph cells located in the pituitary. This rabbit polyclonal antibody will help detect growth hormone in these cell types in uncharacterized tissues.

Human Herpesvirus (type 8)

(latent nuclear antigen)

Clone 13B10 VP-H913 • 1 ml

H, *W* • Working Dilution: 1:25 - 1:50

Human herpesvirus type 8 (HHV8) is the product of viral gene ORF73 and is expressed In Kaposi's sarcoma, multicentric Castleman's disease, angioimmunoblastic lymphadenopathies, and in the mantle zone of large immunoblastic B cells. This clone binds to a portion of the C-terminus of the latent nuclear antigen-1 molecule of HHV8.

Human Spasmolytic Polypeptide

Clone GE16C VP-H915 • 1 ml

H • Working Dilution: 1:25 - 1:50

Human spasmolytic polypeptide (HSP) is a trefoil peptide found prominently in the foveolar and surface epithelium, pyloric glands, and mucous neck cells of the stomach. This clone was generated using a synthetic peptide corresponding to a 16 amino acid C-terminal sequence of the human spasmolytic polypeptide.

Insulin

Clone 2D11-H5 VP-I250 • 1 ml

P, *O* • Working Dilution: 1:75 - 1:150

Insulin is a hormone secreted from the beta cells of the islets of Langerhans in the pancreas. This antibody was generated from insulin conjugated to bovine serum albumin carrier protein.

Involucrin

Clone SY5 VP-I251 • 1 ml

P, *F*, *O* • Working Dilution: 1:100 - 1:200

Involucrin is a cytoplasmic protein expressed in a range of stratified squamous epithelia, including the cornea, that lack a distinct cornified layer. The immunogen used to generate this antibody was human involucrin (120 kDa).

Ki67 Antigen

Rabbit Polyclonal VP-K451 • 0.2 ml

H, *F*, *O* • Working Dilution: 1:1000

Clone MM1 VP-K452 • 1 ml

H, *F*, *C*, *O* • Working Dilution: 1:100 - 1:200

Rabbit Monoclonal

Clone SP6 VP-RM04 • 0.5 ml

H • Working Dilution: 1:100 - 1:200

Ki67 is a cell cycle associated protein expressed from G1 through the end of M phase. The rabbit polyclonal antibody and clone MM1 were each generated using a recombinant fusion protein corresponding to a 1086 bp Ki67 motif-containing cDNA fragment. The rabbit monoclonal antibody was generated using a synthetic peptide from C-terminus of human Ki67 protein.

Lamin A/C

Clone 636 VP-L550 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:10 - 1:20

Lamins are intermediate filament-type proteins located adjacent to the inner nuclear membrane of cells. Human lamina consists of four major types of lamin, namely A, B1, B2 and C. Loss of lamin A expression occurs in small cell lung cancers. This antibody recognizes lamins A and C.

Laminin

Clone LAM-89 VP-L551 • 0.5 ml

T, ***F*** • Working Dilution: 1:50 - 1:100

Laminin is an extracellular matrix glycoprotein found in basement membranes of epithelia, surrounding blood vessels, and nerves in established tissues and in other sites during early embryonic development. This antibody was generated using purified human laminin and shows no crossreaction with collagen type IV, fibronectin, vitronectin or chondroitin sulphate types A, B, and C.

Langerin

Clone 12D6 VP-L552 • 1 ml

H • Working Dilution: 1:100 - 1:200

Langerin is a transmembrane mannose-binding protein found on Langerhans cells. This clone was generated using a recombinant protein of 29 kDa corresponding to the external domain of the langerin molecule.

M2A Antigen (Lymphatic Endothelial Cell Marker)

Clone D2-40 VP-M671 • 0.5 ml

P • Working Dilution: 1:40

This antibody recognizes the M2A oncofetal antigen, a 40 kDa, O-linked sialoglycoprotein, expressed on the cell membrane of lymphatic endothelium, fetal gonocytes, and certain germ cell tumor cell lines. No reactivity is seen on cancer cell lines of human breast, prostate, melanoma, cervix, colon, pharynx, brain, and bone marrow. This antibody has also been shown to be a useful marker for cells of mesothelial origin, including malignant mesothelioma.

Macrophage Marker

Clone MAC387 VP-M640 • 1 ml

T, ***F*** • Working Dilution: 1:100

This antibody recognizes the L1 protein. L1 protein is expressed on neutrophils, monocytes, some reactive macrophages, and squamous mucosal epithelia. The clone was made using a purified monocyte membrane preparation.

Maspin

Clone EAW24 VP-M642 • 1 ml

H • Working Dilution: 1:25 - 1:50

Maspin, an abbreviated name for mammary-specific serpin, is a tumor suppressor protein present in normal breast and prostatic epithelial cells but down-regulated in carcinomas of these cell types. This antibody was made using a recombinant protein corresponding to the N-terminal region of the human maspin molecule.

Mast Cell Tryptase

Clone 10D11 VP-M672 • 1 ml

P • Working Dilution: 1:150 - 1:300

Tryptase is one of several chemical mediators found in mast cells. This antibody will be a useful marker of human mast cells and mast cell tryptase as it offers an advantage over traditional histological methods that require special fixatives to preserve the tryptase-containing granules.

Matrix Metalloproteinase 9

Clone 2C3 VP-M644 • 1 ml

H • Working Dilution: 1:40

Matrix metalloproteinases (MMPs) are a family of zinc-containing proteolytic enzymes. MMP9 is expressed in normal kidney tubules, hepatocytes, spermatids, myocytes, stomach parietal cells, prostatic columnar epithelium, and uterine cells. This antibody was generated using a recombinant protein corresponding to a 134 amino acid portion of the C-terminal region of the mature human MMP9 molecule.

MDM2 Protein

Clone 1B10 VP-M645 • 1 ml

H, ***F*** • Working Dilution: 1:50 - 1:100

This antibody targets the human phosphoprotein homologue of the murine double minute 2 gene product (MDM2). MDM2 amplification is observed in some soft tissue sarcomas, osteosarcomas, and high grade malignant gliomas. This clone was made using a recombinant fusion protein corresponding to a site near the C-terminal of the MDM2 molecule.

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Primary Antibodies (continued)

Melan A

Clone A103 VP-M646 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:25

Melan A, a product of the MART-1 gene, is present in most melanomas. This antibody was generated using human melan A, and recognizes a 20 to 22 kDa protein doublet in melan A mRNA-positive melanoma cell lines. It does not react with melan A mRNA negative cell lines.

Melanoma Marker (HMB45)

Clone HMB45 VP-M647 • 1 ml

T, ***F*** • Working Dilution: 1:30 - 1:60

This antibody recognizes the majority of melanomas and other tumors that show melanoma/melanocytic differentiation including melanocytic Schwannoma clear cell sarcoma. It does not recognize normal adult melanocytes or non-melanocytic cells. Some staining has been reported in prenatal retinal pigment epithelium and junctional and blue nevus cells and variably with fetal/neonatal melanocytes. The immunogen used to generate the antibody was an extract of pigmented melanoma metastases from lymph nodes.

Merosin Laminin Alpha 2 Chain

Clone Mer3/22B2 VP-M648 • 1 ml

F • Working Dilution: 1:50 - 1:100

Merosin is the muscle specific form of laminin and is composed of three chains: alpha 2, beta 1, and gamma 1. Mutations in the gene for the laminin alpha 2 chain of merosin account for a form of muscular dystrophy. This clone reacts with the 300 kDa fragment of merosin.

Mesothelin

Clone 5B2 VP-M649 • 1 ml

H, ***F*** • Working Dilution: 1:20 - 1:40

Mesothelin is a glycoprotein expressed on mesothelial cells in the kidney, some epithelial cells of the trachea, tonsil, and oviduct and in certain cancers. This antibody was generated against a fusion protein corresponding to approximately 100 amino acids which are present in the membrane-bound form of the human mesothelin molecule.

Microphthalmia Transcription Factor (MITF)

Clone 34CA5 VP-M650 • 1 ml

HE, ***F*** • Working Dilution: 1:10 - 1:20

Microphthalmia transcription factor (MITF) exists in several isoforms, MITF-A, -C, -H and -M. This antibody is specific for MITF-M that is expressed in normal and malignant melanocytes.

Minichromosome Maintenance Protein 2

Clone CRCT2.1 VP-M651 • 1 ml

H • Working Dilution: 1:25 - 1:50

Minichromosome Maintenance Protein 7

Clone DCS-141.1 VP-M652 • 1 ml

H, ***W*** • Working Dilution: 1:25 - 1:50

Minichromosome maintenance (MCM) proteins are crucial in eukaryotic DNA replication. Generally, the levels of MCM proteins increase in variable amounts as normal cells move from G_0 into G_1/S phase of the cell cycle. Clones CRCT2.1 and DCS-141.1 are specific for minichromosome maintenance proteins 2 and 7, respectively.

Muc Glycoproteins

Muc-1 Core Glycoprotein

Clone Ma552 VP-M654 • 1 ml

H, ***F*** • Working Dilution: 1:50 - 1:100

Muc-1 Glycoprotein

Clone Ma695 VP-M655 • 1 ml

H, ***F*** • Working Dilution: 1:100

Muc-2 Glycoprotein

Clone Ccp58 VP-M656 • 1 ml

H, ***F*** • Working Dilution: 1:100 - 1:200

Muc-5AC Glycoprotein

Clone CLH2 VP-M657 • 1 ml

H • Working Dilution: 1:50 - 1:100

Muc-6 Glycoprotein

Clone CLH5 VP-M658 • 1 ml

H • Working Dilution: 1:50 - 1:100

Mucins are a family of glycoproteins synthesized and secreted by glandular epithelial tissues. To date, nine distinct epithelial mucin genes (Muc-1, 2, 3, 4, 5AC, 5B, 6, 7 and 8) have been identified. Mucins display differential expression in epithelia with cell-type specificity. Modifications in expression levels and glycosylation patterns of mucins have been described in several cancers.

Muscle Specific Actin

Clone HHF35 VP-M659 • 1 ml

P, ***F***, ***W*** • Working Dilution: 1:100 - 1:200

Actins are protein cytoskeletal components, and are divided into alpha-actins present in muscle tissue, beta and gamma-actins present in non-muscle cells, and a subset of gamma-actins present in muscle cells. This antibody is specific for alpha- and gamma-actins of smooth muscle and reacts with myocardium and skeletal muscle, arterial wall muscle fibers, smooth muscle of the G.I. tract, myometrium, prostatic stroma, and bladder wall.

Myeloperoxidase

Clone 59A5 VP-M661 • 1 ml

P • Working Dilution: 1:75 - 1:150

Myeloperoxidase is a lysosomal enzyme in all myeloid cells including mature granulocytes, neutrophil granulocytes and monocytes in blood, in precursors of granulocytes in the bone marrow, and in Kupffer cells of the liver. This antibody was generated using a recombinant protein corresponding to 101 amino acids from exon 7 of the human myeloperoxidase molecule.

MyoD1 (Rhabdomyosarcoma Marker)

Clone 5.8A VP-M669 • 1 ml

HE, *F*, *O* • Working Dilution: 1:20 - 1:50

MyoD1 is one of several regulatory genes that control myogenic differentiation. The MyoD1 gene protein is not detected in normal adult tissue but is highly expressed in rhabdomyosarcomas. This antibody recognizes an epitope near the C-terminus of the MyoD1 protein.

Myoglobin

Clone MY018 VP-M673 • 1 ml

P • Working Dilution: 1:50 - 1:100

Myoglobin is found in skeletal and cardiac muscle but not smooth muscle. This antibody does not recognize hemoglobin and may help in detecting tissues of striated muscle origin.

Myosin Heavy Chain

Myosin Heavy Chain (developmental)

Clone RNMy2/9D2 VP-M664 • 1 ml

F • Working Dilution: 1:10 - 1:40

Myosin Heavy Chain (fast)

Clone WB-MHCf VP-M665 • 1 ml

F • Working Dilution: 1:10 - 1:40

Myosin Heavy Chain (neonatal)

Clone WB-MHCn VP-M666 • 1 ml

F • Working Dilution: 1:5 - 1:20

Myosin Heavy Chain (slow)

Clone WB-MHCs VP-M667 • 1 ml

F • Working Dilution: 1:20 - 1:80

Myosin is one of the key proteins involved with muscle contraction. It is a rod-like molecule consisting of heavy and light chains. The heavy chain component varies between different muscles and fiber types and some isoforms are developmentally regulated. These antibodies recognize the isoforms indicated.

Neuroblastoma Marker

Clone NB84a VP-N750 • 1 ml

P, *F*, *C* • Working Dilution: 1:100 - 1:200

This antibody reacts with a molecule found in many tissues including most epithelial and endothelial cells. It also recognizes about 90% of all neuroblastomas but not other tumor types, such as leukemia, rhabdomyosarcoma or Wilms' tumor. It does, however, recognize about half of the cases of Ewing's sarcoma.

Neurofilament

Neurofilament 200kDa

Clone RT97 VP-N752 • 1 ml

P, *F* • Working Dilution: 1:50

Neurofilament 200kDa

Clone N52.1.7 VP-N753 • 1 ml

H, *F* • Working Dilution: 1:25 - 1:400

Neurofilament 68kDa

Clone NR4 VP-N754 • 1 ml

H, *F* • Working Dilution: 1:20 - 1:50

Neurofilaments make up the main structural elements of axons and dendrites and are found in neurons, peripheral nerves, and sympathetic ganglion cells. Neurofilaments consist of three major subunits with molecular weights of 68 kDa, 160 kDa and 200 kDa. These antibodies recognize the subunits as indicated.

Neuron Specific Enolase

Clone 5E2 VP-N755 • 1 ml

P, *F*, *W* • Working Dilution: 1:50 - 1:100

Neuron specific enolase is found predominantly in neurons, neuroendocrine cells, smooth and striated muscle cells, megakaryocytes, T cells, and some platelets. Clone 5E2 reacts with the 47 kDa component of the gamma-gamma enolase isoenzyme.

Oct-2

Clone Oct-207 VP-O850 • 1 ml

H, *F* • Working Dilution: 1:25 - 1:50

Oct-2 found most highly in B cells and lymphomas derived from B cells. It has been noted that a lack of Oct-2 and BOB.1 in Reed-Sternberg cells of Hodgkin's disease may indicate a mutation in the Ig gene of these cells. This clone was generated using a recombinant protein corresponding to 129 amino acids of the N-terminus of the human Oct-2 molecule.

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Primary Antibodies (continued)

Osteonectin

Clone 15G12 VP-O851 • 1 ml

H, *W* • Working Dilution: 1:40 - 1:80

Osteonectin, is found in developing teeth and bone as well as steroid-producing cells of the adrenal glands, suprabasal layers of the epidermis, glomeruli of the kidney, bronchi of the lung, megakaryocytes, and large vessels. Osteonectin has been reported in some malignancies, but it has not been detected in a number of different sarcomas, including chondrosarcoma. This antibody recognizes a region within the C-terminus of the human osteonectin molecule.

Osteopontin

Clone OP3N VP-O852 • 1 ml

H • Working Dilution: 1:50 - 1:100

Osteopontin is present in normal breast, endothelial cells, macrophages, myoepithelial cells and epithelium of the G.I. tract, gall bladder, pancreas, urinary and reproductive tracts, lung, and salivary and sweat glands. It has been reported to be present in breast carcinoma and osteosarcomas but not lymphoid tumors. This antibody was raised against a recombinant protein representing a C-terminal region of osteopontin.

Ovarian Cancer Antigen (CA125)

Clone Ov185:1 VP-O853 • 1 ml

H, *F* • Working Dilution: 1:100

CA125 is a protein associated with ovarian epithelial malignancies. It is also present in seminal vesicle carcinoma and anaplastic lymphoma. Expression, however, is not found exclusively in malignant tumors. This antibody appears specific for a repetitive protein determinant expressed in the protein core of the CA125 human ovarian cancer antigen.

p27 Protein

Clone 1B4 VP-P951 • 1 ml

H, *W* • Working Dilution: 1:20 - 1:40

p27 protein, also known as kinase inhibitory protein 1 (Kip1), binds to cyclin E/cdk2 complexes and is found in growth-arrested cells. p27 is found in proliferating cells in only a sequestered form when it cannot interact with cyclin E/cdk2 complexes. This antibody was raised against a recombinant antigen corresponding to the full length p27 molecule.

p53 Protein

Clone IMX25 VP-P952 • 1 ml

H, *W* • Working Dilution: 1:50 - 1:100

Clone PAb 1801 VP-P953 • 1 ml

H, *F*, *W*, *C*, *O* • Working Dilution: 1:40

Clone BP53-12 VP-P954 • 1 ml

P, *F*, *W*, *O* • Working Dilution: 1:50

Rabbit Polyclonal (CM1) VP-P955 • 0.2 ml

P, *F*, *W*, *O* • Working Dilution: 1:50 - 1:100

Rabbit Polyclonal (CM5) VP-P956 • 0.2 ml

H • Working Dilution: 1:500 - 1:1000

Clone DO-1 VP-P957 • 1 ml

H, *F*, *W* • Working Dilution: 1:20 - 1:40

Clone DO-7 VP-P958 • 1 ml

P, *F*, *W*, *C*, *O* • Working Dilution: 1:50 - 1:100

Rabbit Monoclonal

Clone SP5 VP-RM08 • 0.5 ml

P/*H*, *W* • Working Dilution: 1:100

p53 protein is crucial to normal regulation of cell growth and is a suppressor of tumor cell proliferation. A high proportion of breast and colon carcinomas are positive for p53 and this expression correlates with poor prognosis in breast cancer. These antibodies recognize both wild type and mutant forms of p53 protein under denaturing and non-denaturing conditions. Clone IMX25 and p53 CM5 recognize rat and mouse p53. Clone PAb 1801 recognizes a site between amino acids 46-55 of the human p53 molecule. Clones D0-1, D0-7 and BP53-12 recognize amino acid sequence 20-25 of the human p53 molecule. Clone SP5 was raised against the full-length human wild type p53 molecule.

p57 Protein (Kip2)

Clone 25B2 VP-P959 • 1 ml

H • Working Dilution: 1:50 - 1:100

p57 protein is a cyclin dependent kinase inhibitor. The gene encoding for p57 is in a chromosome region that is a common site for loss of heterozygosity in certain diseases. This antibody was raised using a recombinant antigen corresponding to a 116 amino acid region of the N-terminus of the p57 protein.

p63 Protein

Clone 7JUL VP-P960 • 1 ml

HE, *F* • Working Dilution: 1:25 - 1:50

p63 exists as at least six isoforms and is found in several normal tissues including proliferating cells of epithelium, cervix, urothelium, and prostate. It has an altered expression in some cancers. This clone was generated using a recombinant fusion protein corresponding to a region (aa319-410) common to six isoforms of the p63 molecule.

p73 Protein

Clone 24 VP-P961 • 1 ml

H, *F* • Working Dilution: 1:50 (paraffin)
Working Dilution: 1:200 (frozen)

p73 protein has been reported in several cancers, but it is not induced by some DNA damage signals. p73 has six known isoforms and the relative expression levels of each splice variant may modulate p73 activity. This clone was raised using a recombinant protein corresponding to 110 amino acids of the C-terminal region of the human p73 molecule.

Parathyroid Hormone

Clone 105G7 VP-P962 • 1 ml

P • Working Dilution: 1:150 - 1:300

Parathyroid hormone is secreted by chief cells of the parathyroid glands. This antibody was raised using a recombinant protein corresponding to the entire human parathyroid hormone molecule.

Parvalbumin (Alpha)

Clone 2E11 VP-P963 • 1 ml

H • Working Dilution: 1:200 - 1:400

Alpha and beta parvalbumins are expressed in different human tissues with the alpha form highly expressed in extracts of human cerebellum, weakly in kidney and not in skeletal muscle, thymus, lung, placenta, heart, liver, and diaphragm. Reduced expression of alpha parvalbumin has been reported in Purkinje cells in cases of spinocerebellar ataxia-1. This antibody does not detect parvalbumin in preterm placenta thereby indicating its specificity for the alpha form of this protein.

Parvovirus B19

Clone R92F6 VP-P964 • 1 ml

P, *F* • Working Dilution: 1:20 - 1:40

Parvovirus B19 is a single-stranded DNA virus. This clone is specific for the viral antigens VP1 and VP2.

P-Cadherin

Clone 56C1 VP-P965 • 1 ml

H, *F*, *W* • Working Dilution: 1:50 - 1:100

P-Cadherin is only detected in the basal or parabasal layers of stratified epithelia. It has been implicated in tumor progression and certain cancers. This antibody was generated using a recombinant protein corresponding to a region of the external domain of the P-cadherin molecule.

Perforin

Clone 5B10 VP-P967 • 1 ml

H • Working Dilution: 1:20

Perforin is found in cytotoxic T lymphocytes (CTLs), CD5 positive NK cells, CD3 positive large granular lymphocytes, and gamma/delta T cells. This antibody recognizes the external portion of the C-terminus of the perforin molecule.

Peripherin

Clone PJM50 VP-P968 • 1 ml

H, *F*, *W*, *O* • Working Dilution: 1:100 - 1:200

Peripherin is expressed in peripheral neurons including enteric ganglion cells and in neuronal derivatives of the neural crest. This antibody does not stain chromaffin cells, which is an advantage over other neural markers, but is a useful marker for neural crest derived tumors such as neuroblastomas and ganglioneuroblastomas.

Placental Alkaline Phosphatase

Clone 8A9 VP-P969 • 1 ml

H, *F* • Working Dilution: 1:20 - 1:40

Rabbit Monoclonal
Clone SP15 VP-RM12 • 0.5 ml

H • Working Dilution: 1:100

Placental alkaline phosphatase (PLAP) is only found in normal term placenta, endocervix, and oviduct and in ovarian and proximal intestinal tumors. A PLAP-like variant is seen mostly in fetal testis and thymus and some defined tumors. Clone 8A9 stains seminomas and placenta indicating a specificity for both PLAP and PLAP-like enzyme. Clone SP15 reacts with a membrane-bound isoenzyme of PLAP occurring in the placenta during the 3rd trimester of gestation.

Platelet-Derived Endothelial Growth Factor

Clone P-GF.44C VP-P971 • 1 ml

H, *W* • Working Dilution: 1:60 - 1:120

Platelet-derived endothelial growth factor (PDEGF) is seen in macrophages, stromal cells, glial cells, and some epithelia. No expression is seen in gastrointestinal epithelium, smooth muscle, adrenal glands, lung, and testis. This antibody was raised using a recombinant protein corresponding to human thymidine phosphorylase (TP), also known as PDEGF.

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Primary Antibodies (continued)

Polyomavirus (JC/BK viruses)

Clone 3.1.1 VP-P973 • 1 ml

I, *O* • Working Dilution: 1:40 - 1:80

This antibody detects polyomavirus types JC and BK. This antibody does not cross-react with respiratory syncytial virus, parainfluenza virus types 1,2,3 and 4b, adenovirus, varicella-zoster virus, herpes simplex virus types 1 and 2, mumps virus, measles virus, echovirus 19, coxsackie B4 virus, poliovirus types 1,2 and 3, and influenza virus types A and B.

Progesterone Receptor

Clone 1A6 VP-P975 • 1 ml

H, *F*, *W* • Working Dilution: 1:40

Clone 16 VP-P976 • 1 ml

H, *F*, *W* • Working Dilution: 1:100 - 1:200

Rabbit Monoclonal

Clone SP2 VP-RM06 • 0.5 ml

H • Working Dilution: 1:40

Progesterone receptor (PR) is expressed in a variety of different tissues but is found most prominently in the female reproductive tract. A large amount of research has focused on the differential expression of PR in human breast and uterine cancers. PR exists as two isoforms, PRA and PRB. Clone 1A6 reacts with a homologous region of the A and B forms of PR. Clone 16 is specific for a region of the N-terminus of the A form of PR. Clone SP2 was raised using a recombinant protein encoding human progesterone receptor amino acids 412-526.

Progesterone Receptor (A/B Forms)

Clones 16 and SAN27 VP-P977 • 1 ml

H, *F*, *W* • Working Dilution: 1:100 - 1:200

This antibody cocktail consisting of two clones will detect both forms of progesterone receptor in various tissues and cell lines.

Progesterone Receptor (B Form)

Clone SAN27 VP-P987 • 1 ml

H • Working Dilution: 1:100 - 1:200

Progesterone receptor (B Form) is highly expressed in endometrial glandular and stromal nuclei in the proliferative phase of the menstrual cycle and expressed at low levels during the secretory phase and early pregnancy. This clone was generated using a recombinant protein representing the 164 amino acid N-terminal sequence unique to the B form of the progesterone receptor.

Proinsulin

Clone 1G4 VP-P978 • 1 ml

H • Working Dilution: 1:200 - 1:400

Differential staining patterns for proinsulin have been observed in certain tumors. This antibody was made using purified human proinsulin.

Proliferating Cell Nuclear Antigen (PCNA)

Clone PC10 VP-P980 • 1 ml

H/*P*, *W*, *C*, *O* • Working Dilution: 1:100 - 1:200

Proliferating cell nuclear antigen (PCNA) is seen in S phase of the cell cycle and also during DNA synthesis associated with DNA damage repair mechanisms. It may also be detected in non-cycling cells such as those in G_0 phase. This antibody was generated using rat PCNA induced in the protein A expression vector pR1T2T.

Prostate Specific Antigen

Clone PSA 28/A4 VP-P981 • 1 ml

P, *F* • Working Dilution: 1:50 - 1:100

Prostate specific antigen (PSA) is expressed in secretory and ductal epithelium of normal and neoplastic prostatic tissue. Low levels of expression of PSA have been reported in non-prostatic tissues and tumors such as breast carcinomas.

Prostatic Acid Phosphatase

Clone PASE/4LJ VP-P982 • 1 ml

P, *F* • Working Dilution: 1:100

Prostatic acid phosphatase is found in the prostate and seminal fluid. The antigen used for immunization was prostatic acid phosphatase purified from human seminal plasma.

Protein Gene Product 9.5

Clone 10A1 VP-P983 • 1 ml

H, *F*, *W* • Working Dilution: 1:20 - 1:40

Protein gene product (PGP) 9.5 is a neuron specific protein found predominantly in neurons and nerve fibers throughout the central and peripheral nervous system. The immunogen used was a recombinant fusion protein corresponding to the full length of the protein gene product 9.5 molecule.

pS2 Protein

Rabbit Polyclonal VP-P984 • 0.5 ml

P, *F* • Working Dilution: 1:100 - 1:200

pS2 is expressed in normal gastric mucosa, small intestinal mucosa, and normal breast epithelium. It is also detected in gastric carcinoma, gynecological cancers, and predominantly in estrogen receptor positive breast cancers. It has been reported that high expression of pS2 in these breast cancers is a predictor for a favorable prognosis.

Renal Cell Carcinoma Marker

Clone 66.4.C2 VP-R150 • 1 ml

T • Working Dilution: 1:50 - 1:100

The glycoprotein, gp200, is found in normal kidney. It is also localized in breast tubules and ducts, the surface of epididymal tubular epithelia, and in the colloid of the thyroid follicles. Due

to its reported high expression in metastatic renal cell carcinomas it is referred to as a renal cell carcinoma marker. This clone is specific for a proximal nephrogenic renal antigenic site on the carbohydrate domain of gp200.

Respiratory Syncytial Virus

Clones 5H5N, 2G122, 5A6, 1C3 VP-R151 • 1 ml

H, ***F***, ***I*** • Working Dilution: 1:200 - 1:400

There are multiple types and subtypes of respiratory syncytial virus (RSV). This product is a mixture of four antibodies to optimize sensitivity and detection of the various RSV forms. It does not crossreact with tissue culture isolates of influenza virus types A and B, parainfluenza virus types 1, 2, 3 and 4b, adenovirus, herpes simplex virus types 1 and 2, varicella-zoster virus, cytomegalovirus, mumps virus, measles virus, echovirus 19, coxsackie B4 virus, poliovirus types 1, 2 and 3 or negative tissue culture cells used in routine virus isolation.

ret Oncoprotein

Clone 3F8 VP-R152 • 1 ml

H • Working Dilution: 1:20 - 1:40

Ret expression is reported in several regions of the central nervous system; in the developing cranial nerve ganglia and a subset of cells within dorsal root ganglia, in motor neurons in the spinal cord and hindbrain, in neuroretina, and the growing tips of the renal collecting ducts in developing kidney. Mutations of the ret proto-oncogene are implicated in certain developmental abnormalities and tumors. This antibody was raised to the intracellular domain of the molecule, present in all isoforms of the protein.

Retinoblastoma Gene Protein

Clone 13A10 VP-R153 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:25 - 1:50

Retinoblastoma (Rb) gene protein derives its name from a rare tumor of the retina. The Rb gene protein is found in many cell types. This clone was raised to the N-terminal region of the Rb gene protein.

S-100 Protein

Clone S1/61/69 VP-S275 • 1 ml

P, ***F*** • Working Dilution: 1:20 - 1:40

Rabbit Polyclonal VP-S276 • 1 ml

P, ***F*** • Working Dilution: 1:200 - 1:400

S-100 protein is found in neuroectodermal tissue, including nerves and melanocytes, Langerhans cells in skin, and interdigitating reticulum cells in the paracortex of lymph nodes. VP-S275 is not recommended for staining neural elements, but stains almost all malignant melanomas. VP-S276 will identify S-100 in neural elements and other normal and uncharacterized tissues.

Human Securin

Clone DCS-280.2 VP-S279 • 1 ml

H • Working Dilution: 1:25 - 1:50

Human securin is also referred to as pituitary tumor transforming gene-1. Its expression correlates with cell proliferation in a cell cycle dependent manner in normal tissues and several tumor types. This antibody was raised using a recombinant fusion protein corresponding to the GST-Pds1 of the human securin molecule.

Sialyl Lewis^a Antigen (CA19-9)

Clone C241:5:1:4 VP-S280 • 1 ml

H, ***F*** • Working Dilution: 1:200

This antibody specifically recognizes sialyl Lewis^a containing glycolipids and does not cross-react with Lewis^a, Lewis^b or other structurally related molecules. The epitope CA19-9 recognized by this antibody has been reported to be a useful marker of gastrointestinal and pancreatic cancers.

Smooth Muscle Actin, Alpha

Clone sm-1 VP-S281 • 1 ml

P, ***F***, ***W*** • Working Dilution: 1:25 - 1:50

Alpha smooth muscle actin is found in vascular walls, intestinal muscularis mucosae and muscularis propria, and in the stroma of various tissues. The immunogen used to generate this antibody was a synthetic amino terminal decapeptide of the alpha smooth muscle isoform of actin.

Spectrin

Clone RBC2/3D5 VP-S283 • 1 ml

F, ***W*** • Working Dilution: 1:100

Labeling for spectrin is necessary to monitor membrane integrity. This antibody should be used in combination with dystrophin antibodies to ensure the membrane is intact and that the absence of dystrophin staining is real and not due to tissue freezing or handling artifact. It is intended as a positive control for muscle membranes. This clone recognizes the beta chain of spectrin in erythrocytes and muscle.

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Synaptophysin

Clone 27G12 VP-S285 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:100 - 1:200

Rabbit Monoclonal

Clone SP11 VP-RM09 • 0.5 ml

H, ***W*** • Working Dilution: 1:100

Synaptophysin is found in neuronal presynaptic vesicles in brain, spinal cord, retina and similar vesicles of the adrenal medulla, and in neuromuscular junctions. It is also expressed in a wide spectrum of neuroendocrine tumors. Both clone 27G12 and clone SP11 were made using a synthetic peptide corresponding to a region near the C-terminus of the synaptophysin molecule.

Tartrate-Resistant Acid Phosphatase

Clone 26E5 VP-T475 • 1 ml

H • Working Dilution: 1:50 - 1:100

Tartrate-resistant acid phosphatase (TRAP) is found in alveolar macrophages, osteoclasts, spleen, liver, placental decidual cells, and syncytiotrophoblasts. Increased expression has been reported in cases of Gaucher's disease, hairy cell leukemias, and osteoclastomas. The immunogen used to generate this antibody was a recombinant fusion protein corresponding to the N-terminal portion of the TRAP molecule.

Tau

Clone Tau-2 VP-T476 • 1 ml

H • Working Dilution: 1:50 - 1:100

This antibody will help identify the tau protein and positively stain neurofibrillary tangles, neuropil threads, and abnormal neurites in senile plaques of Alzheimer's diseased brain tissue. This clone was raised against the bovine tau protein, and cross-reacts with the phosphorylated form of human tau protein.

Terminal Deoxynucleotidyl Transferase

Clone SEN28 VP-T479 • 1 ml

H, ***W*** • Working Dilution: 1:50 - 1:100

Terminal deoxynucleotidyl transferase (TdT) is found in primitive B and T lymphocytes of normal thymus and bone marrow and in acute lymphoblastic lymphomas and certain leukemias. This clone was raised against a recombinant protein corresponding to the amino terminal region of the terminal deoxynucleotidyl transferase molecule.

Thyrogloblin

Clone ID4 VP-T481 • 1 ml

P, ***F*** • Working Dilution: 1:50 - 1:100

Thyroglobulin is found in follicular epithelial cells of the thyroid. This antibody was made using purified human thyroglobulin.

Thyroid Stimulating Hormone

Clone QB2/6 VP-T482 • 1 ml

T, ***F*** • Working Dilution: 1:50 - 1:100

Thyroid stimulating hormone (TSH) is found in thyrotrophic cells of the pituitary. This antibody is specific for TSH and shows no reactivity with luteinizing hormone, follicle stimulating hormone, and human chorionic gonadotrophin.

Thyroid Transcription Factor-1

Clone SPT24 VP-T483 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:100 - 1:200

Thyroid transcription factor-1 (TTF-1) is expressed in normal adult lung and thyroid and has been reported in some carcinomas of these tissues. The immunogen used to generate this clone was a recombinant protein corresponding to a 123 amino acid fragment of the N-terminal region of the TTF-1 molecule.

Topoisomerase II Alpha

Clone 3F6 VP-T484 • 1 ml

H, ***F***, ***W*** • Working Dilution: 1:20 - 1:40

Topoisomerase II alpha expression is linked to the cell cycle and is associated with the proliferation status of cells. A significant variation in the range of expression of this protein has been reported in many different tumors. This antibody was raised against a recombinant protein corresponding to the C-terminal region of the topoisomerase II alpha molecule.

Toxoplasma gondii P30 Antigen

Clone TP3 VP-T485 • 1 ml

H • Working Dilution: 1:10 - 1:20

Toxoplasma gondii is a protozoan parasite. This antibody will help detect most strains of *Toxoplasma gondii* as it recognizes the highly conserved and prominent P30 surface antigen.

Transforming Growth Factor Beta

Clone TGFB17 VP-T486 • 1 ml

H • Working Dilution: 1:20 - 1:40

This antibody was raised using a recombinant protein corresponding to the full length mature transforming growth factor beta1 molecule. It may however, cross-react with other members of the TGFB family.

Transforming Growth Factor Beta Receptor (type 1)

Clone 8A11 VP-T487 • 1 ml

H • Working Dilution: 1:25 - 1:50

This antibody was raised using a recombinant protein corresponding to the external domain of the human transforming growth factor beta receptor type 1 molecule.

Tyrosinase

Clone T311 VP-T488 • 1 ml

HE, *F* • Working Dilution: 1:25 - 1:50

Tyrosinase is expressed in melanocytes and melanomas. Lack of tyrosinase is associated with certain forms of albinism. The immunogen used to generate this antibody was a recombinant prokaryotic protein corresponding to the tyrosinase molecule.

Tyrosine Hydroxylase

Clone 1B5 VP-T489 • 1 ml

H, *W* • Working Dilution: 1:20 - 1:40

This antibody may be a useful marker of all catecholamine neurons. It was generated using a recombinant protein corresponding to a portion of the carboxyl terminal end of the mouse tyrosine hydroxylase molecule.

Ubiquitin

Clone FPM1 VP-U577 • 1 ml

P • Working Dilution: 1:25 - 1:50

Ubiquitin is a highly conserved polypeptide widely found in eukaryotic cells. This antibody was raised against ubiquitin conjugated with glutaraldehyde cross-linked to keyhole limpet hemocyanin.

Utrophin

Utrophin (N-terminus)

Clone DRP3/20C5 VP-U579 • 2.5 ml

F • Working Dilution: 1:2 - 1:10

Utrophin is a homologue of dystrophin. In normal muscle, utrophin expression is restricted to neuromuscular junctions. In dystrophin-deficient muscle, utrophin may be up-regulated and is also present around the periphery of most muscle fibers. This clone was generated using a fusion protein containing the first 261 amino acids of the published DMDL gene sequence.

Varicella-zoster Virus

Clone C90.2.8 VP-V680 • 1 ml

P, *I* • Working Dilution: 1:25 - 1:50

This antibody is specific for varicella-zoster virus and does not cross-react with respiratory syncytial virus, parainfluenza virus types 1,2,3 and 4b, adenovirus, herpes simplex virus types 1 and 2, influenza virus types A and B, mumps virus, measles virus, echovirus 19, coxsackie B4 virus or poliovirus types 1, 2, and 3.

Villin

Clone CWWB1 VP-V682 • 1 ml

H, *F*, *W* • Working Dilution: 1:100 - 1:200

Villin is found predominantly in epithelial cells that develop a brush border such as those in the intestinal mucosa, gall bladder, kidney proximal tubules, and ductuli efferentes of the testis. It is located in the apical cytoplasm, adjacent to the villus, and hence is a good indicator of cell polarity. This protein is composed of three domains. The first two domains are homologous and the third domain is called the headpiece. This antibody was generated using a recombinant protein corresponding to the C-terminal "headpiece" region of the human villin molecule.

Vimentin

Clone V9 VP-V684 • 1 ml

H, *F*, *W* • Working Dilution: 1:50 - 1:100

Rabbit Monoclonal

Clone SP20 VP-RM17 • 0.5 ml

P/*H* • Working Dilution: 1:100

Vimentin is found in cells of mesenchymal origin such as endothelial cells, fibroblasts, smooth muscle cells, and lymphoid cells. Clone V9 was generated using purified vimentin from porcine eye lens. Clone SP20 was raised using recombinant human vimentin protein.

G

Control Antibodies

These IgG preparations are intended for use as controls for primary antibodies made in rabbit, mouse, rat, and goat. Supplied as lyophilized powders, these antibodies have been purified from pooled serum of healthy adult animals and contain a spectrum of the IgG subclasses present in serum. They should be applied to a control tissue section at the same concentrations as the primary antibody to indicate whether staining is specific for the antigen or is non-specific adsorption of primary antibody to tissue sites.

Product	Catalog Number	Unit Size
Rabbit IgG	I-1000	5 mg
Mouse IgG	I-2000	1 mg
Rat IgG	I-4000	1 mg
Goat IgG	I-5000	5 mg

Key: *P*: Paraffin sections, no pretreatment • *T*: Paraffin, trypsin pretreatment • *H*: Paraffin, high temperature antigen unmasking (citrate) • *HE*: Paraffin, high temperature antigen unmasking (Tris) • *F*: Frozen sections • *I*: Immunofluorescence • *W*: Western blotting • *C*: Flow cytometry • *O*: Other

Secondary Antibodies

Our affinity purified antibodies are of unmatched quality. All secondary antibodies are prepared by immunizing animals using proprietary immunization schedules that produce high affinity antibodies. The antibodies are purified by affinity chromatography. Cross-reactivities that are likely to interfere with specific labeling are removed by solid phase adsorption techniques. Antibodies are conjugated in a manner that ensures the maximum degree of labeling with no alteration in specificity and affinity of the antibody.

These antibodies are subjected to rigorous quality control assays. They can be used for tissue and cell staining, ELISAs, and blots.

Some of these antibodies are supplied in lyophilized form and can be reconstituted with 1 ml of water. With a few exceptions, the recommended dilution for most applications is 1:200.

(H+L) indicates the antibody recognizes both heavy and light chains.

Product	Conjugate	Catalog Number	Unit Size
Mouse			
Anti-Mouse IgG (H+L) made in horse	Unconjugated	AI-2000	1.5 mg
	Biotinylated [d]	BA-2000	1.5 mg
	AMCA	CI-2000	1.5 mg
	Fluorescein	FI-2000	1.5 mg
	Phycoerythrin	EI-2007	1.0 mg
	Texas Red®	TI-2000	1.5 mg
	Alkaline Phosphatase	AP-2000	1.0 ml
	Peroxidase	PI-2000	1.0 mg
	DyLight® 488	DI-2488	1.5 mg
	DyLight® 549	DI-2549	1.5 mg
	DyLight® 594	DI-2594	1.5 mg
	DyLight® 649	DI-2649	1.5 mg
Anti-Mouse IgG (H+L) Kit made in horse - 500 µg AMCA Anti-Mouse IgG - 500 µg Fluorescein Anti-Mouse IgG - 500 µg Texas Red® Anti-Mouse IgG	Fluorescent Kit	FI-2100	1 kit
Anti-Mouse Ig ImmPRESS™ Kit [h]	Peroxidase Polymer	MP-7402	15 ml
			50 ml
Anti-Mouse Ig ImmPRESS™ Kit rat adsorbed [b, h]	Peroxidase Polymer	MP-7422	15 ml
Anti-Mouse M.O.M.™ Ig Reagent [i]	Biotinylated	MKB-2225	0.1 ml
Anti-Mouse IgG (H+L) [b] rat adsorbed made in horse	Unconjugated	AI-2001	0.5 mg
	Biotinylated	BA-2001	0.5 mg
	Fluorescein	FI-2001	0.5 mg
Anti-Mouse IgG (H+L) made in goat	Unconjugated	AI-9200	1.5 mg
	Biotinylated	BA-9200	1.5 mg
Anti-Mouse IgM µ chain specific made in goat	Unconjugated	AI-2020	0.5 mg
	Biotinylated [d]	BA-2020	0.5 mg
	AMCA	CI-2020	0.5 mg
	Fluorescein	FI-2020	0.5 mg
	Texas Red®	TI-2020	0.5 mg

Product	Conjugate	Catalog Number	Unit Size
Rabbit			
Anti-Rabbit IgG (H+L) made in goat	Unconjugated	AI-1000	1.5 mg
	Biotinylated [d]	BA-1000	1.5 mg
	AMCA	CI-1000	1.5 mg
	Fluorescein	FI-1000	1.5 mg
	Phycoerythrin	EI-1007	1.0 mg
	Texas Red®	TI-1000	1.5 mg
	Alkaline Phosphatase	AP-1000	1.0 ml
	Peroxidase	PI-1000	1.0 mg
	DyLight® 488	DI-1488	1.5 mg
	DyLight® 549	DI-1549	1.5 mg
	DyLight® 594	DI-1594	1.5 mg
	DyLight® 649	DI-1649	1.5 mg
Anti-Rabbit IgG (H+L) Kit made in goat - 500 µg AMCA Anti-Rabbit IgG - 500 µg Fluorescein Anti-Rabbit IgG - 500 µg Texas Red® Anti-Rabbit IgG	Fluorescent Kit	FI-1200	1 kit
Anti-Rabbit Ig ImmPRESS™ Kit [h]	Peroxidase Polymer	MP-7401	15 ml
			50 ml
Anti-Rabbit IgG (H+L) made in horse	Unconjugated	AI-1100	1.5 mg
	Biotinylated	BA-1100	1.5 mg
Goat			
Anti-Goat IgG (H+L) [a] made in rabbit	Unconjugated	AI-5000	1.5 mg
	Biotinylated [d]	BA-5000	1.5 mg
	AMCA	CI-5000	1.5 mg
	Fluorescein	FI-5000	1.5 mg
	Texas Red®	TI-5000	1.5 mg
Anti-Goat IgG (H+L) [a] made in horse	Biotinylated	BA-9500	1.5 mg
	Alkaline Phosphatase	AP-9500	1.0 ml
	Peroxidase	PI-9500	1.0 mg
Anti-Goat Ig ImmPRESS™ Kit [a, h]	Peroxidase Polymer	MP-7405	50 ml

Product	Conjugate	Catalog Number	Unit Size
Universal			
Anti-Mouse/Rabbit IgG (H+L) made in horse [d, e]	Biotinylated	BA-1400	2.1 mg
Anti-Mouse/Rabbit Ig ImmPRESS™ Kit [h]	Peroxidase Polymer	MP-7500	15 ml
			50 ml
Anti-Mouse/Rabbit/Goat IgG (H+L), pan specific [f, g] made in horse	Biotinylated	BA-1300	2.2 ml
Rat			
Anti-Rat IgG (H+L) made in rabbit	Unconjugated	AI-4000	1.5 mg
	Biotinylated [d]	BA-4000	1.5 mg
	Fluorescein	FI-4000	1.5 mg
Anti-Rat Ig ImmPRESS™ Kit [h]	Peroxidase Polymer	MP-7404	50 ml
Anti-Rat Ig ImmPRESS™ Kit, mouse adsorbed [c, h]	Peroxidase Polymer	MP-7444	15 ml
Anti-Rat IgG (H+L) [c] mouse adsorbed made in rabbit	Unconjugated	AI-4001	0.5 mg
	Biotinylated	BA-4001	0.5 mg
	Fluorescein	FI-4001	0.5 mg
Anti-Rat IgG (H+L) made in goat	Biotinylated	BA-9400	1.5 mg
	Texas Red®	TI-9400	1.5 mg
Anti-Rat IgG (H+L) [c] mouse adsorbed made in goat	Biotinylated	BA-9401	0.5 mg
Sheep			
Anti-Sheep IgG (H+L) [a] made in rabbit	Unconjugated	AI-6000	1.5 mg
	Biotinylated [d]	BA-6000	1.5 mg
	Fluorescein	FI-6000	1.5 mg
	Texas Red®	TI-6000	1.5 mg
Hamster			
Anti-Hamster IgG (H+L), made in goat	Unconjugated	AI-9100	1.5 mg
	Biotinylated	BA-9100	1.5 mg
	Fluorescein	FI-9100	1.5 mg
	Texas Red®	TI-9100	1.5 mg
Guinea Pig			
Anti-Guinea Pig IgG (H+L), made in goat	Unconjugated	AI-7000	1.5 mg
	Biotinylated [d]	BA-7000	1.5 mg
	Fluorescein	FI-7000	1.5 mg
	Texas Red®	TI-7000	1.5 mg
Cat			
Anti-Cat IgG (H+L) made in goat	Biotinylated	BA-9000	1.5 mg

a - Suitable for use with goat, sheep, and bovine IgG primary antibodies.
b - Designed for use in rat tissues.
c - Designed for use in mouse tissues.
d - Antibodies included in VECTASTAIN® ABC Kits

Product	Conjugate	Catalog Number	Unit Size
Human			
Anti-Human IgG (H+L) made in goat	Unconjugated	AI-3000	1.5 mg
	Biotinylated [d]	BA-3000	1.5 mg
	AMCA	CI-3000	1.5 mg
	Fluorescein	FI-3000	1.5 mg
	Texas Red®	TI-3000	1.5 mg
	Alkaline Phosphatase	AP-3000	1.0 ml
	Peroxidase	PI-3000	1.0 mg
Anti-Human IgA α chain specific made in goat	Unconjugated	AI-3030	0.5 mg
	Biotinylated	BA-3030	0.5 mg
Anti-Human IgE ε chain specific made in goat	Unconjugated	AI-3040	0.5 mg
	Biotinylated	BA-3040	0.5 mg
	Fluorescein	FI-3040	0.5 mg
Anti-Human IgG γ chain specific made in goat	Unconjugated	AI-3080	0.5 mg
	Biotinylated	BA-3080	0.5 mg
	Fluorescein	FI-3080	0.5 mg
Anti-Human IgM μ chain specific made in goat	Unconjugated	AI-3020	0.5 mg
	Biotinylated [d]	BA-3020	0.5 mg
	Fluorescein	FI-3020	0.5 mg
Anti-Human Kappa Chain made in goat	Unconjugated	AI-3060	0.5 mg
	Biotinylated	BA-3060	0.5 mg
	AMCA	CI-3060	0.5 mg
	Fluorescein	FI-3060	0.5 mg
Anti-Human Lambda Chain made in goat	Unconjugated	AI-3070	0.5 mg
	Biotinylated	BA-3070	0.5 mg
	AMCA	CI-3070	0.5 mg
	Fluorescein	FI-3070	0.5 mg
Horse			
Anti-Horse IgG (H+L) made in goat	Biotinylated	BA-8000	1.5 mg
Chicken			
Anti-Chicken IgG (H+L) made in goat	Biotinylated	BA-9010	1.5 mg
Swine			
Anti-Swine IgG (H+L) made in goat	Biotinylated	BA-9020	1.5 mg

e - Universal Anti-Mouse/Rabbit IgG (BA-1400) should be reconstituted with 2 ml water and diluted 1:50 for use.
f - Universal Pan-Specific Anti-Mouse/Rabbit/Goat IgG (BA-1300) should be diluted 1:20.
g - Antibody used in the VECTASTAIN® Universal Quick Kits.
h - Prediluted, Ready-to-Use solutions. See pages A12-A13 for more information on ImmPRESS™ reagents
i - Designed for use in mouse tissues with M.O.M.™ Kit reagents.

Antibodies to Lectins

These antibodies to lectins are produced by hyperimmunizing goats with pure lectins. Following conventional purification steps, specific antibody is isolated by affinity chromatography on lectin-agarose columns. Each anti-lectin is supplied lyophilized in buffered saline.

These highly purified specific antibodies provide excellent intermediate reagents for localizing lectin receptors in tissues and on cells, or following lectin transport in neuronal tracing.

Biotinylated anti-lectins are optimally labeled with biotin. They can be used as intermediates to localize lectin receptors in tissues or on cells, or to follow lectin migration in neuronal transport. In some applications, biotinylated anti-lectins used in conjunction with unlabeled lectins may provide greater sensitivity than using biotinylated lectins alone.

See Section K, "Lectins and Glycobiology Reagents", for full lectin product listing.

Product	Conjugate	Catalog Number	Unit Size
Anti-Concanavalin A, made in goat	Unconjugated	AS-2004	1 mg
Anti-*Dolichos biflorus* agglutinin, made in goat	Unconjugated	AS-2034	1 mg
Anti-*Galanthus nivalis* lectin, made in goat	Unconjugated	AS-2240	1 mg
Anti-*Griffonia (Bandeiraea) simplicifolia* lectin I, made in goat	Unconjugated	AS-2104	1 mg
Anti-*Lens culinaris* agglutinin/*Pisum sativum* agglutinin, made in goat	Unconjugated	AS-2044	1 mg
Anti-Peanut agglutinin (PNA), made in goat	Unconjugated	AS-2074	1 mg
	Biotinylated	BA-0074	0.5 mg
Anti-*Phaseolus vulgaris* agglutinin (E+L), made in goat	Unconjugated	AS-2224	1 mg
	Biotinylated	BA-0224	0.5 mg
Anti-*Phaseolus vulgaris* agglutinin (E+L), made in rabbit	Unconjugated	AS-2300	1 mg
Anti-*Ricinus communis* agglutinin I & II, made in goat	Unconjugated	AS-2084	1 mg
	Biotinylated	BA-0084	0.5 mg
Anti-Soybean agglutinin, made in goat	Unconjugated	AS-2014	1 mg
Anti-*Ulex europaeus* agglutinin I, made in goat	Unconjugated	AS-2064	1 mg
	Biotinylated	BA-0064	0.5 mg
Anti-Wheat Germ agglutinin, made in goat	Unconjugated	AS-2024	1 mg
	Biotinylated	BA-0024	0.5 mg

Anti-Avidin and Anti-Streptavidin

Anti-Avidin and Anti-Streptavidin Reagents

Our antibodies to avidin and streptavidin are produced in goats using our highly purified avidin or streptavidin and isolated by affinity chromatography. Anti-Avidin does not bind streptavidin and Anti-Streptavidin does not recognize avidin. These antibodies provide opportunities to significantly amplify signals in many applications.

Biotinylated Anti-Avidin and Biotinylated Anti-Streptavidin have been widely used as amplifying reagents in immunohistochemistry, *in situ* hybridization, microarray assays, ELISAs, blots, and many other applications. The capability of binding avidin or streptavidin via either biotin binding sites or through antigen binding sites, makes these biotinylated antibodies unique. These antibodies can be used either as part of preformed complexes or in sequence to amplify fluorescent signals. When used in sequence, the target is first labeled with fluorochrome-conjugated avidin or streptavidin, followed by incubation with Biotinylated Anti-Avidin or Biotinylated Anti-Streptavidin, followed by a second layer of fluorochrome-conjugated avidin or streptavidin. This sequence can be repeated. This multi-layered approach introduces more fluorochromes at the target site and can provide a multi-fold amplification over a single layer.

These affinity purified antibodies are also available unconjugated or fluorescein-labeled.

Anti-Avidin Antibodies

Antibody Description	Conjugate	Catalog Number	Unit Size	Stock Concentration	Dilution
Anti-Avidin, made in goat	Biotin	BA-0300	0.5 mg	0.5 mg/ml	1:100
	Unconjugated	SP-2000	1 mg	1 mg/ml	1:200
	Fluorescein	SP-2040	0.5 mg	1 mg/ml	1:50

Anti-Streptavidin Antibodies

Antibody Description	Conjugate	Catalog Number	Unit Size	Stock Concentration	Dilution
Anti-Streptavidin, made in goat	Biotin	BA-0500	0.5 mg	0.5 mg/ml	1:100
	Unconjugated	SP-4000	1 mg	2 mg/ml	1:400
	Fluorescein	SP-4040	0.5 mg	1 mg/ml	1:50

Antibodies to Tags and Labels

Anti-Biotin

The mouse monoclonal Anti-Biotin-M is a high-titer, low background antibody. Provided as a culture supernatant, this antibody is designed to be used for immunohistochemical procedures and *in situ* hybridization. The VECTASTAIN® ABC systems and the ImmPRESS™ Anti-Mouse Ig peroxidase polymer detection system (MP-7401) are sensitive methods for detecting this antibody.

Our polyclonal Anti-Biotin is produced in goats and is purified by affinity chromatography. Several different conjugates of this antibody are available. The unconjugated antibody can be detected with a VECTASTAIN® ABC system or the ImmPRESS™ Anti-Goat Ig peroxidase polymer detection system (MP-7405) in blot, immunohistochemical, and *in situ* hybridization applications.

Agarose-bound Anti-Biotin contains 1 mg of Anti-Biotin per ml of settled 4% agarose gel beads. This product can be used to bind and dissociate biotinylated molecules.

Fluorescein-labeled Anti-Biotin has been optimally conjugated to provide a bright fluorescent signal for detecting biotinylated probes in cells or tissue.

The enzyme-conjugated Anti-Biotin antibodies have been produced with the highest specific activity enzymes in optimal enzyme/antibody ratios. The covalent linkage between the enzyme and antibody is chosen to provide stable, highly active conjugates for immunohistochemical, *in situ* hybridization, enzyme immunoassay, or transfer blot applications.

Conjugate	Catalog Number	Unit Size
Anti-Biotin, made in mouse, unconjugated	MB-9100	1 ml
Anti-Biotin, made in goat, unconjugated	SP-3000	1 mg
Anti-Biotin, made in goat, agarose bound	SP-3030	2 ml
Anti-Biotin, made in goat, alkaline phosphatase	SP-3020	1 ml
Anti-Biotin, made in goat, fluorescein	SP-3040	0.5 mg
Anti-Biotin, made in goat, peroxidase	SP-3010	1 mg

Anti-Digoxigenin/Digoxin (DIG)

Nucleic acid probes are frequently labeled with digoxigenin (DIG), a steroid found in plants. This is particularly common in double-label *in situ* hybridization applications with a biotin-labeled probe. DIG-labeled probes can be visualized using our high affinity, purified antibody conjugates to digoxigenin.

Conjugate	Catalog Number	Unit Size
Anti-Digoxigenin/Digoxin, made in goat, unconjugated	MB-7000	1 mg
Anti-Digoxigenin/Digoxin, made in goat, DyLight® 488 labeled	DI-7488	0.5 mg
Anti-Digoxigenin/Digoxin, made in goat, DyLight® 594 labeled	DI-7594	0.5 mg

Anti-Dinitrophenyl (DNP)

Dinitrophenyl (DNP) is not found endogenously in tissues and is an excellent alternative to biotin for use in applications requiring biotin-free systems. Probes or antibodies labeled with DNP can be detected using alkaline phosphatase labeled DNP or using the biotinylated antibody followed by a streptavidin or avidin system.

Conjugate	Catalog Number	Unit Size
Anti-DNP, made in rabbit, alkaline phosphatase	MB-3100	150 µg
Anti-DNP, made in rabbit, biotinylated	BA-0603	0.5 mg

Anti-Fluorescein

Fluorescein is not found in biological systems. This green fluorescent label can be directly visualized or used as a specific tag and detected with our high affinity, purified antibody reagents shown below. Labeled probes can be used in applications such as *in situ* hybridization and blotting.

Conjugate	Catalog Number	Unit Size
Anti-Fluorescein, made in goat, unconjugated	SP-0601	1 mg
Anti-Fluorescein, made in goat, alkaline phosphatase	MB-2100	150 µg
Anti-Fluorescein, made in goat, biotinylated	BA-0601	0.5 mg
Anti-Fluorescein, made in goat, peroxidase	SP-1910	0.5 mg

Anti-Rhodamine/Texas Red®

Texas Red® is not found endogenously in tissues. This red fluorescent label can be directly visualized or detected with our high affinity, purified antibody reagents shown below. Labeled probes can be used in applications such as *in situ* hybridization and blotting.

Conjugate	Catalog Number	Unit Size
Anti-Rhodamine*, made in goat, unconjugated	SP-0602	1 mg
Anti-Rhodamine*, made in goat, alkaline phosphatase	MB-1920	150 µg
Anti-Rhodamine*, made in goat, biotinylated	BA-0605	0.5 mg

** Binds most rhodamines including Texas Red®*

Antibodies to Fusion Protein Tags

Fusion protein reagents are part of a methodology for purification, detection, and investigation of expressed proteins. The common fusion tags [green fluorescent protein (GFP), poly histidine (polyHis), c-Myc, human influenza virus hemagglutinin (HA), Maltose binding protein (MBP)] can be easily detected with our antibodies against the specific tag.

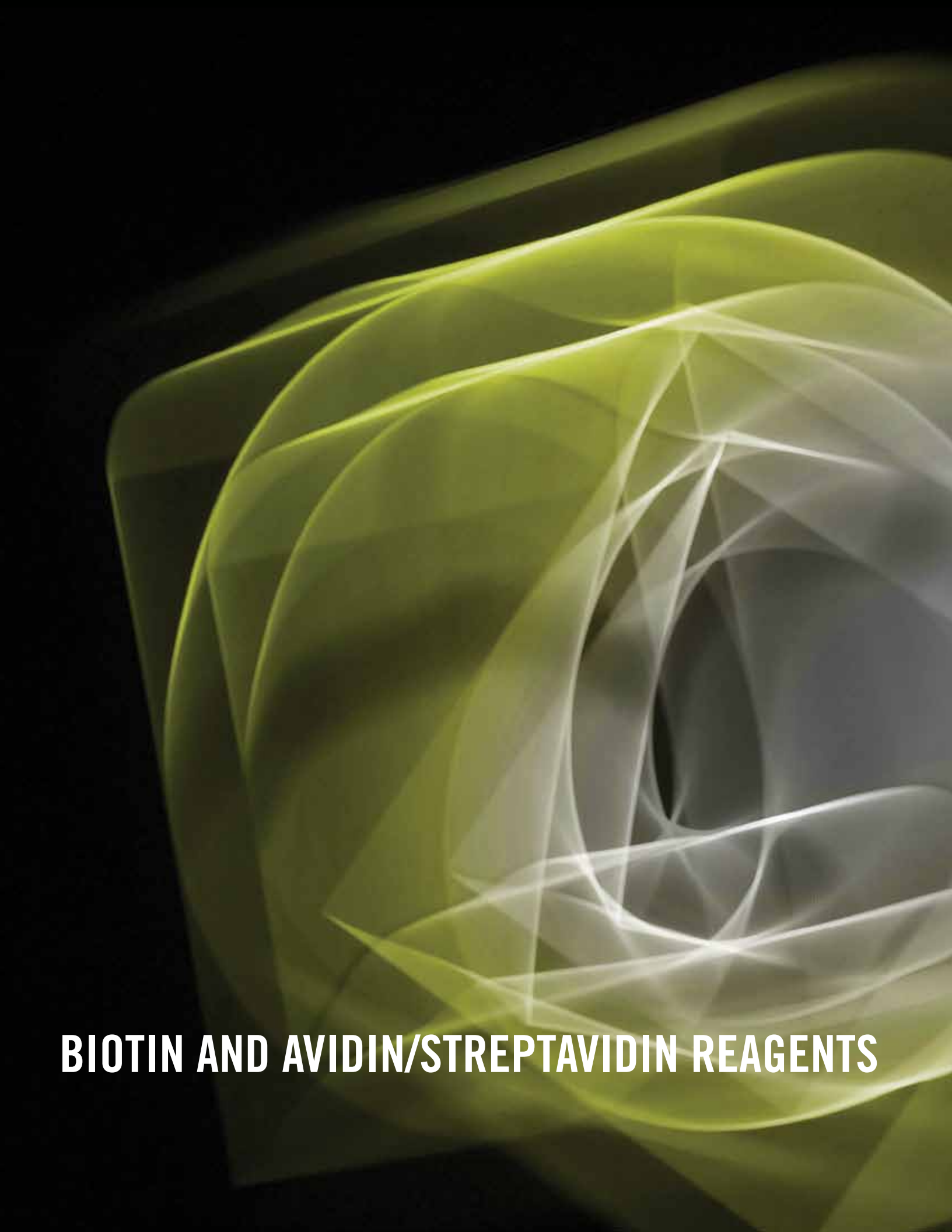
Our biotinylated, affinity-purified polyclonal antibodies (produced in goat) are optimized to detect fusion proteins in western blot detection. They can be used in combination with a VECTASTAIN® ABC-AmP™ detection system or other streptavidin/avidin-based reagents.

For maximum sensitivity, GFP can also be detected using ImmPRESS™ Anti-GFP antibody (made in goat). This reagent is prepared using our proprietary peroxidase micropolymer technology.

Recombinant GFP Standard (MB-0752) contains purified recombinant *Aquorea victorea* GFP (28 kDa) overexpressed in *E. coli*. It is designed for quantitation of GFP fusion protein in the test sample, and can be used as posistive control on western blots in conjuction with Peroxidase-labeled Anti-GFP (MB-0712) or Biotinylated Anti-GFP (BA-0702).

Additional information on fusion protein purification reagents can be found in Section J, "Affinity Binding Matrices", page J6.

Product	Catalog Number	Unit Size
Anti-c-Myc, made in goat, biotinylated	BA-0703	0.25 mg
Anti-GFP, made in goat, biotinylated	BA-0702	0.25 mg
Anti-GFP, ImmPRESS™ (peroxidase), made in goat	MB-0712	100 µl
Recombinant GFP Standard	MB-0752	100 µg
Anti-HA, made in goat, biotinylated	BA-0704	0.25 mg
Anti-MBP, made in goat, biotinylated	BA-0701	0.25 mg
Anti-polyHistidine, made in goat, biotinylated	BA-0705	0.25 mg



BIOTIN AND AVIDIN/STREPTAVIDIN REAGENTS

Biotinylated Products

Biotinylated Secondary Antibodies

Vector Laboratories' affinity-purified antibodies are of unmatched quality for use in immunological techniques. These antibodies are prepared using a proprietary immunization schedule that produces high affinity antibodies. The antibodies are then purified by affinity chromatography, and cross-reactivities that are likely to interfere with specific labeling are removed by solid phase adsorption techniques. The biotinylated secondary antibodies are conjugated to ensure the maximum degree of labeling while not compromising the specificity or affinity of the antibody. These antibodies are subjected to rigorous quality control assays. They can be used for tissue and cell staining, ELISAs, and blots.

Most of these antibodies are supplied lyophilized; reconstitute with 1 ml of water. The recommended dilution for most applications is 1:200.

(H+L) indicates the antibody recognizes both heavy and light chains.

Product	Catalog Number	Unit Size
Anti-Cat IgG (H+L) biotinylated, made in goat	BA-9000	1.5 mg
Anti-Chicken IgG (H+L) biotinylated, made in goat	BA-9010	1.5 mg
Anti-Goat IgG (H+L) *a, d* biotinylated, made in rabbit	BA-5000	1.5 mg
Anti-Goat IgG (H+L) *a* biotinylated, made in horse	BA-9500	1.5 mg
Anti-Guinea Pig IgG (H+L) *d* biotinylated, made in goat	BA-7000	1.5 mg
Anti-Hamster IgG (H+L) biotinylated, made in goat	BA-9100	1.5 mg
Anti-Horse IgG (H+L) biotinylated, made in goat	BA-8000	1.5 mg
Anti-Human IgG (H+L) *d* biotinylated, made in goat	BA-3000	1.5 mg
Anti-Human IgA, biotinylated, α chain specific, made in goat	BA-3030	0.5 mg
Anti-Human IgE, biotinylated, ε chain specific, made in goat	BA-3040	0.5 mg
Anti-Human IgG, biotinylated, γ chain specific, made in goat	BA-3080	0.5 mg
Anti-Human IgM, biotinylated, μ chain specific, made in goat	BA-3020	0.5 mg
Anti-Human Kappa Chain biotinylated, κ chain specific, made in goat	BA-3060	0.5 mg
Anti-Human Lambda Chain biotinylated, λ chain specific, made in goat	BA-3070	0.5 mg

Product	Catalog Number	Unit Size
Anti-Mouse IgG (H+L) *d* biotinylated, made in horse	BA-2000	1.5 mg
Anti-Mouse IgG (H+L) *b* biotinylated, made in horse, rat adsorbed	BA-2001	0.5 mg
Anti-Mouse IgG (H+L) biotinylated, made in goat	BA-9200	1.5 mg
Anti-Mouse IgM *d*, biotinylated, μ chain specific, made in goat	BA-2020	0.5 mg
Anti-Rabbit IgG (H+L) *d* biotinylated, made in goat	BA-1000	1.5 mg
Anti-Rabbit IgG (H+L) biotinylated, made in horse	BA-1100	1.5 mg
Anti-Rat IgG (H+L) *d* biotinylated, made in rabbit	BA-4000	1.5 mg
Anti-Rat IgG (H+L) *c*, biotinylated, made in rabbit, mouse adsorbed	BA-4001	0.5 mg
Anti-Rat IgG (H+L) biotinylated, made in goat	BA-9400	1.5 mg
Anti-Rat IgG (H+L) *c*, biotinylated, made in goat, mouse adsorbed	BA-9401	0.5 mg
Anti-Sheep IgG (H+L) *a, d* biotinylated, made in rabbit	BA-6000	1.5 mg
Anti-Swine IgG (H+L) biotinylated, made in goat	BA-9020	1.5 mg
Universal Anti-Mouse/Rabbit IgG (H+L) *d, e* biotinylated, made in horse	BA-1400	2.1 mg
Universal Pan-Specific Anti-Mouse/ Rabbit/Goat IgG (H+L) *f, g* biotinylated, made in horse	BA-1300	2.2 ml

a - Suitable for use with goat, sheep, and bovine IgG primary antibodies.

b - Designed for use in rat tissues.

c - Designed for use in mouse tissues.

d - Antibodies included in VECTASTAIN® ABC Kits.

e - Universal Anti-Mouse/Rabbit IgG (BA-1400) should be reconstituted with 2 ml water and diluted 1:50 for use. Do not use the Universal antibody to stain rodent or rabbit tissue because of cross reactivity with endogenous IgG.

f - Universal Pan-Specific Anti-Mouse/Rabbit/Goat IgG (BA-1300) should be diluted 1:20. Do not use this Pan-Specific antibody to stain rodent, rabbit, goat, sheep, or bovine tissue because of cross reactivity with endogenous IgG.

g - Antibody used in the VECTASTAIN® Universal Quick Kits.

Biotinylated Lectins

These conjugates are prepared from our affinity-purified lectins and are optimally labeled with biotin. Essentially free of inactive lectin conjugates and containing no free biotin, these biotinylated lectins are ideal for examining glycoconjugates using a biotin/avidin or biotin/streptavidin system. Biotinylated lectins can also be used in the isolation of lymphokines and other products of mitogenic stimulation.

Biotinylated Lectin Screening Kits

Kit I consists of 1 mg of the following biotinylated lectins: Con A, DBA, PNA, RCA_{120}, SBA, UEA I and WGA.
Kit II consists of 1 mg of the following biotinylated lectins: GSL I, LCA, PHA-E, PHA-L, PSA, SJA and succinylated WGA.
Kit III consists of 0.5 mg of the following biotinylated lectins: DSL, ECL, GSL II, Jacalin, LEL, STL and VVL.

These kits provide a panel of biotinylated lectins with a variety of sugar specificities. These reagents can be used with the VECTASTAIN® ABC reagent or any avidin or streptavidin conjugate for detection.

Product	Catalog Number	Unit Size
Biotinylated Lectin Kit I	BK-1000	1 kit
Biotinylated Lectin Kit II	BK-2000	1 kit
Biotinylated Lectin Kit III	BK-3000	1 kit

Biotinylated Anti-Lectins

High affinity antibodies are produced by immunizing goats with our purified lectins. Specific antibodies are isolated by affinity chromatography on lectin-agarose columns, and then optimally labeled with biotin. Biotinylated anti-lectins can be used as intermediates to localize lectin receptors in tissues or on cells or to follow lectin migration in neuronal transport. In some applications, biotinylated anti-lectins, used in conjunction with unlabeled lectins, may provide greater sensitivity than using biotinylated lectins alone.

Biotinylated Anti-Lectins

Product	Catalog Number	Unit Size
Anti-Peanut agglutinin (PNA), biot	BA-0074	0.5 mg
Anti-*Phaseolus vulgaris* agglutinin (E+L), biot	BA-0224	0.5 mg
Anti-*Ricinus communis* agglutinin I & II, biot	BA-0084	0.5 mg
Anti-*Ulex europaeus* agglutinin I, biot	BA-0064	0.5 mg
Anti-Wheat Germ agglutinin, biot	BA-0024	0.5 mg

Biotinylated Lectins

Product	Catalog Number	Unit Size
Aleuria aurantia lectin (AAL), biot	B-1395	1 mg
Amaranthus caudatus lectin (ACL, ACA), biot	B-1255	2 mg
Bauhinia purpurea lectin (BPL), biot	B-1285	2 mg
Concanavalin A (Con A), biot	B-1005	5 mg
Succinylated Concanavalin A, biot	B-1005S	5 mg
Datura stramonium lectin (DSL), biot	B-1185	2 mg
Dolichos biflorus agglutinin (DBA), biot	B-1035	5 mg
Erythrina cristagalli lectin (ECL, ECA), biot	B-1145	5 mg
Euonymus europaeus lectin (EEL), biot	B-1335	2 mg
Galanthus nivalis lectin (GNL), biot	B-1245	2 mg
Griffonia (Bandeiraea) simplicifolia lectin I (GSL I, BSL I), biot	B-1105	2 mg
GSL I - isolectin B_4, biot	B-1205	0.5 mg
Griffonia (Bandeiraea) simplicifolia lectin II (GSL II, BSL II), biot	B-1215	2 mg
Hippeastrum hybrid lectin (HHL, AL), biot	B-1385	2 mg
Jacalin, biot	B-1155	5 mg
Lens culinaris agglutinin (LCA, LcH), biot	B-1045	5 mg
Lotus tetragonolobus lectin (LTL), biot	B-1325	2 mg
Lycopersicon esculentum lectin (LEL, TL), biot	B-1175	1 mg
Maackia amurensis lectin I (MAL I, MAL), biot	B-1315	2 mg
Maackia amurensis lectin II (MAL II, MAH), biot	B-1265	1 mg
Maclura pomifera lectin (MPL), biot	B-1345	2 mg
Narcissus pseudonarcissus lectin (NPL, NPA), biot	B-1375	2 mg
Peanut agglutinin (PNA), biot	B-1075	5 mg
Phaseolus vulgaris erythroagglutinin (PHA-E), biot	B-1125	2 mg
Phaseolus vulgaris leucoagglutinin (PHA-L), biot	B-1115	2 mg
Pisum sativum agglutinin (PSA), biot	B-1055	5 mg
Psophocarpus tetragonolobus lectin I (PTL I, WBA I), biot	B-1365	2 mg
Psophocarpus tetragonolobus lectin II (PTL II, WBA II), biot	B-1405	2 mg
Ricinus communis agglutinin I (RCA I, RCA_{120}), biot	B-1085	5 mg
Ricinus communis agglutinin II (RCA II, RCA_{60}, ricin) [a], biot	B-1095	5 mg
Sambucus nigra lectin (SNA, EBL), biot	B-1305	2 mg
Solanum tuberosum lectin (STL, PL), biot	B-1165	2 mg
Sophora japonica agglutinin (SJA), biot	B-1135	2 mg
Soybean agglutinin (SBA), biot	B-1015	5 mg
Ulex europaeus agglutinin I (UEA I), biot	B-1065	2 mg
Vicia villosa lectin (VVL, VVA), biot	B-1235	2 mg
Wheat Germ agglutinin (WGA), biot	B-1025	5 mg
Succinylated Wheat Germ agglutinin, biot	B-1025S	5 mg
Wisteria floribunda lectin (WFA, WFL), biot	B-1355	2 mg

[a] *This is a toxic lectin.*

biot=biotinylated

Some lectins may have other names in common usage.

See Section K, "Lectins and Glycobiology Reagents", for full lectin product listing.

Biotinylated Products (continued)

Biotinylated Molecular Weight Markers

DNA Molecular Weight Markers

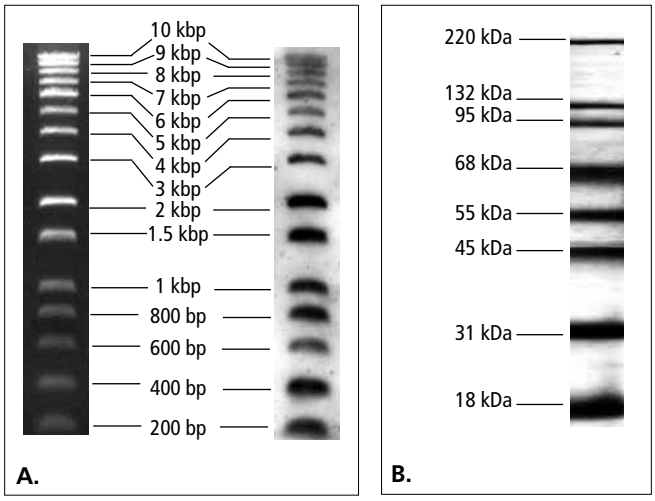
This product (SP-1400) is an electrophoresis standard consisting of 15 bands between 0.2 and 10 kilobase pairs. Bands are regularly spaced so that sizes are easy to identify. The biotinylated marker can be transferred to a membrane and detected using an avidin or streptavidin-based system.

Markers are supplied ready-to-use containing loading dye so that electrophoresis can be monitored.

Protein Molecular Weight Markers

This product (MB-1302) provides ideal standards for western blots. This product consists of eight distinct biotinylated protein bands with a molecular weight range from 19 kDa to 222 kDa. It is designed to be run in an SDS-PAGE under denaturing conditions. This mixture of biotinylated proteins can be run alongside the sample under study and, following transfer, developed with any avidin and streptavidin-based detection system.

Product	Catalog Number	Unit Size	Number of Applications
Protein Molecular Weight Markers, Biotinylated	SP-1400	250 µl	50
DNA Molecular Weight Markers, Biotinylated	MB-1302	25 µg	50



A) *Biotinylated DNA molecular weight marker visualized by ethidium bromide staining (left) and, after transfer to nylon membrane, by the UltraSNAP™ Detection System (right).*
B) *Biotinylated protein molecular weight marker separated by 7.5% denaturing PAGE, transferred to nitrocellulose, and detected with VECTASTAIN® ABC-AP using BCIP/NBT substrate.*

Biotinylated Protein A

Staphylococcus aureus, Cowan strain

Biotinylated Protein A (B-2001), isolated from a special strain of S. *aureus,* is a 41 kDa protein which binds to the Fc portion of most immunoglobulins. This interaction is neither species specific nor isotype specific, as Protein A has been shown to bind IgG from a variety of species. Protein A can also interact with IgA, IgM and subclasses of IgG from different species.

Biotinylated Protein A is useful to detect or localize immunoglobulins on cell surfaces or in tissue sections. Used with the appropriate avidin or streptavidin conjugates, immunoglobulins can be studied with brightfield, fluorescence or electron microscopy. Biotinylated Protein A can also be used to detect positive clones in monoclonal antibody production by an avidin or streptavidin enzyme conjugate.

Biotinylated Protein A is used in many systems as a substitute for a biotinylated second antibody. However, in some applications, Biotinylated Protein A may bind to endogenous immunoglobulin in the tissue producing background. In addition, Biotinylated Protein A may not bind to the species or class of primary antibody being employed. For routine immunohistochemical staining we recommend using biotinylated anti-immunoglobulins.

Biotinylated Bovine Serum Albumin (BSA)

Biotinylated Bovine Serum Albumin (BSA) (B-2007) is produced from our ultrapure immunohistochemical grade bovine serum albumin. This biotinylated protein has several uses: as a reagent for measuring binding capacity of avidin or streptavidin matrices, to amplify biotin/avidin detection systems, or as a positive control in dot blot assays.

Biotinylated Agarose

Biotin covalently attached to agarose beads will bind avidin or streptavidin essentially irreversibly. Biotinylated Agarose (B-2011) has a binding capacity exceeding 5 mg of avidin or streptavidin per ml of settled beads and can be used for the rapid removal of avidin or streptavidin conjugates from solution.

Product	Catalog Number	Unit Size
Biotinylated Protein A	B-2001	1 mg
Biotinylated Bovine Serum Albumin (BSA)	B-2007	10 mg
Biotinylated Agarose	B-2011	5 ml

Biotinylated Antibodies against Fusion Protein Tags

These biotinylated, affinity-purified polyclonal goat antibodies are optimized to detect fusion proteins in western blots. They can be detected with a VECTASTAIN® ABC-AmP™ detection system or other streptavidin/avidin-based reagents. These antibodies complement the FusionAid™ reagents for purification, detection, and investigation of proteins of interest. For additional fusion protein reagents please see Section J, "Affinity Binding Matrices", page J6.

Conjugate	Catalog Number	Unit Size
Biotinylated Anti-MBP Antibody	BA-0701	0.25 mg
Biotinylated Anti-c-Myc Antibody	BA-0703	0.25 mg
Biotinylated Anti-HA Antibody	BA-0704	0.25 mg
Biotinylated Anti-polyHistidine Antibody	BA-0705	0.25 mg
Biotinylated Anti-GFP Antibody	BA-0702	0.25 mg

Biotinylated Enzymes

The high specific activities of these enzymes are retained during our biotinylation procedure resulting in reagents that provide excellent sensitivity. These biotinylated enzymes can be used in conventional sandwich techniques employing avidin or streptavidin.

Conjugate	Catalog Number	Unit Size
Alkaline Phosphatase [a], biotinylated	B-2005	1 mg
Horseradish Peroxidase [a], biotinylated	B-2004	5 mg
β-Galactosidase [b], biotinylated	B-2008	100 U

[a] *These are not the same products used in the VECTASTAIN® ABC systems.*

[b] *One unit is 1 µmole o-nitrophenyl-ß-D-galactopyranoside hydrolyzed per min at 37 °C, pH 7.3.*

Biotinylated Neuronal Tracers

NEUROBIOTIN™ Tracer

NEUROBIOTIN™ Tracer (SP-1120) is an amino derivative of biotin that can be used as an intracellular label for cells, particularly neurons. It is used for visualizing neural architecture and for the identification of gap junction coupling.

Key advantages of NEUROBIOTIN™ Tracer over biocytin and other neuronal labels:

- better solubility
- more efficiently iontophoresed
- remains in cell longer
- non-toxic
- can be fixed with formalin or glutaraldehyde

NEUROBIOTIN™ Tracer can be used in many types of preparations including *in vivo*, whole mounts, slice preparations, or cultured cells. It can be delivered by many routes such as intracellular electrodes, microinjection, cut-loading, or scrape-loading. NEUROBIOTIN™ Tracer can be detected using biotin/avidin or biotin/streptavidin systems with either chromogenic (see Section A, "Immunohistochemistry") or fluorescence (see Section B, "Immunofluorescence") visualization methods.

Biotinylated Dextran Amines

Used as anterograde tracers, our Biotinylated Dextran Amines are of approximately 10,000 MW and are conjugated with either fluorescein (BDA-F; SP-1130) or Texas Red® (BDA-TR; SP-1140). These tracers can be effectively introduced by iontophoretic or pressure injection methods. The injection site can then be examined easily with a fluorescence microscope. Details of labeled fibers and fibrillar termini can be observed after detection with a biotin/avidin or biotin/streptavidin system such as a VECTASTAIN® Elite® ABC Kit and a peroxidase substrate (see Section A, "Immunohistochemistry"). In addition, Biotinylated Dextran Amines can be used in double labeling protocols in conjunction with anterograde or retrograde HRP labeling or combined with immunohistochemistry.

Conjugate	Catalog Number	Unit Size
NEUROBIOTIN™ Tracer	SP-1120	50 mg
Biotinylated Dextran Amine-Fluorescein	SP-1130	10 mg
Biotinylated Dextran Amine-Texas Red®	SP-1140	10 mg

Anti-Biotin Products

Our mouse monoclonal **Anti-Biotin-M (MB-9100)** is a high-titer, high affinity, low background antibody. Provided as a culture supernatant, this antibody is designed to be used for immunohistochemical procedures and *in situ* hybridization. The VECTASTAIN® ABC system and the ImmPRESS™ Anti-Mouse Ig polymer detection system (MP-7401) are sensitive methods for detecting this antibody.

Our high affinity polyclonal **Anti-Biotin antibody (SP-3000)** is produced by hyperimmunizing goats with biotin and is purified by affinity chromatography. Several different conjugates of this antibody are available. The unconjugated antibody can be detected with a VECTASTAIN® ABC system or the ImmPRESS™ Anti-Goat Ig polymer detection system (MP-7405) for blot, immunohistochemical and *in situ* hybridization applications.

Agarose bound Anti-Biotin (SP-3030) contains 1 mg of Anti-Biotin per ml of settled 4% agarose beads. This product can be used to bind and dissociate biotinylated molecules.

Fluorescein labeled Anti-Biotin (SP-3040) is optimally conjugated to provide a bright fluorescent signal for detecting biotinylated probes in cells or tissue sections.

Alkaline Phosphatase conjugated Anti-Biotin (SP-3020) and **Peroxidase conjugated Anti-Biotin (SP-3010)** antibodies are produced with the highest specific activity enzymes in optimal enzyme/antibody ratios. The covalent linkage between the enzyme and antibody is chosen to provide stable, highly active conjugates for immunohistochemical, *in situ* hybridization, enzyme immunoassay, or transfer blot applications.

Conjugate	Catalog Number	Unit Size
Anti-Biotin, made in mouse, Unconjugated	MB-9100	1 ml
Anti-Biotin, made in goat, Unconjugated	SP-3000	1 mg
Anti-Biotin, made in goat, Agarose bound	SP-3030	2 ml
Anti-Biotin, made in goat, Fluorescein conjugated	SP-3040	0.5 mg
Anti-Biotin, made in goat, Alkaline Phosphatase conjugated	SP-3020	1 ml
Anti-Biotin, made in goat, Peroxidase conjugated	SP-3010	1 mg

Biotin Quantitation Kit

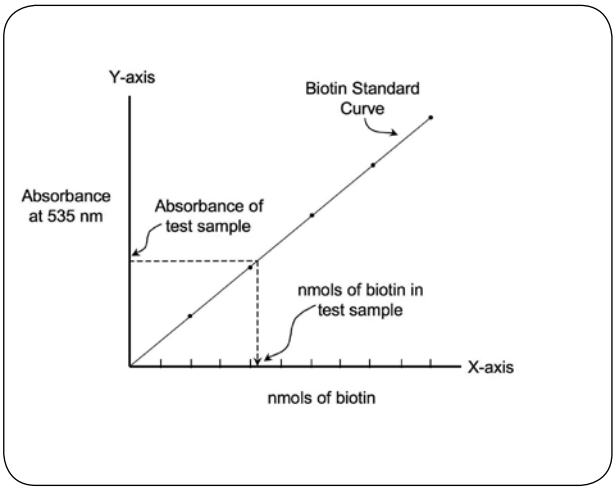
Quant*Tag™ Biotin Kit

The Quant*Tag™ Biotin Kit (BDK-2000) is designed to determine the amount of free biotin in solution or the number of biotins attached to nucleic acids, proteins, or other macromolecules. This kit can be used to determine accurately the labeling efficiency of biotin-labeled molecules.

Unlike conventional biotin quantitation methods like the HABA assay, no predigestion of protein or nucleic acids is required, saving time and increasing accuracy. The Quant*Tag™ Biotin Kit, more sensitive than the HABA assay, is able to detect less than 1 nmol of biotin. Quant*Tag™ Kit reagents chemically react with free or bound biotin producing a colored product that can be quantified using a spectrophotometer. The absorbance is measured in the visible spectrum allowing the use of plastic cuvettes or microtitre plates.

The Quant*Tag™ Biotin Kit is quick and easy to use, and the assay can be completed in 30 minutes. A biotin standard is included. The kit contains sufficient reagents to perform from 25 to 250 tests depending on assay size.

Product	Catalog Number	Unit Size
Quant*Tag™ Biotin Kit	BDK-2000	1 kit



The test sample is reacted with the kit reagents and the included standards containing known amounts of biotin. The absorbance readings of the standards are plotted producing a standard curve. The absorbance of the test sample is located on the standard curve indicating the amount of biotin present.

Avidin and Streptavidin Reagents

Avidin and streptavidin reagents are powerful tools in detection and purification applications of biotinylated proteins, nucleic acids and other macromolecules. Conjugated to enzymes, they are suitable for use in solid-phase assays, tissue/cell staining systems, and blotting applications. The fluorochrome conjugates can be employed in immunofluorescence, *in situ* hybridization detection, and flow cytometry. Agarose bound avidin and streptavidin are ideal for isolation of biotinylated macromolecules.

For a complete listing and discussion of our biotin/avidin-based VECTASTAIN® systems, please see Section A, "Immunohistochemistry".

Unconjugated and Enzyme Conjugates

Avidin. Avidin is a 68 kDa glycoprotein with an extraordinarily high affinity ($10^{15}\ M^{-1}$) for the small molecular weight vitamin, biotin. Because this affinity is over one million times higher than that of antibodies for most antigens, the binding of avidin with biotin (unlike antibody-antigen interactions) is essentially irreversible. In addition to this high affinity, the biotin/avidin system can be effectively exploited because avidin has four binding sites for biotin, and most proteins (including antibodies and enzymes) can be conjugated with several molecules of biotin. These aspects provide the potential for macromolecular complexes to be formed between avidin and biotinylated enzymes.

Over the years we have found that different methods employed in the preparation of avidin can result in distinct "forms" of avidin. These forms of avidin are optimized for use in different applications.

Avidin D – has very low non-specific binding properties. Fluorescent conjugates made with Avidin D are recommended for routine immunofluorescence applications.

Avidin DN – exhibits very low non-specific binding to nucleic acids. Conjugates of Avidin DN can be used to detect biotin-labeled DNA or RNA probes for *in situ* hybridization or nucleic acid blot techniques.

Avidin DCS – is a cell sorter grade with reduced non-specific binding for use in cell sorting and other applications. Fluorescent conjugates of Avidin DCS have a high fluor/protein ratio with increased efficiency of fluorescence.

Streptavidin. Streptavidin is a 60 kDa non-glycosylated protein composed of four identical subunits, each of which has a binding site for biotin, and is purified from the microorganism *Streptomyces avidinii*. This protein resembles egg white avidin in its biotin binding properties. In most applications, streptavidin and avidin conjugates are interchangeable.

The avidin and streptavidin enzyme conjugates are produced with the highest specific activity enzymes in optimal ratios. Specific covalent linkages are chosen to provide stable, highly active conjugates.

Avidin and Enzyme Conjugates

Product	Catalog Number	Unit Size	Stock Concentration	Dilution
Avidin D	A-2000	10 mg	N/A	N/A
Avidin DN	A-3100	1 mg	N/A	N/A
Alkaline Phosphatase Avidin D [a]	A-2100	100 U	100 U/ml	1:500
Horseradish Peroxidase Avidin D, concentrate	A-2004	5 mg	5 mg/ml	1:1000
Horseradish Peroxidase Avidin D, R.T.U.	A-2704	100 ml	ready-to-use	prediluted
Beta-Galactosidase Avidin D [b]	A-2300	100 U	100 U/ml	1:500
Glucose Oxidase Avidin D	A-2200	5 mg	5 mg/ml	1:1000

[a] *one unit is 1 µmole p-nitrophenylphosphate hydrolyzed per min. at 37 °C, pH 9.8.*

[b] *one unit is 1 µmole o-nitrophenyl-ß-D-galactopyranoside hydrolyzed per min. at 37 °C, pH 7.3.*

Streptavidin and Enzyme Conjugates

Product	Catalog Number	Unit Size	Stock Concentration	Dilution
Streptavidin	SA-5000	1 mg	N/A	N/A
Alkaline Phosphatase Streptavidin	SA-5100	1 ml	1 mg/ml	1:500
Horseradish Peroxidase Streptavidin, concentrate	SA-5004	1 mg	1 mg/ml	1:200
Horseradish Peroxidase Streptavidin, R.T.U.	SA-5704	100 ml	ready-to-use	prediluted

Avidin and Streptavidin Reagents (continued)

Fluorochrome-Conjugated Avidin and Streptavidin

These fluorochrome conjugates can be used to detect biotinylated macromolecules in applications such as immunofluorescence, *in situ* hybridization, or flow cytometry.

Using a biotin/avidin or biotin/streptavidin detection system results in an additional layer of amplification over a directly conjugated secondary antibody. These reagents possess very low non-specific binding properties and extremely high affinity for biotin.

Our fluorochrome-conjugated avidin and streptavidin are made using the fluors – AMCA, fluorescein, rhodamine, phycoerythrin, or Texas Red® – as well as the new DyLight® dyes.

DyLight® dyes offer several advantages including greater photostability, pH independence, and brighter fluorescence. We have conjugated these fluorochromes to streptavidin for use in a variety of applications, in particular, cell- and tissue-based immunofluorescent detection. The DyLight® conjugates are completely stable from pH 4 to pH 9, making them compatible with many buffers and diluents.

Phycoerythrin is a very bright red-orange fluorescent protein. Our newly improved Phycoerythrin Streptavidin (SA-5207) is significantly brighter than previous conjugates. Phycoerythrin Streptavidin can be used on microarrays, in cell sorting, and in tissue staining.

The dilution factors shown below are for most applications.

Fluorochrome-Conjugated Avidin

Product	Color (Ex/Em)	Catalog Number	Unit Size	Concentration	Dilution
AMCA Avidin D	Blue (350/450)	A-2008	5 mg	5 mg/ml	1:250
Fluorescein Avidin D	Green (495/515)	A-2001	5 mg	5 mg/ml	1:500
Fluorescein Avidin DN	Green (495/515)	A-3101	1 mg	1 mg/ml	1:100
Fluorescein Avidin DCS	Green (495/515)	A-2011	1 mg	2 mg/ml	1:200
Rhodamine Avidin D	Red (550/575)	A-2002	5 mg	5 mg/ml	1:333
Rhodamine Avidin DCS	Red (550/575)	A-2012	1 mg	2 mg/ml	1:200
$Rhodamine_{600}$ Avidin D	Red (575/600)	A-2005	5 mg	5 mg/ml	1:250
Texas Red® Avidin D	Red (595/615)	A-2006	5 mg	2.5 mg/ml	1:250
Texas Red® Avidin DCS	Red (595/615)	A-2016	1 mg	2 mg/ml	1:200
Fluorescent Avidin Kit - 0.5 mg AMCA Avidin D - 0.5 mg Fluorescein Avidin DCS - 0.5 mg Texas Red® Avidin D	 Blue (350/450) Green (495/515) Red (595/615)	A-1100	1 kit	0.5 mg/ml ea	 AMCA-Av 1:25 Fl-Av 1:50 TR-Av 1:50

Fluorochrome-Conjugated Streptavidin

Product	Color (Ex/Em)	Catalog Number	Unit Size	Concentration	Dilution
AMCA Streptavidin	Blue (350/450)	SA-5008	1 mg	1 mg/ml	1:100
DyLight® 488 Streptavidin	Green (493/518)	SA-5488	1 mg	1 mg/ml	1:100
Fluorescein Streptavidin	Green (495/515)	SA-5001	1 mg	1 mg/ml	1:100
DyLight® 549 Streptavidin	Orange (556/571)	SA-5549	1 mg	1 mg/ml	1:100
Phycoerythrin Streptavidin	Orange (565/574)	SA-5207	1 mg	1 mg/ml	1:100
DyLight® 594 Streptavidin	Red (592/617)	SA-5594	1 mg	1 mg/ml	1:100
Texas Red® Streptavidin	Red (595/615)	SA-5006	1 mg	1 mg/ml	1:100
DyLight® 649 Streptavidin	Far Red (655/670)	SA-5649	1 mg	1 mg/ml	1:100
Fluorescent Streptavidin Kit - 250 µg AMCA Streptavidin - 250 µg Fluorescein Streptavidin - 250 µg Texas Red® Streptavidin	 Blue (350/450) Green (495/515) Red (595/615)	SA-1200	1 Kit	0.5 mg/ml ea	 AMCA-Sav 1:50 FL-Sav 1:50 TR-Sav 1:50

Anti-Avidin and Anti-Streptavidin Reagents

Our antibodies to avidin and streptavidin are produced in goats using our highly purified avidin or streptavidin and isolated by affintiy chromatography. Anti-Avidin does not bind streptavidin and Anti-Streptavidin does not recognize avidin. These antibodies provide opportunities to significantly amplify signals in many applications.

Biotinylated Anti-Avidin and Biotinylated Anti-Streptavidin have been widely used as amplifying reagents in immunohistochemistry, *in situ* hybridization, biochip assays, ELISAs, blots, and many other applications. The capability of binding avidin or streptavidin via either biotin binding sites or through antigen binding sites, makes these biotinylated antibodies unique. These antibodies can be used either as part of preformed complexes or in sequence to amplify fluorescent signals. When used in sequence, the target is first labeled with fluorochrome-conjugated avidin or streptavidin, followed by incubation with Biotinylated Anti-Avidin or Biotinylated Anti-Streptavidin, followed by a second layer of fluorochrome-conjugated avidin or streptavidin. This sequence can be repeated. This multi-layered approach introduces more fluorochromes at the target site and can provide a multi-fold amplification over a single layer.

These affinity purified antibodies are also available unconjugated or fluorescein-labeled.

Anti-Avidin Antibodies

Antibody Description	Conjugate	Catalog Number	Unit Size	Stock Concentration	Dilution
Anti-Avidin, made in goat	Biotinylated	BA-0300	0.5 mg	0.5 mg/ml	1:100
	Unconjugated	SP-2000	1 mg	1 mg/ml	1:200
	Fluorescein conjugated	SP-2040	0.5 mg	1 mg/ml	1:50

Anti-Streptavidin Antibodies

Antibody Description	Conjugate	Catalog Number	Unit Size	Stock Concentration	Dilution
Anti-Streptavidin, made in goat	Biotinylated	BA-0500	0.5 mg	0.5 mg/ml	1:100
	Unconjugated	SP-4000	1 mg	2 mg/ml	1:400
	Fluorescein conjugated	SP-4040	0.5 mg	1 mg/ml	1:50

Avidin and Streptavidin Reagents (continued)

Avidin and Streptavidin/Biotin Blocking

Avidin/Biotin Blocking Kit

Avidin/Biotin Blocking Kit (SP-2001) blocks all endogenous biotin, biotin receptors, and avidin binding sites present in tissues. This kit is designed for use with biotin/avidin detection systems such as the VECTASTAIN® ABC Kits if avidin or biotinylated reagents bind non-specifically to tissues or proteins. This blocking kit consists of 18 ml of Avidin D and 18 ml of biotin in convenient dropper bottles.

Streptavidin/Biotin Blocking Kit

Streptavidin/Biotin Blocking Kit (SP-2002) blocks all endogenous biotin, biotin receptors, and streptavidin binding sites present in tissues. This kit is designed for use with biotin/streptavidin detection systems such as the VECTASTAIN® Universal *Quick* kits if streptavidin or biotinylated reagents bind non-specifically to tissues or proteins. This blocking kit consists of 18 ml of streptavidin and 18 ml of biotin in convenient dropper bottles.

Product	Catalog Number	Unit Size
Avidin/Biotin Blocking Kit	SP-2001	1 kit
Streptavidin/Biotin Blocking Kit	SP-2002	1 kit

Agarose Bound Avidin and Streptavidin

Agarose Avidin D

Agarose Avidin D (A-2010) is prepared by conjugating Avidin D to heat stable, cross-linked 4% agarose gel beads. To ensure minimal steric interference and low nonspecific binding, Avidin D is conjugated through a hydrophilic spacer arm. The procedure we have developed for coupling Avidin D to agarose optimally preserves the biotin binding activity of the Avidin D. Unlike cyanogen bromide coupling, our procedure does not produce conjugates which can be leached from the gel with solutes such as Tris buffer. Our procedure also does not generate charged groups on the gel that can bind proteins nonspecifically. Protein concentration is 2 mg Avidin D per ml of settled agarose beads. The product is supplied as a 1:1 suspension in buffer. The binding capacity is 2 mg of biotinylated IgG per ml of settled beads.

This product can be used to separate biotinylated macromolecules from unbiotinylated materials or for solid-phase binding assays.

Agarose Streptavidin

Agarose Streptavidin (SA-5010) is prepared by conjugating streptavidin to heat stable, cross-linked 4% agarose gel beads. To ensure minimal steric interference and low non-specific binding, streptavidin is conjugated through a hydrophilic spacer arm. The procedure we have developed for coupling streptavidin to agarose preserves the biotin binding activity of the streptavidin. Unlike cyanogen bromide coupling our procedure does not produce conjugates which can be leached from the gel with solutes such as Tris buffer. Our procedure also does not generate charged groups on the gel that can bind proteins nonspecifically. Protein concentration is 1 mg streptavidin per ml settled agarose beads. The product is supplied as a 1:1 suspension in buffer. The binding capacity is approximately 1 mole biotinylated HRP per mole of streptavidin.

This product can be used to separate biotinylated macromolecules from unbiotinylated materials or for solid-phase binding assays.

Product	Catalog Number	Unit Size
Agarose Avidin D	A-2010	5 ml
Agarose Streptavidin	SA-5010	2 ml

VECTREX® Matrices for Nucleic Acid Applications

The VECTREX® matrix is optimized for immobilization of nucleic acids. This matrix consists of particles of a highly cross-linked sugar polymer that has a very large surface area and low non-specific binding of nucleic acids. Unlike agarose, which may retain small molecules in its pores, the VECTREX® particle will retain molecules only through specific affinity interaction on the surface of the particle. The VECTREX® matrix is dense and sediments readily without centrifugation.

VECTREX® Avidin D

VECTREX® Avidin D (A-2020) is used for irreversible binding of biotinylated nucleic acids. This product consists of Avidin D covalently linked to the surface of the VECTREX® matrix.

VECTREX® Avidin D is useful in applications such as subtractive hybridization. A probe that is biotinylated with PHOTOPROBE®, FastTag®, or 3′ or 5′ EndTag™ Labeling Systems is hybridized to a mixture of nucleic acids in solution. After hybridization, the mixture is incubated with a suspension of VECTREX® Avidin D or passed through a column containing VECTREX® Avidin D. The DNA or RNA that is not associated with the biotinylated probe is left in solution or passes through the column. The biotinylated probe and the nucleic acid complementary to the probe are thus separated from the non-complementary sequences. This matrix is supplied as a 1:1 suspension in buffer. The binding capacity is at least 22 ng of biotin-labeled λ DNA per µl of 1:1 suspension.

VECTREX® Avidin DLA

VECTREX® Avidin DLA (MB-2021) is used for reversible binding of biotinylated nucleic acids. VECTREX® Avidin DLA is made by coupling our VECTREX® matrix to a modified form of Avidin D that has a significantly reduced binding affinity for biotin. The affinity is such that, unlike VECTREX® Avidin D, biotinylated protein, nucleic acids, or other molecules can be eluted with biotin. The matrix is supplied as a 1:1 suspension in buffer. The binding capacity is at least 25 ng of biotin-labeled λ DNA per µl of 1:1 suspension.

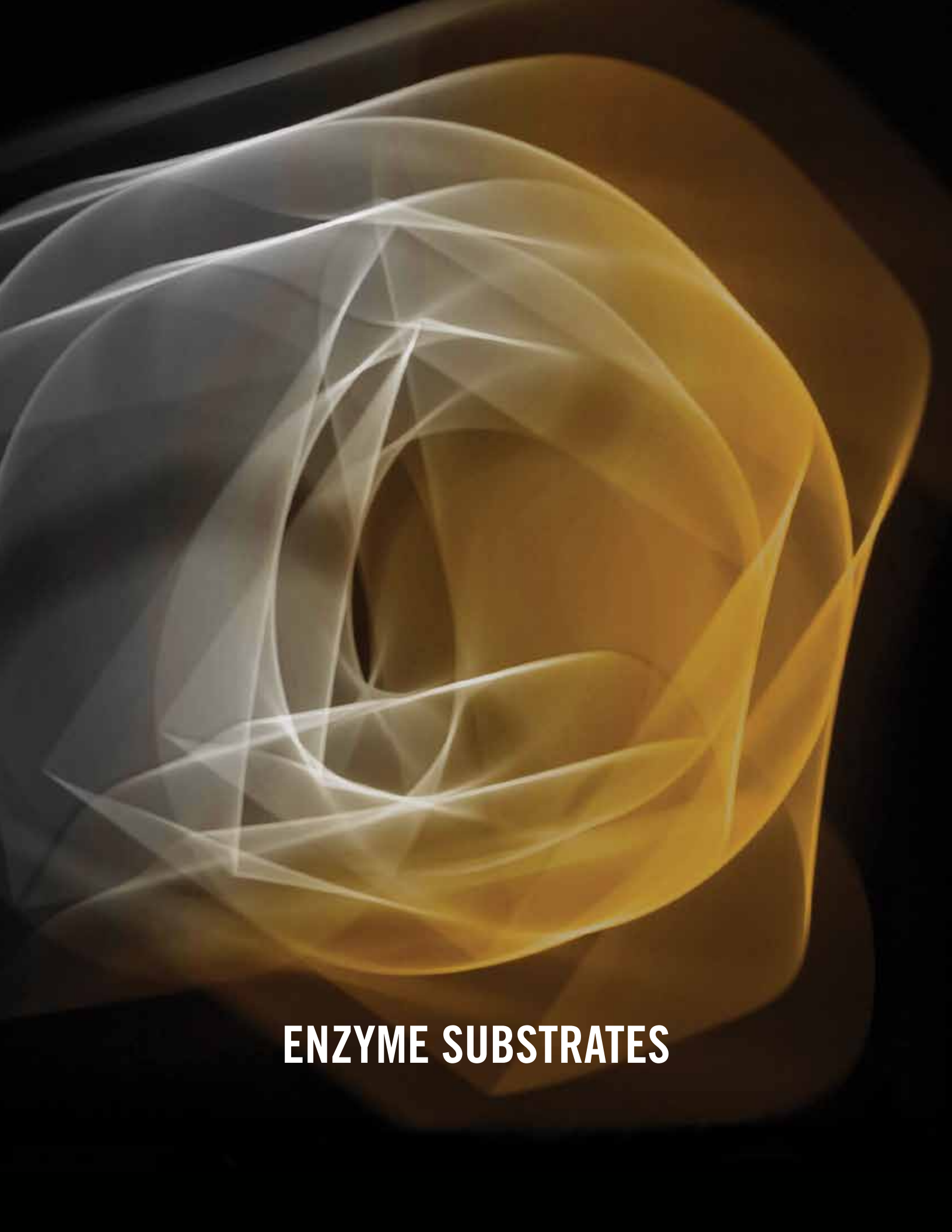
VECTREX® Avidin DLA Binding and Elution Kit

VECTREX® Avidin DLA Binding and Elution Kit (MB-1397) contains 1 ml of settled VECTREX® Avidin DLA, Binding buffer and biotin solution for elution.

H

VECTREX® Matrices

Product	Catalog Number	Unit Size	Kit Contents
VECTREX® Avidin D	A-2020	1 ml	N/A
VECTREX® Avidin DLA	MB-2021	1 ml	N/A
VECTREX® Avidin DLA Binding and Elution Kit	MB-2022	1 Kit	- 1 ml of settled VECTREX® Avidin DLA - binding buffer (3.0 ml of 10x CENT buffer) - elution solution (2.0 ml of 25 mM biotin solution)



ENZYME SUBSTRATES

Introduction

Vector Laboratories offers many enzyme substrate kits for use with peroxidase, alkaline phosphatase, and glucose oxidase detection systems that can be used in a wide variety of applications.

All Vector Laboratories substrate kit reagents are supplied in convenient dropper bottles promoting ease of handling of chromogens and eliminating wait times for mixing and dissolving powders or tablets.

Choose a substrate that matches the enzyme in your detection system. Choosing the optimal substrate for your application will depend on several considerations:

Sensitivity. Substrates provide varying degrees of sensitivities (see sensitivity comparison chart below). For example, ImmPACT™ peroxidase substrates are about 2-4 times more sensitive than the original peroxidase substrate kits, and the alkaline phosphatase substrate BCIP/NBT can achieve increased sensitivities with longer incubation times.

Color. Color contrast is essential in multiple antigen labeling applications, in pigmented tissues such as melanomas, and in counterstained tissues. Color choices also depend on personal preference.

Visualization. Viewing options include brightfield, fluorescence, darkfield, electron microscopy, and spectral imaging.

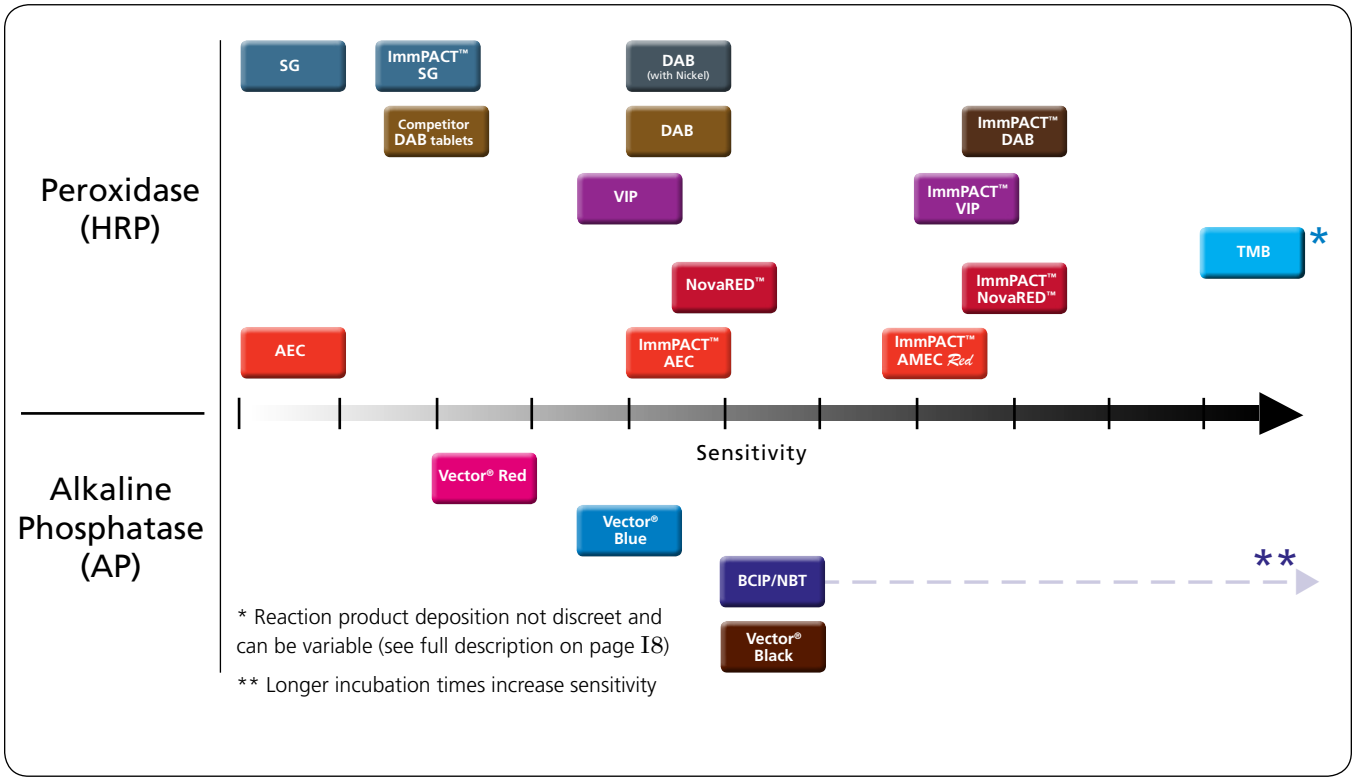
Heat resistance. For IHC/ISH double labeling applications, the heat resistant substrate is applied first in IHC, followed by ISH detection that includes a heat denaturation step.

Additional Information

- Substrate properties and suitable applications are summarized in the following tables. Full enzyme substrate descriptions, photomicrographs, and ordering information are on pages I5-I13.
- **Counterstain compatibility of substrates** can be found in Section A, "Immunohistochemistry" page A25.
- **Substrate compatibility in multiple antigen labeling** applications can be found in Section A, "Immunohistochemistry" page A22.

Relative Sensitivity of Substrates in Immunohistochemistry

HRP substrates were used with the ImmPRESS™ Polymer Detection Reagent. AP substrates were used with the VECTASTAIN® ABC-AP Kit.



Substrates at a Glance

Properties

Substrate	Color	Catalog Number	Mounting	Fluorescent	Contrast in Pigmented Tissue	Multiple Labeling	Heat Resistant
Peroxidase							
DAB	Brown	SK-4100	Non-aqueous or Aqueous			●	●
DAB +Ni	Gray-Black	SK-4100	Non-aqueous			●	
ImmPACT™ DAB	Brown	SK-4105	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		●	●	
AEC	Red	SK-4200	Aqueous		●	●	
ImmPACT™ AEC	Red	SK-4205	Aqueous		●	●	
ImmPACT™ AMEC *Red*	Red	SK-4285	Aqueous		●	●	
TMB	Blue (450 & 650 nm)	SK-4400	Non-aqueous				
4-CN	Blue-gray	SK-4300					
ABTS	Green (405 nm)	SK-4500					
DuoLuX™ for HRP	Chemiluminescent (453 nm)	SK-6604		●			
Alkaline Phosphatase							
Vector® Red	Magenta	SK-5100	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			●	●
pNPP	Yellow (405-420 nm)	SK-5900					
DuoLuX™ for AP	Chemiluminescent (453 nm)	SK-6605		●			
Glucose Oxidase							
GO Kit I (NBT)	Indigo	SK-3100	Non-aqueous or Aqueous				
GO Kit II (TNBT)	Black	SK-3200	Non-aqueous				

Substrates at a Glance (continued)

Applications

Substrate	Color	Catalog Number	IHC/ICC	ISH	Blots	ELISAs	Microscopy			
							Brightfield	Darkfield	Electron	Spectral Imaging
Peroxidase										
DAB	Brown	SK-4100	●	●	●		●	●	●	●
DAB +Ni	Gray-Black	SK-4100	●	●	●		●	●	●	●
ImmPACT™ DAB	Brown	SK-4105	●	●	●		●	●	●	●
Vector® VIP	Purple	SK-4600	●	●	●		●	●	●	●
ImmPACT™ VIP	Purple	SK-4605	●	●			●	●	●	●
Vector® SG	Blue-Gray	SK-4700	●	●	●		●	●	●	●
ImmPACT™ SG	Blue-Gray	SK-4705	●	●			●	●	●	●
Vector® NovaRED™	Red	SK-4800	●	●	●		●	●		●
ImmPACT™ NovaRED™	Red	SK-4805	●	●			●	●		●
AEC	Red	SK-4200	●	●	●		●			●
ImmPACT™ AEC	Red	SK-4205	●	●			●			●
ImmPACT™ AMEC *Red*	Red	SK-4285	●	●			●			●
TMB	Blue (450 & 650 nm)	SK-4400	●	●	●	●	●			●
4-CN	Blue-Gray	SK-4300			●					
ABTS	Green (405 nm)	SK-4500				●				
DuoLuX™ for HRP	Chemiluminescent (453 nm)	SK-6604			●	●				
Alkaline Phosphatase										
Vector® Red	Magenta	SK-5100	●	●	●		●			●
Vector® Blue	Blue	SK-5300	●	●	●		●			●
Vector® Black	Brown-Black	SK-5200	●		●		●			
BCIP/NBT	Indigo	SK-5400	●	●	●		●			●
pNPP	Yellow (405-420 nm)	SK-5900				●				
DuoLuX™ for AP	Chemiluminescent (453 nm)	SK-6605			●	●				
Glucose Oxidase										
GO Kit I (NBT)	Indigo	SK-3100	●				●			
GO Kit II (TNBT)	Black	SK-3200	●				●			

Abbreviations used in figure legends: AP – alkaline phosphatase; HRP – horseradish peroxidase; m – mouse monoclonal antibody; g – goat antibody; rm – rabbit monoclonal antibody; rp – rabbit polyclonal antibody; s – sheep polyclonal antibody

Enzyme Substrate Kits

Peroxidase Enzyme Substrates

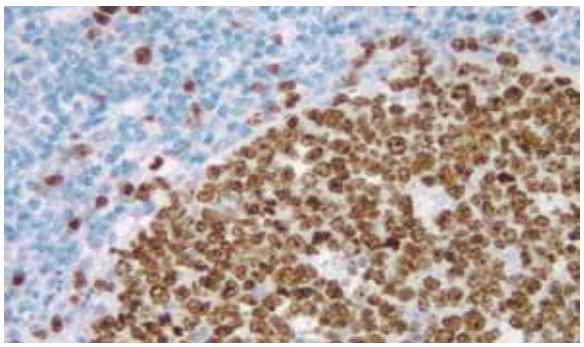
Vector Laboratories offers two lines of peroxidase substrates. Our original substrate kits provide sensitivity greater than conventional substrates and are known for their consistency and reliability. The reagents in the peroxidase substrate kits are supplied as concentrated stock solutions in convenient dropper bottles. The newest line, the ImmPACT™ substrates, provides the following advantages:

- 2-4 fold greater sensitivity than original substrate kits
- Supplied with stock solutions in dropper bottles and optimized diluent
- Optimized specifically for immunohistochemistry applications
- Working solution stable for up to 14 days at 4 °C

DAB Substrates

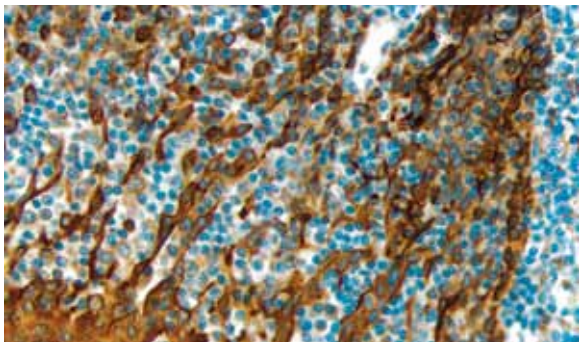
DAB (3, 3'-diaminobenzidine) produces a dark brown reaction product and can be used for both immunohistochemical and blotting applications. DAB is effective as a single label or as a second color for multiple antigen labeling (see chart on page A22). Because of its heat-resistance, DAB can be used in IHC/ISH double labeling applications. With the aid of imaging systems and software, the spectral profile of DAB can be distinguished from our other proprietary enzyme substrates in applications where antigens are co-localized. Sections stained with DAB can also be viewed by darkfield and electron microscopy. Slides developed with DAB can be dehydrated, cleared, and permanently mounted. The DAB reaction product can be intensified with DAB Enhancing Solution (H-2200).

DAB Substrate Kit (SK-4100)
This kit contains stock solutions in convenient dropper bottles. This kit includes nickel, providing the option of changing the reaction product from brown to gray-black and increasing sensitivity in blot applications. Slides developed with DAB/Ni should be dehydrated, cleared, and permanently mounted. This product can also be used on blots. Ordering information on page I9.



Tonsil: Ki67 (m), ImmPRESS™ Reagent (HRP; Universal), Vector® DAB (brown), Hematoxylin QS (blue).

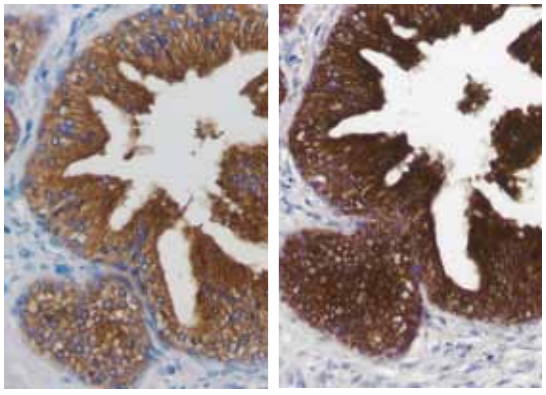
ImmPACT™ DAB Substrate (SK-4105)
This product is an entirely new formula for a diaminobenzidine-based peroxidase substrate. ImmPACT™ DAB produces a dark brown reaction product that is crisper and is 3-4 times more sensitive than conventional DAB substrates. It can be used for both manual and automated staining methods. The ImmPACT™ DAB working solution is stable for at least five days at room temperature and at least two weeks when stored at 4 °C. This product consists of 120 ml of diluent and a convenient concentrated stock solution of highly purified DAB. This product can also be used on blots. Ordering information on page I9.



Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ DAB (brown), Hematoxylin QS (blue).

DAB Enhancing Solution

DAB Enhancing Solution (H-2200) intensifies the DAB reaction product in stained sections. A ten second exposure to this solution darkens the reaction product. Ordering information on page I9.



Prostate stained with Prostate Specific Antigen antibody (m), ImmPRESS™ Reagent (HRP) and Vector® DAB (brown; left). Vector® DAB stain enhanced with DAB Enhancing Solution (right).

Enzyme Substrate Kits (continued)

Vector® VIP Substrates

Vector® VIP and ImmPACT™ VIP substrates both produce intense, violet reaction products, and can be used as alternatives to DAB or as a second color for multiple antigen labeling (see chart on page A22). Vector® VIP and ImmPACT™ VIP substrates also provide excellent color contrast in pigmented tissues such as melanoma. With the aid of imaging systems and software, the spectral profile of both substrates can be distinguished from other enzyme substrates in applications where antigens are co-localized. Sections stained with either substrate can be viewed by darkfield and electron microscopy. Vector® VIP and ImmPACT™ VIP substrates can be used for both manual and automated staining methods. Stained sections should be dehydrated, cleared, and permanently mounted.

Vector® VIP Substrate Kit (SK-4600)
This kit contains stock solutions in convenient dropper bottles. The sensitivity of Vector® VIP substrate is equivalent to Vector® DAB (SK-4100). This product can also be used on blots. Ordering information on page I9.

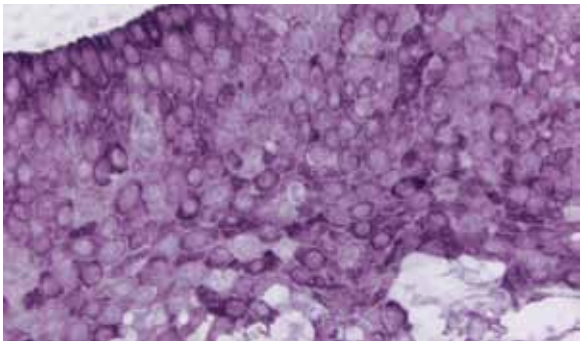
ImmPACT™ VIP Substrate (SK-4605)
This product utilizes Vector Laboratories' novel ImmPACT™ technology to generate an intense violet reaction product that is 2-4 times more sensitive than Vector® VIP substrate kit (SK-4600). This kit consists of 120 ml of diluent and concentrated stock solutions of ImmPACT™ VIP reagents in convenient dropper bottles. This substrate formulation is not recommended for use on blots. Working solutions are stable for up to 14 days at 4 °C. Ordering information on page I9.

Vector® SG Substrates

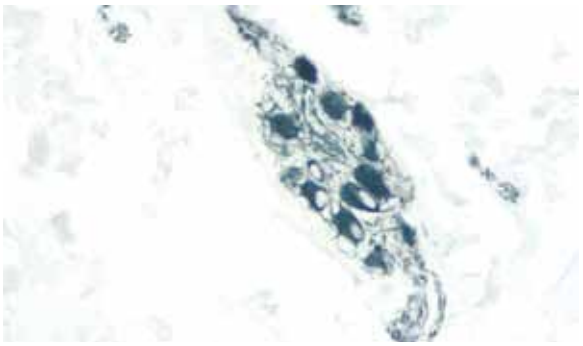
Vector® SG and ImmPACT™ SG substrates produce blue-gray reaction products that can be used as a single label or as a second color for multiple antigen labeling (see chart on page A22). With the aid of imaging systems and software, the spectral profile of both substrates can be distinguished from other enzyme substrates in applications where antigens are co-localized. Sections stained with either substrate also can be viewed by darkfield or electron microscopy. Vector® SG and ImmPACT™ SG substrates can be used for both manual and automated staining methods. Stained sections can be dehydrated, cleared, and permanently mounted.

Vector® SG Substrate Kit (SK-4700)
This kit contains stock solutions in convenient dropper bottles. The sensitivity of Vector® SG subsrate is equivalent to AEC. This product can also be used on blots. Ordering information on page I9.

ImmPACT™ SG Substrate (SK-4705)
This product utilizes Vector Laboratories' novel ImmPACT™ technology to generate an intense blue-gray reaction product that is 2-3 times more sensitive than Vector® SG substrate kit (SK-4700). This product consists of 120 ml of diluent and concentrated stock solutions of ImmPACT™ SG reagents in convenient dropper bottles. This substrate formulation is not recommended for use on blots. Working solutions are stable for up to 14 days at 4 °C. Ordering information on page I9.



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



Colon: Peripherin (m), ImmPRESS™ Reagent (HRP), ImmPACT™ SG (blue-gray).

Vector® NovaRED™ Substrates

Vector® NovaRED™ and ImmPACT™ NovaRED™ substrates produce red reaction products. Unlike AEC, sections stained with either substrate should be dehydrated, cleared, and permanently mounted. Both substrates are useful as alternatives to DAB or as a second color for multiple antigen labeling (see chart on page A22). Vector® NovaRED™ and ImmPACT™ NovaRED™ substrates also provide excellent color contrast in pigmented tissue such as melanoma. With the aid of imaging systems and software, the spectral profile of both substrates can be distinguished from other enzyme substrates in applications where antigens are co-localized. Sections stained with either substrate also can be viewed by darkfield microscopy. Vector® NovaRED™ and ImmPACT™ NovaRED™ substrates can be used for both manual and automated staining methods.

Vector® NovaRED™ Substrate Kit (SK-4800)
This kit contains stock solutions in convenient dropper bottles. The sensitivity of Vector® NovaRED™ substrate is equivalent to Vector® DAB (SK-4100), and 4 times greater than AEC. This product can also be used on blots. Ordering information on page I9.

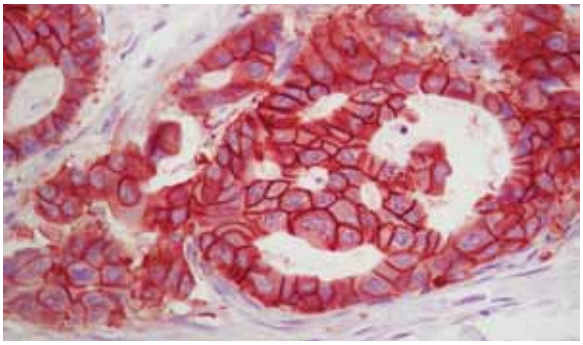
ImmPACT™ NovaRED™ Substrate (SK-4805)
This product utilizes Vector Laboratories' novel ImmPACT™ technology to generate an intense red reaction product that is 2-4 times more sensitive than Vector® NovaRED™ substrate kit (SK-4800). This product consists of 120 ml of diluent and concentrated stock solutions of ImmPACT™ NovaRED™ reagents in convenient dropper bottles. This substrate formulation is not recommended for use on blots. Working solutions are stable for up to 14 days at 4 °C. Ordering information on page I9.

AEC Substrates

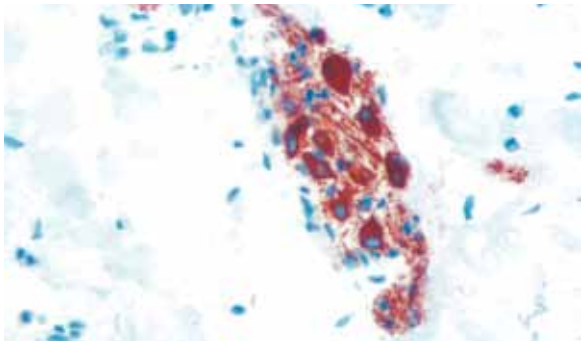
AEC (3-amino-9-ethylcarbazole) and ImmPACT™ AEC both produce red reaction products that can be used as a single label or as a second color for multiple antigen labeling (see chart on page A22). AEC and ImmPACT™ AEC can be used for both manual and automated staining methods. Stained slides must be aqueously mounted because these reaction products are soluble in organic solvents. Sections stained with AEC are stable for at least 2 years when mounted in VectaMount™ AQ (H-5501).

AEC Substrate Kit (SK-4200)
This kit contains stock solutions in convenient dropper bottles. This product can also be used on blots. Ordering information on page I9.

ImmPACT™ AEC (SK-4205)
This product utilizes Vector Laboratories' novel ImmPACT™ technology to generate a crisper, brighter red reaction product that is 3-5 times more sensitive than conventional AEC substrates such as AEC Substrate Kit (SK-4200). This product consists of 120 ml of diluent and concentrated stock solutions of ImmPACT™ AEC reagents in convenient dropper bottles. ImmPACT™ AEC must be aqueously mounted and is stable for at least 2 years when mounted in VectaMount™ AQ (H-5501). This product is not recommended for use on blots. Working solutions are stable for up to 14 days at 4 °C. Ordering information on page I9.



HER2 positivity in metastatic adenocarcinoma: HER2 (rp), ImmPRESS™ Reagent (HRP; Universal), ImmPACT™ NovaRED™ (red), Hematoxylin QS (blue). (Image courtesy of Dr. GM Reynolds, Centre for Liver Research, University of Birmingham, U.K.)



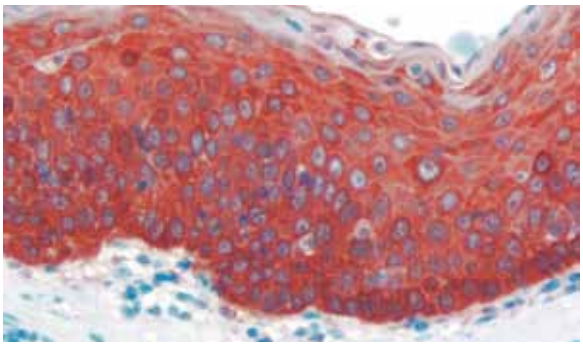
Colon: Peripherin (m), ImmPRESS™ Reagent (HRP), ImmPACT™ AEC (red), Hematoxylin QS (blue).

Enzyme Substrate Kits (continued)

ImmPACT™ AMEC *Red*

ImmPACT™ AMEC *Red* (SK-4285) utilizes Vector Laboratories' ImmPACT™ technology to generate a crisper, brighter red reaction product that is 5 -10 times more sensitive than conventional AEC substrates and can be used as a single label or as a second color for multiple antigen labeling (see chart on page A22). ImmPACT™ AMEC *Red* can be used for both manual and automated staining methods. Stained slides must be aqueously mounted because the reaction product is soluble in organic solvents. Sections stained with ImmPACT™ AMEC *Red* are stable for at least 2 years when mounted in VectaMount™ AQ (H-5501).

ImmPACT™ AMEC *Red* consists of 120 ml of diluent and concentrated stock solutions of ImmPACT™ AMEC *Red* reagents in convenient dropper bottles. This product is not recommended for use on blots. Working solutions are stable for up to 14 days at 4 ˚C. Ordering information on page I9.



Tonsil: Cytokeratin AE1/AE3 (m), ImmPRESS™ Reagent (HRP), ImmPACT™ AMEC Red (red), Hematoxylin QS (blue).

4-CN Substrate Kit

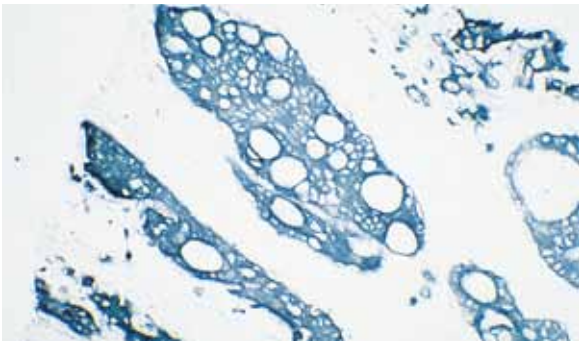
4-chloro-1-naphthol

The 4-CN Substrate Kit (SK-4300) produces an insoluble blue/gray reaction product and can be used for development of nitrocellulose blots. This substrate is not recommended for immunohistochemical applications.

TMB Substrate

3,3',5,5'-tetramethylbenzidine

TMB Substrate Kit (SK-4400) contains reagents in convenient dropper bottles necessary to produce either a precipitating blue reaction product or a soluble blue reaction product. This kit contains a stabilizing solution which, when added to the working solution, allows the development of a blue precipitate. This sensitive substrate can be used for blotting, immunohistochemical, and *in situ* hybridization applications. TMB stained slides can be dehydrated and permanently mounted. The soluble blue product, produced in the absence of the stabilizing solution, is useful for ELISA assays. Ordering information on page I9.



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

ABTS Substrate Kit

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

The reaction product of the ABTS Substrate Kit (SK-4500) is soluble in aqueous solutions. Because of its reproducibility and high extinction coefficient, this substrate is recommended for microtiter plate enzyme immunoassays. The green color produced in assays can be seen by eye or measured at 405 nm with conventional plate readers.

DuoLuX™ Chemiluminescent/ Chemifluorescent Substrate

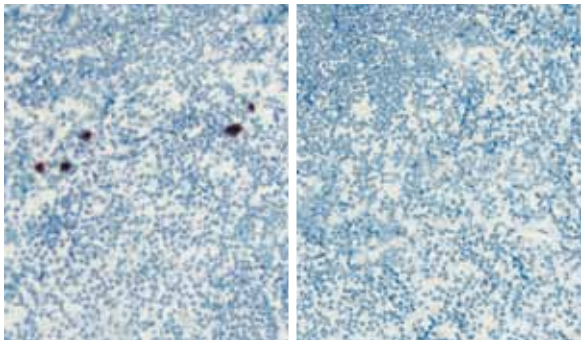
See pages I12-I13 for product information.

Endogenous Peroxidase Blocking

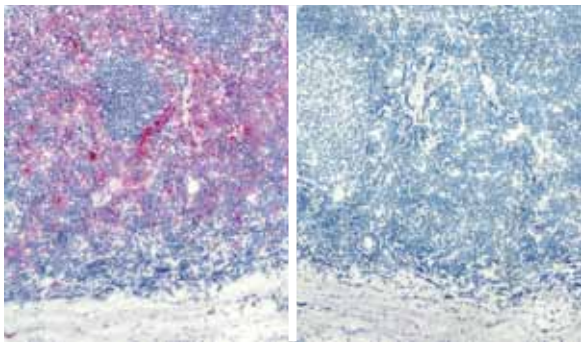
BLOXALL™ Endogenous Peroxidase/Alkaline Phosphatase Blocking Solution

- Inhibits endogenous peroxidase, pseudoperoxidase, and alkaline phosphatase activities
- Ready-to-use in convenient dropper bottle
- Simple one-step protocol
- Compatible with formalin-fixed, paraffin-embedded tissue sections, frozen tissue sections, and cell preparations
- More effective than conventional blocking methods

Tissues can contain endogenous peroxidase, pseudoperoxidase, and/or alkaline phosphatase activity that will produce background staining if an alkaline phosphatase and/or peroxidase detection system and corresponding substrates are used. **BLOXALL™** Endogenous Peroxidase/Alkaline Phosphatase Blocking Solution (SP-6000) inactivates endogenous peroxidase, pseudoperoxidase, and alkaline phosphatase in formalin-fixed, paraffin-embedded tissue sections, frozen tissue sections, and cell preparations in a single 10 minute incubation step. This reagent is provided as a ready-to-use reagent in a convenient dropper bottle.



Endogenous peroxidase activity in frozen, acetone-fixed tonsil demonstrated with ImmPACT™ DAB peroxidase substrate (left). This activity is blocked using BLOXALL™ Solution (right).



Endogenous alkaline phosphatase activity in frozen, acetone-fixed tonsil demonstrated with Vector® Red alkaline phosphatase substrate (left). This activity is blocked using BLOXALL™ Solution (right).

Peroxidase Substrates and Associated Reagents

Product	Color	Catalog Number	Unit Size	Mounting	Working Solution
DAB Substrate Kit	Brown	SK-4100	1 Kit	Non-aqueous or Aqueous	300 ml
DAB with Ni Substrate Kit	Gray-Black	SK-4100	1 Kit	Non-aqueous	300 ml
ImmPACT™ DAB Substrate	Brown	SK-4105	120 ml	Non-aqueous or Aqueous	120 ml
Vector® VIP Substrate Kit	Purple	SK-4600	1 Kit	Non-aqueous	300 ml
ImmPACT™ VIP Substrate	Purple	SK-4605	120 ml	Non-aqueous	120 ml
Vector® SG Substrate Kit	Blue-Gray	SK-4700	1 Kit	Non-aqueous or Aqueous	300 ml
ImmPACT™ SG Substrate	Blue-Gray	SK-4705	120 ml	Non-aqueous or Aqueous	120 ml
Vector NovaRED™ Substrate Kit	Red	SK-4800	1 Kit	Non-aqueous	300 ml
ImmPACT™ NovaRED™ Substrate	Red	SK-4805	120 ml	Non-aqueous	120 ml
AEC Substrate Kit	Red	SK-4200	1 Kit	Aqueous	300 ml
ImmPACT™ AEC Substrate	Red	SK-4205	120 ml	Aqueous	120 ml
ImmPACT™ AMEC *Red* Substrate	Red	SK-4285	120 ml	Aqueous	120 ml
TMB Substrate Kit	Blue	SK-4400	1 Kit	Non-aqueous	300 ml
4-CN Substrate Kit	Blue-Gray	SK-4300	1 Kit	N/A	300 ml
ABTS Substrate Kit	Green (405 nm)	SK-4500	1 Kit	N/A	300 ml
DuoLuX™ Chemiluminescent/Chemifluorescent Substrate	Chemiluminescent (453 nm)	SK-6604	200 ml	N/A	200 ml
DAB Enhancing Solution	N/A	H-2200	30 ml	N/A	30 ml
BLOXALL™ Blocking Solution	N/A	SP-6000	100 ml	N/A	100 ml

Enzyme Substrate Kits (continued)

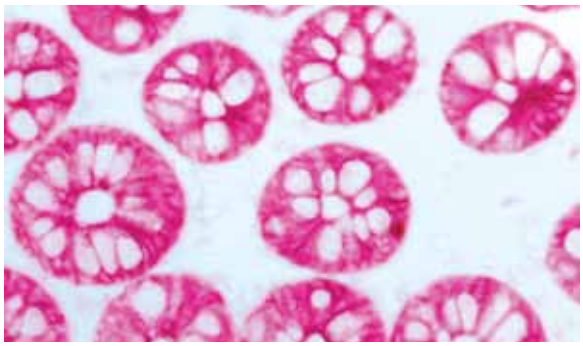
Alkaline Phosphatase Enzyme Substrates

Vector® Red Substrate Kit

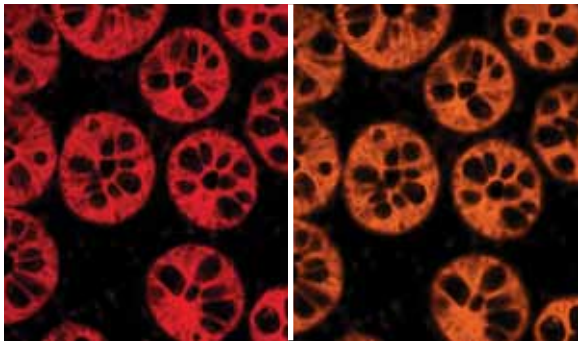
Vector® Red Substrate Kit (SK-5100) produces a magenta heat stable reaction product that is also fluorescent. Sections stained with the Vector® Red substrate can be dehydrated, cleared, and permanently mounted. This substrate can be used singly or in combination with other alkaline phosphatase or peroxidase substrates for multiple label applications (see chart on page A22). Because of its stability under ISH conditions, Vector® Red can be used in IHC/ISH double labeling applications. With the aid of imaging systems and software, the spectral profile of this substrate can be distinguished from other enzyme substrates in applications where antigens are co-localized.

The bright red to orange fluorescence of the Vector® Red reaction product can be visualized using Dy594, Texas Red® or TRITC filter systems. This fluorescence is especially bright in dehydrated, permanently mounted sections. The fluorescence is stable and does not photobleach like most fluorescent dyes. This substrate can also be used for *in situ* hybridization or on blots, where it has a wider dynamic range than other substrates.

This kit contains stock solutions in convenient dropper bottles.



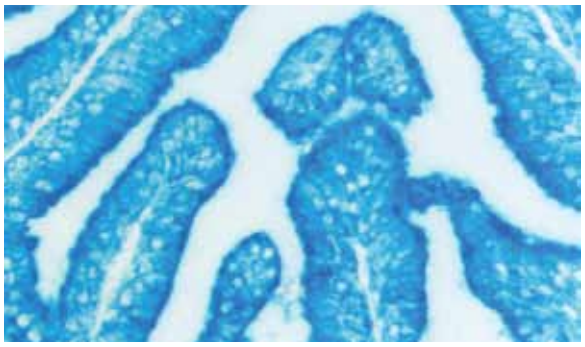
Colon: Cytokeratin AE1/AE3 (m), Vector® AP detection system, Vector® Red (magenta).



Colon: Cytokeratin AE1/AE3 (m), Vector® AP detection system, Vector® Red (red fluorescence) viewed with a Texas Red® filter cube (left) and TRITC filter cube (right).

Vector® Blue Substrate Kit

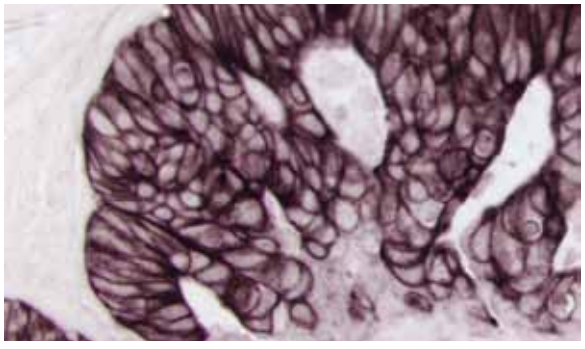
The Vector® Blue Substrate Kit (SK-5300) produces a blue, heat stable reaction product. This substrate can be used singly or in combination with other alkaline phosphatase or peroxidase substrates for multiple label applications (see chart on page A22). With the aid of imaging systems and software, the spectral profile of this substrate can be distinguished from other enzyme substrates in applications where antigens are co-localized. Vector® Blue is also fluorescent. Sections stained with the Vector® Blue substrate can be dehydrated, cleared, and permanently mounted; however, non-xylene clearing agents and non-xylene based mounting media (such as VectaMount™, H-5000) must be used because the reaction product is soluble in xylene. Vector® Blue can also be used on blots in applications such as ELISPOT, singly or in combination with a peroxidase substrate such as AEC. This kit contains stock solutions in convenient dropper bottles.



Prostate: Prostate Specific Antigen (m), VECTASTAIN® ABC-AP Kit, Vector® Blue (blue).

Vector® Black Substrate Kit

Vector® Black Substrate Kit (SK-5200) forms a brown-black precipitate that can be dehydrated, cleared, and permanently mounted. It can be used in immunohistochemical or blotting applications. This kit contains stock solutions in convenient dropper bottles.

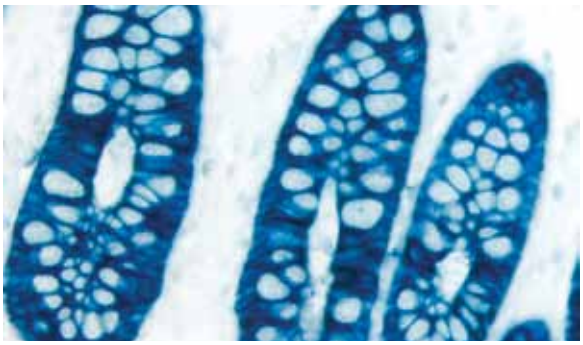


Colon Carcinoma: Pan-Cytokeratin (m), VECTASTAIN® ABC-AP Kit, Vector® Black (brown-black).

BCIP/NBT Substrate Kit

5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium

BCIP/NBT Substrate Kit (SK-5400) forms an indigo, heat stable reaction product that can be dehydrated, cleared, and permanently mounted. This substrate can be used singly or in combination with other alkaline phosphatase or peroxidase substrates for multiple label applications (see chart on page A22). This substrate can also be used for developing blots or for *in situ* hybridization. At 20-30 minute development times, the sensitivity of this substrate is equivalent to the other alkaline phosphatase substrates. However, BCIP/NBT will continue to develop from several hours to overnight making it one of the most sensitive chromogenic substrates. This kit contains stock solutions in convenient dropper bottles.



Colon: Cytokeratin AE1/AE3 (m), Vector® AP detection system, BCIP/NBT (indigo).

p-Nitrophenylphosphate Substrate

pNPP

p-Nitrophenylphosphate Substrate (SK-5900) is ideal for ELISA using alkaline phosphatase enzyme detection systems. The reagent is supplied in a stabilized concentrate. The bright yellow color of the soluble reaction product is measured at 405-420 nm, providing an excellent reagent for microtitration plate readers.

Endogenous Alkaline Phosphatase Blocking

BLOXALL™ Endogenous Peroxidase/Alkaline Phosphatase Blocking Solution

See pages I9 for product information.

Levamisole Solution

Tissues can contain endogenous alkaline phosphatase activity that will produce background staining if an alkaline phosphatase detection system and substrate are used. To eliminate this problem, Levamisole Solution (SP-5000), an inhibitor of alkaline phosphatase, is provided in a convenient dropper bottle and can be added to the alkaline phosphatase substrate solution. Levamisole does not inhibit the isoenzyme used for either the VECTASTAIN® ABC-AP reagents or other alkaline phosphatase conjugates. 100x Concentrate.

DuoLuX™ Chemiluminescent/ Chemifluorescent Substrate

See pages I12-I13 for product information.

Glucose Oxidase Enzyme Substrates

Product	Catalog Number	Unit Size
Glucose Oxidase NBT (Indigo)	SK-3100	1 Kit
Glucose Oxidase TNBT (Black)	SK-3200	1 Kit

These substrate kits form indigo (NBT, SK-3100) or black (TNBT, SK-3200) reaction products when used with glucose oxidase enzyme systems. Additional information can be found in Section A, "Immunohistochemistry" pages A18-A25.

Alkaline Phosphatase Substrates and Associated Reagents

Product	Color	Catalog Number	Unit Size	Mounting	Total Working Solution
Vector® Red Substrate Kit	Magenta	SK-5100	1 Kit	Non-aqueous or Aqueous	200 ml
Vector® Blue Substrate Kit	Blue	SK-5300	1 Kit	Non-aqueous* or Aqueous	200 ml
Vector® Black Substrate Kit	Brown-Black	SK-5200	1 Kit	Non-aqueous	200 ml
BCIP/NBT Substrate Kit	Indigo	SK-5400	1 Kit	Non-aqueous or Aqueous	200 ml
pNPP Substrate	Yellow (405-420 nm)	SK-5900	18 ml	N/A	360 ml
DuoLuX™ Chemiluminescent/ Chemifluorescent Substrate	Chemiluminescent (453 nm)	SK-6605	100 ml	N/A	100 ml
BLOXALL™ Blocking Solution	N/A	SP-6000	100 ml	N/A	100 ml
Levamisole Solution	N/A	SP-5000	18 ml	N/A	1800 ml

** Not compatible with xylene-based clearing agents and mounting media*

Chemiluminescent/Chemifluorescent Substrates

DuoLuX™ Chemiluminescent/ Chemifluorescent Substrate

Product	Catalog Number	Unit Size
DuoLuX™ - Peroxidase	SK-6604	200 ml
DuoLuX™ - Alkaline Phosphatase	SK-6605	100 ml

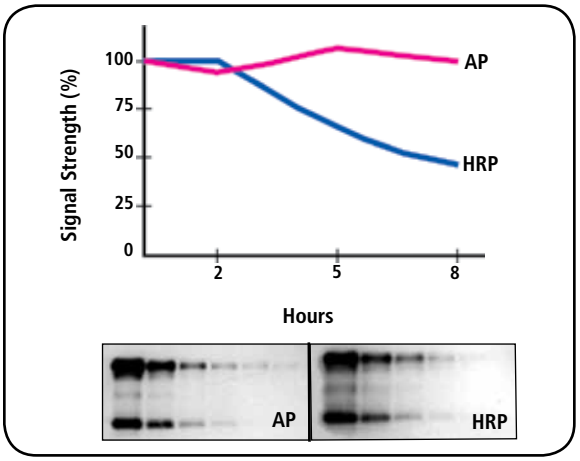
The DuoLuX™ Chemiluminescent/Chemifluorescent Substrate is a unique formula based on acridan chemistry. The DuoLuX™ Substrate provides the following advantages:

- Very high sensitivity
- Prolonged and intense light emission
- Chemiluminescent or fluorescent detection
- Permanent fluorescence

The DuoLuX™ Chemiluminescent/Chemifluorescent Substrate is supplied for either horseradish peroxidase or alkaline phosphatase development and can be used for western, Southern, northern, or dot blots, or for ELISA applications. This substrate is about 10 times more sensitive than the alkaline phosphatase substrate BCIP/NBT and about 100 times more sensitive than the peroxidase substrate DAB.

Chemiluminescent properties

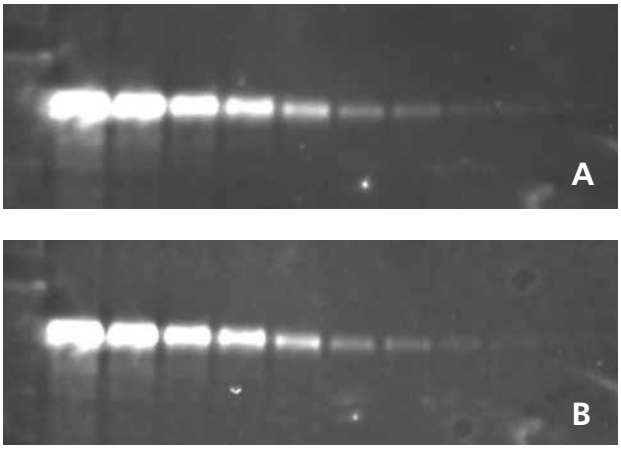
Light emission of the DuoLuX™ Chemiluminescent/ Chemifluorescent Substrate occurs continuously over a long period of time for both the peroxidase and alkaline phosphatase substrates. With either substrate formula, reacted DuoLuX™ Substrate luminesces in the blue range with a peak emission at 453 nm. Unlike other chemiluminescent substrates, blots can be reexposed to film many times over an 8 hour period to optimize band intensities or resolution.



Serial dilutions (1:2) of mouse IgG were resolved by PAGE, transferred onto nitrocellulose membrane and detected with the DuoLuX™ Substrate using either the Alkaline Phosphatase-based VECTASTAIN® ABC-AmP™ Kit (lower left) or Peroxidase Streptavidin (lower right). Graph shows prolonged light emission characteristics of the DuoLuX™ Substrate with each enzyme.

Fluorescent properties

In addition to its chemiluminescent properties, the reaction product of the DuoLuX™ Substrate is also fluorescent. Fluorescence can be recorded with a digital imaging system or a conventional camera months after chemiluminescence has faded. The excitation maximum is at 405 nm, but other wavelengths (254 nm and 365 nm) also excite. Maximum fluorescent emission occurs at 453 nm. For fluorescence detection, nitrocellulose is recommended due to the intrinsic fluorescence of nylon. Acquisition of fluorescent signal requires a much shorter exposure time than chemiluminescence, often a fraction of a second.



Western blot visualized by fluorescence using the VECTASTAIN® ABC-AmP™ Kit with the DuoLuX™ Substrate. Serial dilutions (1:2) of rabbit IgG heavy chain were resolved by PAGE, transferred onto nitrocellulose membrane, and detected with biotinylated anti-rabbit IgG, followed by the VECTASTAIN® ABC-AmP™ Kit with the DuoLuX™ Substrate. [A] Fluorescent detection by image acquisition (1 second). [B] Fluorescent image of the same blot as shown in [A] obtained 12 months later. (Same 1 second acquisition time.)

The DuoLuX™ Chemiluminescent/Chemifluorescent Substrate for peroxidase (SK-6604) is supplied in two bottles, consisting of 100 ml of the DuoLuX™ Substrate (Reagent 1) and 100 ml of a peroxidase converter solution (Reagent 2). Reagents 1 and 2 are mixed in equal volumes just prior to use, providing 200 ml of peroxidase substrate working solution.

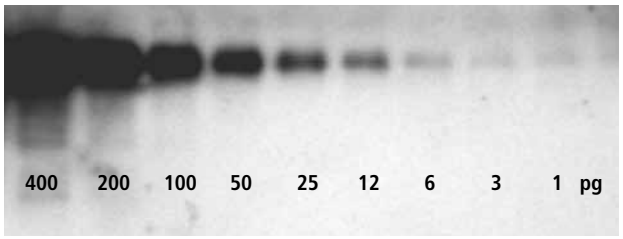
The DuoLuX™ Chemiluminescent/Chemifluorescent Substrate for alkaline phosphatase (SK-6605) is supplied in ready-to-use form, consisting of 100 ml of substrate solution.

Applications

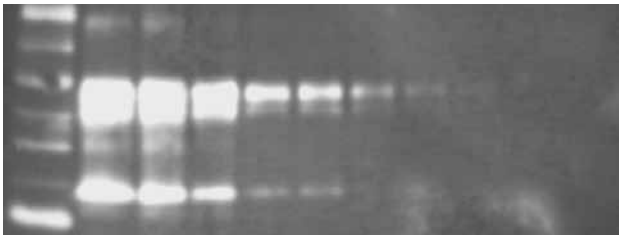
The DuoLuX™ Chemiluminescent/Chemifluorescent Substrate is ideal for development of protein and nucleic acid blots. Complete kits are available for western and Southern/northern blot development (VECTASTAIN® ABC-AmP™ system and Vector® UltraSNAP™ Kit, respectively.)

Protein detection

For western blot applications using chemiluminescent detection, the sensitivity using either alkaline phosphatase or peroxidase is approximately 1 pg of protein. Film exposure times using peroxidase are generally 5-30 seconds; exposure times for alkaline phosphatase are 1-5 minutes. Using fluorescence detection, sensitivities are less than 10 pg of protein. For western blots, DuoLuX™ Chemiluminescent/ Chemifluorescent Substrate can be used on either nitrocellulose or PVDF membranes but, in general, nitrocellulose is preferred.



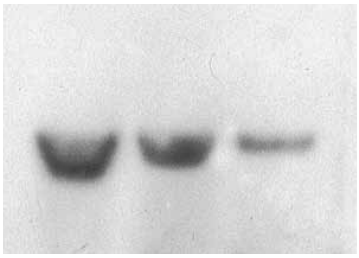
Western blot visualized by chemiluminescence. Serial dilutions of rabbit IgG heavy chain were resolved by electrophoresis, transferred onto nitrocellulose membrane and detected with biotinylated anti-rabbit IgG, using the VECTASTAIN® ABC-AmP™ Kit with the DuoLuX™ Substrate. Chemiluminescent detection was captured by exposure to film.



Western blot visualized by fluorescence. Serial dilutions (1:2) of mouse IgG were resolved by PAGE, transferred onto nitrocellulose membrane, and detected with biotinylated anti-mouse IgG, Peroxidase Streptavidin and the DuoLuX™ Substrate. Fluorescent image was obtained in 0.07 seconds.

Nucleic acid detection

For developing Southern, northern, or dot blots using alkaline phosphatase, typical film exposure times range from 30 seconds to 10 minutes with sensitivity ranging from 100 fg to 10 pg of nucleic acid. Using peroxidase, exposure times are somewhat shorter and sensitivities are slightly less than those for alkaline phosphatase. Both nitrocellulose or nylon membranes can be used.



Southern blot of the E. coli phosphoenolpyruvate carboxylase gene. Various dilutions of a 1.3 kb PCR product of the E. coli ppc gene was labeled with PHOTOPROBE® (LA) Biotin and hybridized to immobilized Tsp509 I-digested E. coli genomic DNA. Probe was detected using Alkaline Phosphatase Streptavidin followed by DuoLuX™ Substrate visualized by chemiluminescence.

Detection Kits

The **VECTASTAIN® ABC-AmP™** kits contain the DuoLuX™ Substrate and specially formulated reagents to optimally detect proteins on western blots using an amplified alkaline phosphatase system (see Section E, "Blot and Gel Detection", page E5 for a complete description).

The **UltraSNAP™ kit** contains the DuoLuX™ Substrate and specially formulated reagents to provide optimal detection of biotin labeled nucleic acids on blots using an alkaline phosphatase system (see Section E, "Blot and Gel Detection", page E9 for a complete description).


AFFINITY BINDING MATRICES

Agarose Matrices

Vector Laboratories' affinity binding matrices are made using 4% agarose gel beads that have been crosslinked to increase flow rates, reduce compressibility, and increase heat stability. Our affinity binding matrices are used in various solid phase applications such as binding studies, affinity purification, or sample clean-up. The bead diameter ranges in size from 45-165 microns. They are stable in solutions at pH 3-11, as well as in many organic solvents.

Agarose Protein A

Protein A isolated from *Staphylococcus aureus*, Cowan strain, is attached through a stable linkage to heat stable, cross-linked 4% agarose gel beads. The method of attachment allows for maximum immunoglobulin binding capacity and ease of elution. Agarose Protein A (SP-0050) can be used for immunoglobulin purification, immunoprecipitation of antigens, or other applications involving specific immunoglobulin binding. Protein concentration is 2 mg/ml of settled agarose beads and is supplied as a 1:1 suspension in buffer. The binding capacity exceeds 10 mg Human IgG per ml of settled beads.

Biotinylated Agarose

Biotin covalently attached to agarose beads will bind avidin or streptavidin essentially irreversibly. Biotinylated Agarose (B-2011) has a binding capacity exceeding 5 mg avidin or streptavidin per ml of gel and can be used for the rapid removal of avidin or streptavidin conjugates from solution.

Agarose Streptavidin

Agarose Streptavidin (SA-5010) is prepared by conjugating streptavidin to heat stable, cross-linked 4% agarose gel beads. To ensure minimal steric interference and low nonspecific binding, streptavidin is conjugated through a hydrophilic spacer arm. The procedure we have developed for coupling streptavidin to agarose preserves the biotin binding activity of the streptavidin. Unlike cyanogen bromide coupling, our procedure does not produce conjugates which can be leached from the gel with solutes such as Tris buffer. Our procedure also does not generate charged groups on the gel that can bind proteins nonspecifically. Protein concentration is 1 mg streptavidin per ml settled agarose beads. The product is supplied as a 1:1 suspension in buffer. The binding capacity is approximately 1 mole biotinylated HRP per mole of streptavidin.

This product can be used to separate biotinylated macromolecules from unbiotinylated materials or for solid-phase binding assays.

Agarose Avidin D

Agarose Avidin D (A-2010) is prepared by conjugating Avidin D to heat stable, cross-linked 4% agarose gel beads. To ensure minimal steric interference and low nonspecific binding, Avidin D is conjugated through a hydrophilic spacer arm. The procedure we have developed for coupling Avidin D to agarose optimally preserves the biotin binding activity of the Avidin D. Unlike cyanogen bromide coupling, our procedure does not produce conjugates which can be leached from the gel with solutes such as Tris buffer. Our procedure also does not generate charged groups on the gel that can bind proteins nonspecifically. Protein concentration is 2 mg Avidin D per ml of settled agarose beads. The product is supplied as a 1:1 suspension in buffer. The binding capacity is greater than 2 mg of biotinylated IgG per ml of settled beads.

This product can be used to separate biotinylated macromolecules from unbiotinylated materials or for solid-phase binding assays.

Agarose Anti-Biotin

The Agarose-bound Anti-Biotin (SP-3030) contains 1 mg of Anti-Biotin per ml of settled 4% agarose gel beads. This product can be used to bind and dissociate biotinylated molecules.

Unconjugated Agarose Beads

Unconjugated Agarose Beads (AG-1000) are the same beads used for all of our agarose-conjugated products. These 4% agarose gel beads are crosslinked to increase flow rates, to reduce compressibility, and to prevent melting in hot solutions. These beads can be used as a negative control in binding studies with other agarose conjugates, for prebinding applications, or for diluting our agarose-conjugated products. This product is supplied as a 1:1 suspension in buffer.

Agarose Matrices

Product	Catalog Number	Unit Size (vol. settled gel)
Agarose Protein A	SP-0050	5 ml
Biotinylated Agarose	B-2011	5 ml
Agarose Streptavidin	SA-5010	2 ml
Agarose Avidin D	A-2010	5 ml
Agarose Anti-Biotin	SP-3030	2 ml
Unconjugated Agarose Beads	AG-1000	10 ml

Agarose Bound Lectins

Lectin affinity chromatography is a simple and widely used technique for the isolation of a variety of glycoconjugates. The glycoconjugate is allowed to bind to the immobilized lectin, and the unbound residual material is removed by washing. The bound glycoconjugates are generally eluted with a solution of a sugar known to inhibit binding of the particular lectin. Soluble glycoproteins, hormones, antigens, polysaccharides, detergent-solubilized membrane-bound glycoconjugates, cell surface receptors, blood group substances, viral glycoproteins, histocompatibility antigens, lymphokines, enzymes, lymphocyte markers, serum proteins, and oncofetal antigens are only a few of the substances that have been purified using immobilized lectins.

Our immobilized lectins are prepared using our affinity-purified lectins. Heat stable, cross-linked 4% agarose beads with a molecular weight exclusion limit of about $2x10^7$ daltons are used as the solid-phase matrix to which the lectins are covalently coupled. The attachment of the lectins to the beads is carefully controlled to preserve lectin activity and minimize conformational changes of the bound lectins that might result in nonspecific ionic or hydrophobic interactions. The technique we have developed to couple lectins to agarose beads inserts a hydrophilic spacer arm between the lectin and the matrix.

This coupling method provides several advantages over the traditional cyanogen bromide procedure:

- Maximum carbohydrate binding activity of the coupled lectins is retained
- Linkage is stable over a range of pH values
- Conjugated proteins are not leached off the beads by Tris or other routinely used buffers
- No residual charges are present after conjugation. This minimizes non-specific binding to the matrix.

Our agarose bound lectins are supplied at a constant concentration of lectin per ml of settled beads. The concentration for each lectin is selected to achieve the highest glycoconjugate binding capacity per mg of lectin present in the beads. Each lot is tested for its binding capacity using glycoproteins known to bind the lectin. This provides a guideline for the user and assures the quality of our agarose bound lectins.

Eluting sugars can be found on page J5.

Agarose Bound Lectins

Product	Catalog Number	Protein Concentration	Eluting Sugar	Unit Size (vol. settled gel)
Aleuria aurantia Lectin (AAL), agarose bound	AL-1393	2 mg lectin/ml gel	100 mM L-fucose	2 ml
Aleuria aurantia Lectin (AAL), VECTREX® bound[a]	MB-1396	N/A	500 mM L-fucose	1 ml
Aleuria aurantia Lectin (AAL), VECTREX® bound[a], Binding and Elution Kit	MB-1397	N/A	500 mM L-fucose	1 Kit
Concanavalin A (Con A), agarose bound	AL-1003	6 mg lectin/ml gel	200 mM α-methylmannoside/ 200 mM α-methylglucoside mixture	10 ml
				100 ml
Succinylated Concanavalin A, agarose bound	AL-1003S	6 mg lectin/ml gel	200 mM α-methylmannoside	2 ml
Datura stramonium Lectin (DSL), agarose bound	AL-1183	3 mg lectin/ml gel	Chitin hydrolysate	2 ml
Dolichos biflorus Agglutinin (DBA), agarose bound	AL-1033	3 mg lectin/ml gel	200 mM *N*-acetylgalactosamine	2 ml
Erythrina cristagalli Lectin (ECL, ECA), agarose bound	AL-1143	3 mg lectin/ml gel	200 mM lactose	2 ml
Galanthus nivalis Lectin (GNL), agarose bound	AL-1243	3 mg lectin/ml gel	100 mM-200 mM α-methylmannoside	2 ml
				5 ml
Griffonia (Bandeiraea) simplicifolia Lectin I (GSL I, BSL I), agarose bound	AL-1103	4 mg lectin/ml gel	200 mM galactose/ 200 mM *N*-acetylgalactosamine mixture	2 ml
Griffonia (Bandeiraea) simplicifolia Lectin II (GSL II, BSL II), agarose bound	AL-1213	3 mg lectin/ml gel	Chitin hydrolysate or 200 mM *N*-acetylglucosamine	2 ml

[a]*This lectin is bound to a VECTREX® matrix. See page J7 for applications.*

Continued on following page.

Agarose Bound Lectins (continued)

Product	Catalog Number	Protein Concentration	Eluting Sugar	Unit Size (vol. settled gel)
Jacalin, agarose bound	AL-1153	4 mg lectin/ml gel	800 mM galactose or 100 mM melibiose	2 ml
				5 ml
				10 ml
Lens culinaris Agglutinin (LCA, LcH), agarose bound	AL-1043	3 mg lectin/ml gel	200 mM α-methylmannoside/ 200 mM α-methylglucoside mixture	2 ml
				5 ml
				10 ml
Lotus tetragonolobus Lectin (LTL), agarose bound	AL-1323	3 mg lectin/ml gel	50 mM – 100 mM L-fucose	2 ml
Lycopersicon esculentum (Tomato) Lectin (LEL, TL), agarose bound	AL-1173	2 mg lectin/ml gel	Chitin hydrolysate	2 ml
Peanut Agglutinin (PNA), agarose bound	AL-1073	5 mg lectin/ml gel	200 mM galactose	2 ml
				5 ml
Phaseolus vulgaris Erythroagglutinin (PHA-E), agarose bound	AL-1123	3 mg lectin/ml gel	100 mM acetic acid	2 ml
Phaseolus vulgaris Leucoagglutinin (PHA-L), agarose bound	AL-1113	3 mg lectin/ml gel	100 mM acetic acid	2 ml
Pisum sativum Agglutinin (PSA), agarose bound	AL-1053	3 mg lectin/ml gel	200 mM α-methylmannoside/ 200 mM α-methylglucoside mixture	2 ml
Ricinus communis Agglutinin I (RCA I, RCA_{120}), agarose bound	AL-1083	4 mg lectin/ml gel	200 mM galactose or lactose	2 ml
				5 ml
				10 ml
Ricinus communis Agglutinin II (RCA II, RCA_{60}, ricin), agarose bound	AL-1093	4 mg lectin/ml gel	200 mM galactose or lactose	2 ml
Sambucus nigra Lectin (SNA, EBL), agarose bound	AL-1303	3 mg lectin/ml gel	500 mM lactose in buffered saline followed by 500 mM lactose in acetic acid	2 ml
Soybean Agglutinin (SBA), agarose bound	AL-1013	4 mg lectin/ml gel	200 mM *N*-acetylgalactosamine	2 ml
Ulex europaeus Agglutinin I (UEA I), agarose bound	AL-1063	2 mg lectin/ml gel	50 mM-100 mM L-fucose	2 ml
Vicia villosa Lectin (VVL, VVA), agarose bound	AL-1233	3 mg lectin/ml gel	200 mM *N*-acetylgalactosamine	2 ml
Wheat Germ Agglutinin (WGA), agarose bound	AL-1023	7 mg lectin/ml gel	500 mM *N*-acetylglucosamine or Chitin Hydrolysate	2 ml
				5 ml
				10 ml
Succinylated Wheat Germ Agglutinin, agarose bound	AL-1023S	3 mg lectin/ml gel	Chitin hydrolysate or 500 mM *N*-acetylglucosamine with salt and/or acid elution generally required	2 ml
				5 ml
Wisteria floribunda Lectin (WFA, WFL), agarose bound	AL-1353	3 mg lectin/ml gel	200 mM *N*-acetylgalactosamine	2 ml

Chitin Hydrolysate

Chitin Hydrolysate (SP-0090) can be used as an inhibitor of lectin-conjugate binding or for eluting glycoproteins bound to agarose lectins. This mixture is an inhibitor for the following lectins: DSL, GSL II, LEL, STL, and WGA.

Chitin Hydrolysate is supplied as a highly concentrated solution of *N*-acetylglucosamine (glcNAc) and glcNAc oligomers in nearly saturated sodium chloride.

Sugars

A variety of sugars are provided for use as inhibitors of lectin-conjugate binding or for eluting glycoproteins or other glycoconjugates from columns of agarose lectins. These sugars are supplied as dry powders. If reconstituted in 5 ml of water or buffer the resulting sugar solutions are 400 mM, except for L-fucose and *N*-acetylgalactosamine, which are 100 mM.

Product	Catalog Number	Unit Size	Stock Concentration*	Weight of Sugar
Chitin Hydrolysate	SP-0090	10 ml	N.A.	N.A.
Sugars				
N-acetylgalactosamine	S-9001	5 ml	100 mM	111 mg
N-acetylglucosamine	S-9002	5 ml	400 mM	442 mg
galactose	S-9003	5 ml	400 mM	360 mg
lactose	S-9004	5 ml	400 mM	721 mg
α-methylmannoside	S-9005	5 ml	400 mM	388 mg
α-methylglucoside	S-9006	5 ml	400 mM	388 mg
L-fucose	S-9007	5 ml	100 mM	82 mg
N-acetylneuraminic acid (sialic acid)	S-9008	5 ml	400 mM	619 mg

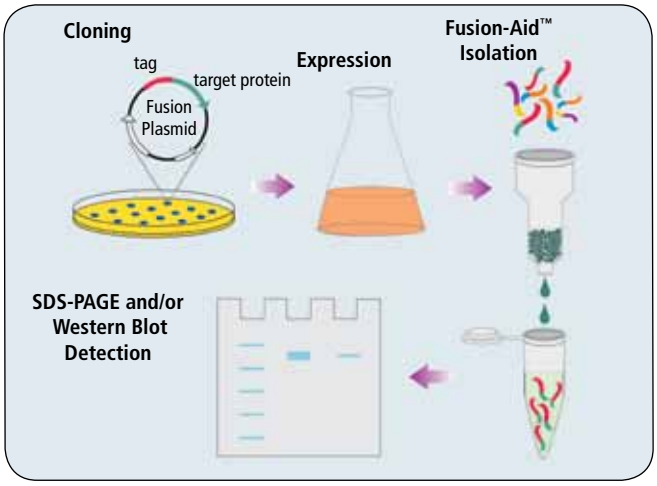
** Stock concentration if reconstituted in 5 ml*

Fusion Protein Purification

Fusion Proteins. Creating fusion proteins using recombinant DNA techniques is the preferred method to purify or detect many proteins of interest.

Two proteins that are commonly fused to proteins of interest are green fluorescent protein (GFP) and maltose binding protein (MBP). Alternatively, proteins of interest are fused to a specific peptide sequence rather than an entire protein. This peptide sequence defines an epitope that can be readily detected with a specific antibody. Two commonly used peptide tags are derived from human influenza virus hemagglutinin (HA) and c-Myc.

Biotinylated antibodies to these common fusion protein tags can be found in Section G, "Antibodies" page G33.

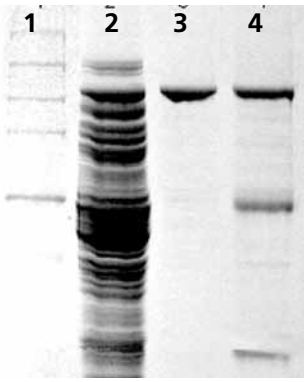


Flow chart of fusion protein expression, purification and analysis. Proteins of interest can be easily isolated using fusion tag-specific antibodies conjugated to agarose beads.

Fusion Protein Purification. The Fusion-Aid™ Kits are designed to immunoprecipitate/isolate fusion proteins containing the appropriate protein tag. The Fusion-Aid™ Kit has affinity-purified primary antibodies coupled directly to agarose beads. In contrast to traditional methods, this eliminates the contamination of the isolated proteins with coprecipitating primary antibodies. The spin columns provided with the kit also facilitate rapid immunoprecipitation and maximum protein recovery (see figure below).

Vector® Fusion-Aid™ Kits contain the following:

- 0.5 ml agarose beads coupled with affinity-purified antibody to the specific tag
- 5 spin columns
- 5 collection tubes



Coomassie-stained SDS-PAGE
lane 1: *M.W. Marker.*

lane 2: *Crude lysate with the HA fusion protein.*

lane 3: *Eluate from Fusion-Aid™ HA Kit beads.*

lane 4: *Eluate using traditional method (polyclonal goat anti-HA was added to crude lysate, applied to agarose protein A and eluted).*

Vector® Fusion-Aid™ Kits

Product	Catalog Number	Unit Size	Kit Contents
Vector® Fusion–Aid™ MBP	MB-0731	1 Kit	- 0.5 ml agarose beads coupled with affinity-purified antibody to MBP - 5 spin columns - 5 collection tubes
Vector® Fusion–Aid™ GFP	MB-0732	1 Kit	- 0.5 ml agarose beads coupled with affinity- purified antibody to GFP - 5 spin columns - 5 collection tubes
Vector® Fusion–Aid™ c-Myc	MB-0733	1 Kit	- 0.5 ml agarose beads coupled with affinity-purified antibody to c-Myc - 5 spin columns - 5 collection tubes
Vector® Fusion–Aid™ HA	MB-0734	1 Kit	- 0.5 ml agarose beads coupled with affinity-purified antibody to HA - 5 spin columns - 5 collection tubes

VECTREX® Matrices for Nucleic Acid Applications

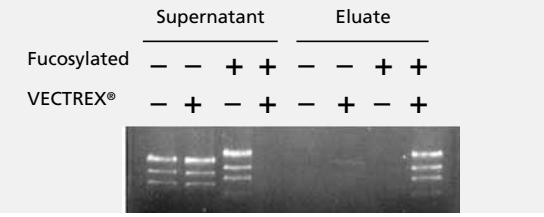
The VECTREX® matrix is optimized for immobilization of nucleic acids. This matrix consists of particles of a highly cross-linked sugar polymer that has a very large surface area and low non-specific binding for nucleic acids. Unlike agarose, which may retain small molecules in its pores, the VECTREX® particle will retain molecules only through specific affinity interaction on the surface of the particles. The VECTREX® matrix is dense and sediments readily even without centrifugation.

VECTREX® AAL

VECTREX® AAL (MB-1396) is used for reversible binding of fucose-labeled nucleic acids. Binding and elution can be performed at physiological pH and salt concentrations. VECTREX® AAL contains *Aleuria aurantia* lectin and will bind nucleic acids labeled with fucose using either the FastTag® system, or 3′ or 5′ EndTag™ Labeling Systems (see pages F4-F7). Elution is easily accomplished under non-denaturing conditions with L-fucose. The matrix is supplied as a 1:1 suspension in buffer. The binding capacity is at least 25 ng fucose-labeled λ DNA per µl 1:1 suspension.

VECTREX® AAL Binding and Elution Kit

VECTREX® AAL Binding and Elution Kit (MB-1397) contains 1 ml of hydrated VECTREX® AAL, binding buffer, and L-fucose elution solution.



Reversible binding of FastTag® Fucose-labeled λ Hind III DNA to VECTREX® AAL. Labeled (+) or unlabeled (-) DNA was incubated with VECTREX® AAL (+) or binding buffer (-). After binding, DNA was eluted with L-fucose. Supernatants and eluates were fractionated by gel electrophoresis.

VECTREX® Avidin D

VECTREX® Avidin D (A-2020) is used for irreversible binding of biotinylated nucleic acids. This product consists of Avidin D covalently linked to the surface of the VECTREX® matrix.

VECTREX® Avidin D is useful in applications such as subtractive hybridization. A probe that is biotinylated with PHOTOPROBE®, FastTag®, or 3′ or 5′ EndTag™ Labeling Systems is hybridized to a mixture of nucleic acid in solution. After hybridization, the mixture is incubated with a suspension of VECTREX® Avidin D or passed through a column containing VECTREX® Avidin D. The DNA or RNA that is not associated with the biotinylated probe is left in solution or passes through the column. The biotinylated probe and the nucleic acid complementary to the probe are thus separated from the non-complementary sequences. This matrix is supplied as a 1:1 suspension in buffer. The binding capacity is at least 22 ng of biotin-labeled λ DNA per µl of 1:1 suspension.

VECTREX® Avidin DLA

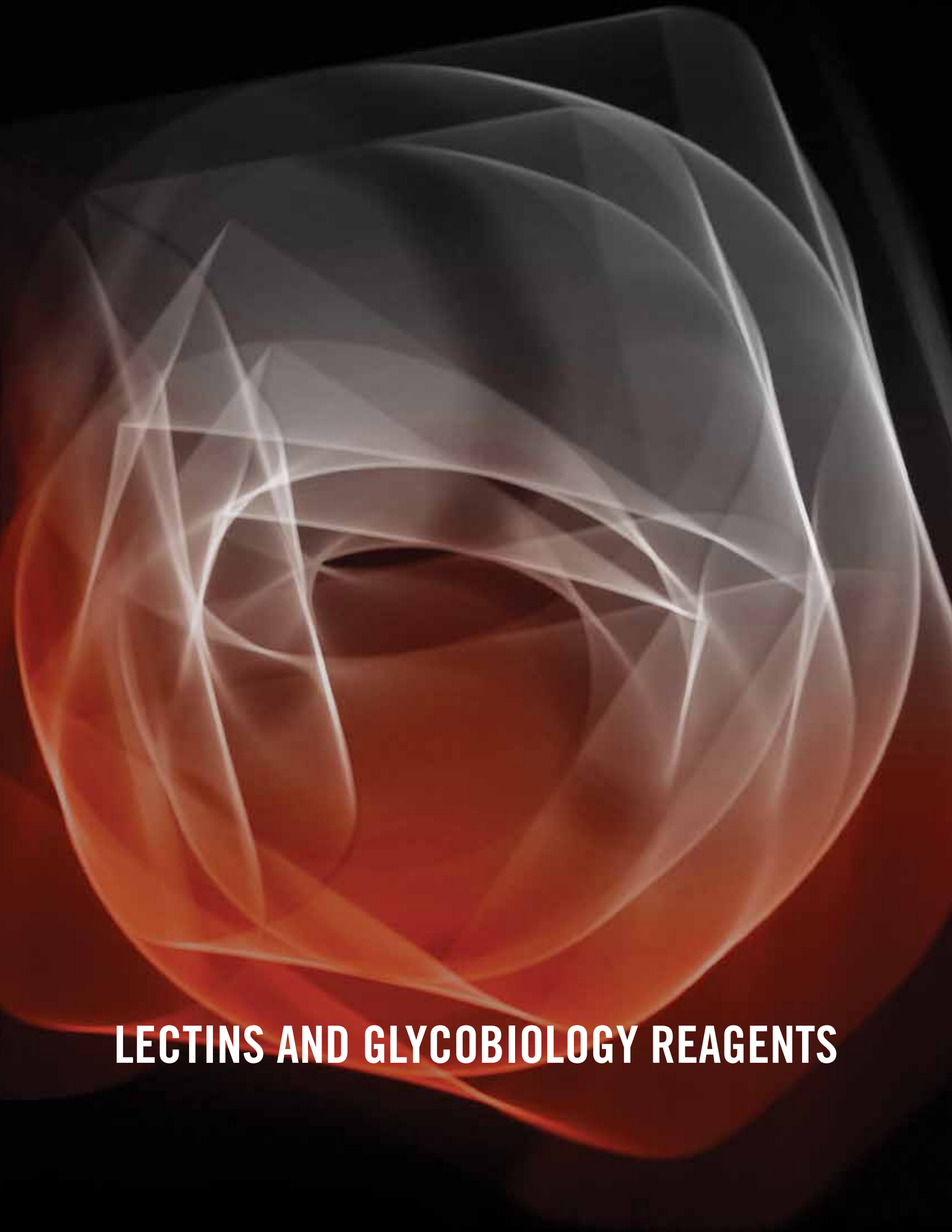
VECTREX® Avidin DLA (MB-2021) is used for reversible binding of biotinylated nucleic acids. VECTREX® Avidin DLA is made by coupling our VECTREX® matrix to a modified form of Avidin D that has a significantly reduced binding affinity for biotin. The affinity is such that, unlike VECTREX® Avidin D, biotinylated protein, nucleic acids, or other molecules can be eluted with biotin. The matrix is supplied as a 1:1 suspension in buffer. The binding capacity is at least 25 ng of biotin-labeled λ DNA per µl of 1:1 suspension.

VECTREX® Avidin DLA Binding and Elution Kit

VECTREX® Avidin DLA Binding and Elution Kit (MB-1397) contains 1 ml of settled VECTREX® Avidin DLA, Binding buffer and biotin solution for elution.

VECTREX® Matrices

Product	Catalog Number	Unit Size (vol. settled matrix)	Kit Contents
VECTREX® Avidin D	A-2020	1 ml	N/A
VECTREX® Avidin DLA	MB-2021	1 ml	N/A
VECTREX® Avidin DLA Binding and Elution Kit	MB-2022	1 Kit	1 ml of settled VECTREX® Avidin DLA binding buffer (3.0 ml of 10x CENT buffer) elution solution (2.0 ml of 25 mM biotin solution)
VECTREX® AAL	MB-1396	1 ml	N/A
VECTREX® AAL Binding and Elution Kit	MB-1397	1 Kit	1 ml of settled VECTREX® AAL binding buffer (3.0 ml of 10x TENT binding buffer) elution solution (1.0 ml of 1.25 M L-fucose solution)


LECTINS AND GLYCOBIOLOGY REAGENTS

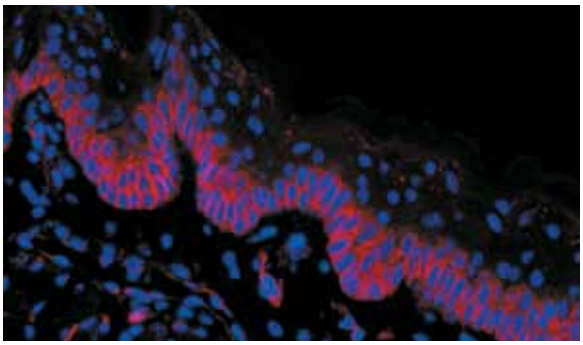
Introduction

Since the 1880's, it has been known that extracts from certain plants could agglutinate red blood cells. In the 1940's, agglutinins were discovered which could "select" types of cells based on their blood group activities. Although "lectin" was originally coined to define agglutinins that could discriminate among types of red blood cells, today the term is used more generally and includes sugar-binding proteins from many sources regardless of their ability to agglutinate cells. Lectins have been found in plants, viruses, microorganisms, and animals but despite their ubiquity, in many cases their biological function is unclear.

Most lectins are multimeric, consisting of non-covalently associated subunits. It is this multimeric structure that gives lectins their ability to agglutinate cells or form precipitates with glycoconjugates in a manner similar to antigen-antibody interactions. This unique group of proteins has provided researchers with powerful tools to explore a myriad of biological structures and processes. Because of the specificity that each lectin has toward a particular carbohydrate structure, even oligosaccharides with identical sugar compositions can be distinguished or separated. The affinity between a lectin and its receptor may vary a great deal due to small changes in the carbohydrate structure of the receptor. These properties enable the researcher to discriminate between structures, isolate a specific glycoconjugate, cell, or virus from a mixture, or study one process among several. Another property of some lectins is an ability to induce mitosis in cells that are normally not dividing. This property has been exploited extensively in an attempt to understand the process of lymphocyte blastogenesis and the biochemical and structural alterations associated with mitogenesis.

Lectins have been purified by "conventional" procedures including salt-induced crystallization, ethanol precipitation, ion exchange chromatography and gel filtration, or by affinity chromatography. The former methods rely on the physicochemical properties of the proteins for separation while affinity chromatography depends on the specific interaction between the lectin and a carbohydrate structure attached to an inert matrix. We employ both "conventional" procedures and affinity chromatography for each of our lectins. Purification is monitored and final product is assessed by immunoprecipitation with antisera, agglutination titre, polyacrylamide gel electrophoresis, and binding activity to specific affinity columns, providing the assurance that our customers have the best lectins available.

Thousands of articles on lectins have been published examining hundreds of different aspects and uses of lectins. In this catalog, only a few of the uses and properties of each lectin are described. However, both general and specific information can be found in the excellent books and articles listed on our website.



Mouse Tongue: endothelial cells stained with Dylight® 594-labeled Griffonia simplicifolia Lectin, Isolectin B_4 (red fluorescence). Mounted with VECTASHIELD® Hard•Set™ with DAPI (blue fluorescence).

Agarose Bound Lectins

Lectin affinity chromatography is a simple and widely used technique for the isolation of a variety of glycoconjugates. The glycoconjugate is allowed to bind to the immobilized lectin, and the unbound residual material is removed by washing. The bound glycoconjugates are generally eluted with a solution of a sugar known to inhibit binding of the particular lectin. Soluble glycoproteins, hormones, antigens, polysaccharides, detergent-solubilized membrane-bound glycoconjugates, cell surface receptors, blood group substances, viral glycoproteins, histocompatibility antigens, lymphokines, enzymes, lymphocyte markers, serum proteins, and oncofetal antigens are only a few of the substances that have been purified using immobilized lectins.

Our immobilized lectins are prepared using our affinity-purified lectins. Heat stable, cross-linked 4% agarose beads with a molecular weight exclusion limit of about $2x10^7$ daltons are used as the solid-phase matrix to which the lectins are covalently coupled. The attachment of the lectins to the beads is carefully controlled to preserve lectin activity and minimize conformational changes of the bound lectins that might result in nonspecific ionic or hydrophobic interactions. The technique we have developed to couple lectins to agarose beads inserts a hydrophilic spacer arm between the lectin and the matrix.

This coupling method provides several advantages over the traditional cyanogen bromide procedure:

- Maximum carbohydrate binding activity of the coupled lectins is retained
- Linkage is stable over a range of pH values
- Conjugated proteins are not leached off the beads by Tris or other routinely used buffers
- No residual charges are present after conjugation. This minimizes non-specific binding to the matrix.

Our agarose bound lectins are supplied at a constant concentration of lectin per ml of settled beads. The concentration for each lectin is selected to achieve the highest glycoconjugate binding capacity per mg of lectin present in the beads. Each lot is tested for its binding capacity using glycoproteins known to bind the lectin. This provides a guideline for the user and assures the quality of our agarose bound lectins.

Labeled Lectins

Some lectins can tolerate a higher degree of conjugation and still remain fully active, soluble, and retain low nonspecific binding properties, while others cannot. Each of our labeled lectins has an appropriate number of fluorochromes or biotins bound that provides the optimum staining characteristics for that lectin. These conjugates are supplied essentially free of unconjugated fluorochromes or biotins, preserved with sodium azide.

Specificity Guide for Lectins

Sugar *	Lectin †
Fucose	AAL, LTL, UEA I
Galactose	ACL, ECL, EEL, GSL I, GSL I-B_4, Jacalin, MAL I, PNA, RCA I, RCA II, SBA
Glucose	Con A, LCA, PSA
Mannose	Con A, GNL, HHL, LCA, NPL, PSA
N-Acetylgalactosamine	BPL, DBA, GSL I, MPL, PTL, RCA I, RCA II, SJA, SBA, VVA, WFA
N-Acetylglucosamine	DSL, GSL II, LEL, STL, WGA
Sialic Acid	MAL II, SNA
Complex Structures	PHA-E, PHA-L

* This table shows the primary sugar specificity of each lectin. Most lectins have additional structural requirements for binding, so this table should only be used as a quick reference guide.

† Lectins in each box are listed in alphabetical order. Please refer to text and cited references for other properties and recommended inhibiting/eluting sugars.

Table of Lectin Properties

Lectin	Common Abbreviation	Source	Mol. Wt. (kDa)	Number of Subunits	pI	ε 0.1% 280 nm	Glycoprotein	Metal Ions Present
Aleuria aurantia	AAL	*Aleuria aurantia* mushrooms	72	2	9	2.97	No	--
Amaranthus caudatus	ACL, ACA	*Amaranthus caudatus* seeds	66-70	2	6.7 - 7.7	1.60	No	No
Bauhinia purpurea	BPL, BPA	*Bauhinia purpurea alba* (Camel's Foot Tree) seeds	195	4	4.6 - 6	1.75	Yes	No
Concanavalin A	Con A	*Canavalia ensiformis* (Jack Bean) seeds	104	4	6.3 - 7	1.20	No	Ca^{++}, Mn^{++}
Succinylated Concanavalin A	Succinylated Con A	*Canavalia ensiformis* (Jack Bean) seeds	56	2	< 4.4	1.2	No	Ca^{++}, Mn^{++}
Datura stramonium	DSL	*Datura stramonium* (Thorn Apple, Jimson Weed) seeds	86	1	> 9	0.80	Yes	No
Dolichos biflorus	DBA	*Dolicos biflorus* (Horse Gram) seeds	111	4	4.6 - 5	1.22	Yes	Ca^{++}, Mn^{++}, Mg^{++} Zn^{++}
Erythrina cristagalli	ECL, ECA	*Erythrina cristagalli* (Coral Tree) seeds	54	2	6.3 - 6.5	1.30	Yes	Ca^{++}, Mn^{++}, Zn^{++}
Euonymus europaeus	EEL	*Eunonymus europaeus* (Spindle Tree) seeds	140	4	4.4	2.40	Yes	Ca^{++}, Zn^{++}
Galanthus nivalis	GNL	*Galanthus nivalis* (Snowdrop) bulbs	50	4	3.5 - 4	1.90	No	No
Griffonia (Bandeiraea) simplicifolia I	GSL I, BSL I	*Griffonia (Bandeiraea) simplicifolia* seeds	114	4	5 - 6.5	1.40	Yes	Ca^{++}, Mn^{++}
Griffonia (Bandeiraea) simplicifolia I Isolectin B_4	GSL I - B_4	*Griffonia (Bandeiraea) simplicifolia* seeds	114	4	6 - 6.2	1.4	Yes	Ca^{++}, Mn^{++}
Griffonia (Bandeiraea) simplicifolia II	GSL II, BSL II	*Griffonia (Bandeiraea) simplicifolia* seeds	113	2	5 - 6	1.25	Yes	Ca^{++}, Mn^{++}
Hippeastrum hybrid	HHL, AL	*Hippeastrum* hybrid (Amaryllis) bulbs	50	4	4.7 - 5.1	1.85	No	No
Jacalin	Jacalin	*Artocarpus integrifolia* (Jackfruit) seeds	66	4	7.8	1.50	Yes	No
Lens culinaris	LCA, LcH	*Lens culinaris* (lentil) seeds	50	4	7.6 - 8.4	1.25	No	Ca^{++}, Mn^{++}
Lotus tetragonolobus	LTL	*Lotus tetragonolobus, Tetragonolobus purpurea* (Winged Pea, Asparagus Pea) seeds	107	4	7.3, 7.6, 7.9 & 8.2	1.51	Yes	Ca^{++}, Mn^{++}
Lycopersicon esculentum	LEL, TL	*Lycopersicon esculentum* (tomato) fruit	71	1	>9	0.76	Yes	--
Maackia amurensis I	MAL I, MAL	*Maackia amurensis* seeds	130	2	4.7	1.40	Yes	No
Maackia amurensis II	MAL II, MAH	*Maackia amurensis* seeds	130	2	4.7	1.33	Yes	No
Maclura pomifera	MPL	*Maclura pomifera* (Osage Orange) seeds	44	4	4.8 - 5.3	1.40	No	No
Narcissus pseudonarcissus	NPL, NPA, DL	*Narcissus pseudonarcissus* (Daffodil) bulbs	59	4	4.2 - 4.6	1.87	No	No

Lectin	Mitogenic Activity	Blood Group Specificity	Preferred Sugar Specificity	Inhibitor or Eluting Sugar	Special Application
Aleuria aurantia	No	Non-specific	Fucα6GlcNAc	L-Fuc	Tumor cell marker detection
Amaranthus caudatus	Yes	O>B>A (-SA) T antigen	Galβ3GalNAc	desialyzed fetuin	T-antigen probe
Bauhinia purpurea	Yes	A,B,O (-SA)	Galβ3GalNAc	Lactose	Detection of Reed Sternberg cells of Hodgkin's disease; binds N-Antigen of human erythrocytes
Concanavalin A	Yes	Non-specific	αMan, αGlc	MeαMan+ MeαGlc	Polylysine and histone conjugated as vectors to transfer genes to airway epithelial cells; insulin-like activity
Succinylated Concanavalin A	Yes	None	αMan, αGlc	MeαMan+ MeαGlc	Growth inhibitor of 3T3 mouse fibroblasts
Datura stramonium	Yes	A, B, O	$(GlcNAc)_{2-4}$	Chitin hydrolysate	Marker for mouse peritoneal cells
Dolichos biflorus	No	A_1>>A_2	αGalNAc	GalNAc	Distinguishes A_1 from A_2 human red blood cells; binds to rat macrophages in lung tissues
Erythrina cristagalli	Yes	A (–SA)	Galβ4GlcNAc	Lactose	Isolation of human natural killer cells
Euonymus europaeus	Yes	O (–SA), B	Galα3Gal	Lactose	Endothelial cell binding
Galanthus nivalis	No	Rabbit	αMan	MeαMan	Detection of HIV and SIV glycoprotein; isolation of mouse IgM; virus isolation
Griffonia (Bandeiraea) simplicifolia I	No	B>>A1	αGal, αGalNAc	Gal/GalNAc	Endothelial cell marker (for mouse)
Griffonia (Bandeiraea) simplicifolia I Isolectin B_4	No	B	αGal	Gal or Raffinose	Non-primate endothelial cell marker; neuronal marker
Griffonia (Bandeiraea) simplicifolia II	No	A (-SA)>>B (-SA)	α or βGlcNAc	Chitin hydrolysate or GlcNAc	Selective staining of Golgi apparatus; marker for uterine blood vessels and certain carcinomas
Hippeastrum hybrid	No	Rabbit	αMan	MeαMan	
Jacalin	Yes	O (+SA), T antigen	Galβ3GalNAc	Gal or Melibiose	Purification of human IgA
Lens culinaris	Yes	Non-specific	αMan, αGlc	MeαMan+ MeαGlc	
Lotus tetragonolobus	No	O<A2	αFuc	L-Fuc	Distinguishes between pathogenic and non-pathogenic trypanosomes
Lycopersicon esculentum	No	Non-specific	$(GlcNAc)_{2-4}$	Chitin hydrolysate	Perfusion studies in mouse (binding to vascular endothelium)
Maackia amurensis I	Yes	Non-specific	Galβ4GlcNAc	Lactose	
Maackia amurensis II	Yes	Non-specific	Neu5Acα3Galβ4GalNAc	Human Glycophorin	
Maclura pomifera	Yes	A, B, O (-SA)	Galβ3GalNAc	Gal	Binding to rat lymphoid cells
Narcissus pseudonarcissus	No	Rabbit	αMan	MeαMan	Detection of beginning of apoptosis of human cell lines

Sugar Abbreviations:

Fuc	L-Fucose
Gal	D-Galactose
GalNAc	*N*-Acetylgalactosamine
Glc	D-Glucose
GlcNAc	*N*-Acetylglucosamine
Man	Mannose
MeαGlc	α-Methylglucoside
MeαMan	α-Methylmannoside
Neu5Ac	*N*-Acetylneuraminic acid (sialic acid)
SA	Sialic Acid

Table of Lectin Properties (continued)

Lectin	Common Abbreviation	Source	Mol. Wt. (kDa)	Number of Subunits	pI	ε 0.1% 280 nm	Glycoprotein	Metal Ions Present
Peanut	PNA	*Arachis hypogaea* peanuts	110	4	5.5 - 6.5	0.89	No	Ca^{++}, Mg^{++}
Phaseolus vulgaris Erythroagglutinin (PHA-E)	PHA-E	*Phaseolus vulgaris* (Red Kidney Bean) seeds	126	4	6 - 8	1.16	Yes	Ca^{++}, Mn^{++}
Phaseolus vulgaris Agglutinin (PHA-E+L)	PHA-E+L	*Phaseolus vulgaris* (Red Kidney Bean) seeds	126	4	5.2 - 6.2	1.16	Yes	Ca^{++}, Mn^{++}
Phaseolus vulgaris Leucoagglutinin (PHA-L)	PHA-L	*Phaseolus vulgaris* (Red Kidney Bean) seeds	126	4	4.2 - 4.8	1.16	Yes	Ca^{++}, Mn^{++}
Pisum sativum	PSA	*Pisum sativum* (Pea) seeds	53	4	6.0 - 6.7	1.20	Trace	Ca^{++}, Mn^{++}
Psophocarpus tetragonolobus I	PTL I, WBA I	*Psophocarpus tetragonolobus* (Winged Bean) seeds	57	2	8.0	0.95	Yes	--
Psophocarpus tetragonolobus II	PTL II, WBA II	*Psophocarpus tetragonolobus* (Winged Bean) seeds	46	2	6.0	1.20	Yes	--
Ricinus communis I	RCA I, RCA_{120}	*Ricinus communis* (Castor Bean) seeds	120	2	7.8	1.17	Yes	No
Ricinus communis II, ricin	RCA II, RCA_{60}, ricin	*Ricinus communis* (Castor Bean) seeds	60	1	7.1	1.17	Yes	No
Ricin A Chain	Ricin A Chain	RCA_{60}	28	1	7.5	0.7	Yes	No
Ricin B Chain	Ricin B Chain	RCA_{60}	32	1	4.5	1.64	Yes	No
Sambucus nigra	SNA, EBL	*Sambucus nigra* (Elderberry) bark	140	4	5.4 - 5.8	1.50	Yes	No
Solanum tuberosum	STL, PL	*Solanum tuberosum*, (potato) tubers	100	2	>9	0.80	Yes	No
Sophora japonica	SJA	*Sophora japonica* (Japanese Pagoda Tree) seeds	133	2	4.9 - 5.6	1.67	Yes	Ca^{++}, Mn^{++}
Soybean	SBA	*Glycine max* (soybean) seeds	120	4	5.8 - 6	1.33	Yes	Ca^{++}, Mn^{++}
Ulex europaeus I	UEA I	*Ulex europaeus* (Furze Gorse) seeds	63	2	4.5 - 5.1	1.30	Yes	Ca^{++}, Mn^{++}, Zn^{++}
Vicia villosa	VVL, VVA	*Vicia villosa* (Hairy Vetch) seeds	144*	4	5.5 - 6.2	0.78	Yes	Ca^{++}, Mn^{++}
Wheat Germ	WGA	*Triticum vulgaris* (wheat germ)	36	2	>9	1.46	No	Ca^{++}
Succinylated Wheat Germ	Succinylated WGA	*Triticum vulgaris* (wheat germ)	36	2	<3	1.46	No	Ca^{++}
Wistera floribunda	WFA, WFL	*Wisteria floribunda* (Japanese Wisteria) seeds	116	4	5.2 - 5.8	0.89	Yes	--

** Literature values reported; 102 kDa - 144 kDa*

Lectin	Mitogenic Activity	Blood Group Specificity	Preferred Sugar Specificity	Inhibitor or Eluting Sugar	Special Application
Peanut	No	T antigen (M, N)	Galβ3GalNAc	Gal	Detection of relocalization of Tag antigen in large bowel carcinoma
Phaseolus vulgaris Erythroagglutinin (PHA-E)	Yes	A(-SA)	Galβ4GlcNAcβ2Manα6 (GlcNAcβ4) (GlcNAcβ4Manα3) Manβ4	bovine thyroglobulin, acetic acid	Binding to central nervous system cells (HNK-1 antigen)
Phaseolus vulgaris Agglutinin (PHA-E+L)	Yes	A(-SA)	See PHA-E/PHA-L	bovine thyroglobulin, acetic acid	Binding to central nervous system cells (HNK-1 antigen)
Phaseolus vulgaris Leucoagglutinin (PHA-L)	Yes	–	Galβ4GlcNAcβ6(GlcNAc β2Manα3)Manα3	bovine thyroglobulin, acetic acid	Anterograde neuronal tracing; metastatic tumor marker; lymphocyte mitogen for lymphokine production
Pisum sativum	Yes	Non-specific	αMan, αGlc	MeαMan+ MeαGlc	Separation of lymphoblastic leukemia antigen in kidney cells; purification of feline T-lymphocytes from peripheral blood
Psophocarpus tetragonolobus I	No	Rabbit, O(-SA)	GalNAc, Gal	GalNAc	Staining of mouse M-cells; blood vessel staining of A and B blood group individuals
Psophocarpus tetragonolobus II	No	O(-SA)	GalNAc, Gal	GalNAc	Blood vessel staining (human) of blood group O individuals
Ricinus communis I	No	Non-specific	Gal	Gal or Lactose	Labeling of receptors on sprouting rat neurons
Ricinus communis II, ricin	No	Non-specific	Gal, GalNAc	Gal or Lactose	TOXIC - Used in rat neuronal retrograde transport (suicide transport)
Ricin A Chain	No	–	–	–	Produce hybrid toxins
Ricin B Chain	Yes	–	Gal	Gal or Lactose	Used to potentiate antibody ricin A chain conjugates for tumor toxicity
Sambucus nigra	No	Non-specific	Neu5Acα6Gal/GalNAc	Lactose in buffered saline & acetic acid	Used to distinguish sialylated oligosaccharides bound by human A influenza virus
Solanum tuberosum	No	Non-specific	$(GlcNAc)_{2-4}$	Chitin hydrolysate	Staining of prostate cancer cell line; bacterial cell wall binding
Sophora japonica	No	A>B>O(-SA)	βGalNAc	GalNAc	Distinguishes between pathogenic and non pathogenic trypanosomes
Soybean	Yes	A>O>B	α>βGalNAc	GalNAc	Stem cell separation
Ulex europaeus I	No	O>A2	αFuc	L-Fuc	Human endothelial cell marker
Vicia villosa	No	Tn antigen	GalNAc	GalNAc	Staining of neurons on human cerebral cortex, detection of Tn and Cad antigens
Wheat Germ	Yes	A,B,O	GlcNAc	Chitin hydrolysate or GlcNAc with acid or salt	Insulin receptor purification, neuronal tracing; bacterial cell wall binding
Succinylated Wheat Germ	No	A,B,O	GlcNAc	Chitin hydrolysate or GlcNAc with acid or salt	Differential binding to intrahepatic blood vessels
Wistera floribunda	Yes	Non-specific	GalNAc	GalNAc , acetic acid	Serotyping α-hemolytic streptococci

Sugar Abbreviations:

Fuc	L-Fucose
Gal	D-Galactose
GalNAc	*N*-Acetylgalactosamine
Glc	D-Glucose
GlcNAc	*N*-Acetylglucosamine
Man	Mannose
MeαGlc	α-Methylglucoside
MeαMan	α-Methylmannoside
Neu5Ac	*N*-Acetylneuraminic acid (sialic acid)
SA	Sialic Acid

Lectins

Aleuria Aurantia Lectin (AAL)

This lectin is a dimer of two identical subunits of about 36 kDa each. Unlike *Ulex europaeus* and *Lotus tetragonolobus* lectins which prefer (α -1,2) linked fucose residues, *Aleuria aurantia* lectin binds preferentially to fucose linked (α -1,6) to *N*-acetylglucosamine or to fucose linked (α -1,3) to *N*-acetyllactosamine related structures. AAL also reversibly binds fucose attached to nucleic acids (see page J7).

Inhibiting/Eluting Sugar: 100 mM L-fucose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1390	2 mg
Biotin	B-1395	1 mg
Alkaline Phosphatase	MB-4100	150 µg
Agarose (2 mg lectin/ml gel)	AL-1393	2 ml
VECTREX® AAL	MB-1396	1 ml
VECTREX® AAL Binding and Elution Kit	MB-1397	1 kit

Amaranthus Caudatus Lectin (ACL, ACA)

This lectin is a 66 kDa to 70 kDa dimer composed of two subunits of about 35 kDa. Four bands are resolved by isoelectric focusing. Unlike most lectins, ACL binds best in mildly acidic conditions. Although this lectin preferentially binds to oligosaccharides containing the galactosyl (β-1,3) *N*-acetylgalactosamine structure (T-antigen), tissue staining patterns are markedly different than those obtained with either peanut agglutinin or Jacalin. *Amaranthus caudatus* lectin also appears to tolerate sialic acid substitution at the 3 position of galactose in the "T" antigen.

Inhibitor: Desialyzed fetuin or bovine submaxillary mucin

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1250	5 mg
Fluorescein	FL-1251	2 mg
Biotin	B-1255	2 mg

Bauhinia Purpurea Lectin (BPL)

BPL binding appears to be highest for glycoconjugates containing galactosyl (β-1,3) *N*-acetylgalactosamine structures but oligosaccharides with a terminal α-linked *N*-acetylgalactosamine can also bind. Although binding specificity is similar to that of peanut agglutinin, tissue staining patterns of these two lectins are distinct.

Inhibiting/Eluting Sugar: 100 mM lactose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1280	5 mg
Fluorescein	FL-1281	2 mg
Biotin	B-1285	2 mg

Concanavalin A (Con A)

Con A recognizes α-linked mannose present as part of a "core oligosaccharide" in many serum and membrane glycoproteins. At neutral and alkaline pH, Con A exists as a tetramer of four identical subunits; below pH 5.6, Con A dissociates into active dimers of 52 kDa. Acetylation, succinylation, or other derivatizations can also produce stable forms with dimeric structures. (See succinylated Con A). "Nicks" in the sequence are often present in the purest preparations due to hydrolytic damage within the seeds.

Con A requires calcium or manganese ions at each of its four saccharide binding sites. Although these divalent metal ions are bound tightly to the polypeptide structure, buffers which can bind calcium (such as phosphate) generally should be avoided in diluting Con A, since a gradual loss in activity may occur.

Inhibiting/Eluting Sugar: mixture of 200 mM α-methylmannoside/200 mM α-methylglucoside

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1000	500 mg
Fluorescein	FL-1001	25 mg
Rhodamine	RL-1002	25 mg
Agarose (6 mg lectin/ml gel)	AL-1003	10 ml
		100 ml
Biotin	B-1005	5 mg

Succinylated Concanavalin A

Succinylated Con A is made from our highly purified Con A and has many unusual properties such as failure to induce patch and cap formation, and, at high concentrations, does not show a diminished mitogenic effect. In addition, succinylated Con A affects cyclic nucleotide levels in lymphocytes differently than does native Con A.

Inhibiting/Eluting Sugar: 200 mM α-methylmannoside

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1000S	25 mg
Fluorescein	FL-1001S	10 mg
Agarose (6 mg lectin/ml gel)	AL-1003S	2 ml
Biotin	B-1005S	5 mg

Datura Stramonium Lectin (DSL)

DSL contains two chains of 40 kDa and 46 kDa joined by disulfide bonds. This lectin is free of a reported 32 kDa contaminant protein. The carbohydrate binding site recognizes (β-1,4) linked *N*-acetylglucosamine oligomers, preferring chitobiose or chitotriose over a single *N*-acetylglucosamine residue. This lectin binds well in the acidic pH range but its affinity decreases above pH 8.0.

DSL also binds well to *N*-acetyllactosamine and oligomers containing repeating *N*-acetyllactosamine sequences. A branched pentasaccharide including two *N*-acetyllactosamine disaccharides linked to mannose (β-1,6) and (β-1,2) was reported to be the most potent inhibitor of agglutination.

Inhibiting/Eluting Sugar: Chitin Hydrolysate

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1180	5 mg
Fluorescein	FL-1181	2 mg
Agarose (3 mg lectin/ml gel)	AL-1183	2 ml
Biotin	B-1185	2 mg

Dolichos Biflorus Agglutinin (DBA)

Dolichos biflorus agglutinin has a molecular weight of 111 kDa and consists of 4 subunits of approximately equal size. It has been used to establish secretor status in blood group A individuals by hemagglutination inhibition techniques and for blood typing.

Inhibiting/Eluting Sugar: 200 mM N-acetylgalactosamine

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1030	5 mg
		10 mg
Fluorescein	FL-1031	2 mg
		5 mg
Rhodamine	RL-1032	2 mg
Agarose (3 mg lectin/ml gel)	AL-1033	2 ml
Biotin	B-1035	5 mg

Erythrina Cristagalli Lectin (ECL, ECA)

Erythrina cristagalli lectin consists of two different subunits of approximately 28 kDa and 26 kDa. The carbohydrate structure to which ECL binds is frequently found in membrane and serum glycoproteins of mammalian origin. Sialic acid substitution on this structure appears to prevent the lectin from binding. This specificity offers an opportunity to utilize agarose bound ECL to isolate or fractionate mammalian glycoproteins.

This lectin has been reported to be useful for the isolation of human natural killer (NK) cells using a negative selection panning technique (protocol available upon request or on our website). Human NK cells appear to lack accessible surface carbohydrate structures required for binding ECL and, unlike other mononuclear cells, do not adhere to ECL-coated culture dishes. Since this procedure involves a negative selection panning technique, a high recovery of viable NK cells can be obtained. The adherent cells can also be recovered by incubation in galactose or lactose.

Inhibiting/Eluting Sugar: 200 mM lactose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1140	10 mg
Fluorescein	FL-1141	5 mg
Agarose (3 mg lectin/ml gel)	AL-1143	2 ml
Biotin	B-1145	5 mg

Euonymus Europaeus Lectin (EEL)

EEL consists of six closely related lectins with isoelectric points between pH 4.3 and pH 4.7. Most of the 35 kDa subunits appear to consist of two disulfide linked chains of about 17 kDa. This lectin has a carbohydrate binding specificity toward type 1 or type 2 chain blood group B structures but will bind other oligosaccharides containing galactosyl (α-1,3) galactose. Unlike *Ulex europaeus* and *Lotus tetragonolobus* lectins, EEL has a high affinity toward type 1 chain blood group H structures. This lectin has been reported to bind to endothelial cells from human and non-human sources and recognizes carbohydrate structures on the surface of stimulated murine peritoneal lymphoid cells.

Inhibiting/Eluting Sugar: 500 mM lactose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1330	5 mg
Fluorescein	FL-1331	2 mg
Biotin	B-1335	2 mg

Lectins (continued)

Galanthus Nivalis Lectin (GNL)

Galanthus nivalis lectin, unlike most mannose-specific lectins, is not a metalloprotein and does not require Ca^{++} or Mn^{++} for binding.

Binding seems to be preferentially directed toward structures containing (α-1,3) mannose residues. Also in contrast to most mannose-binding lectins, GNL will not bind α-linked glucose. Reports indicate that this lectin binds rat and mouse IgM but not IgG. The only protein from human serum reported to bind to this lectin is α_2-macroglobulin. GNL binds to many viral glycoproteins.

Inhibiting/Eluting Sugar: 100 mM - 200 mM α-methylmannoside

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1240	5 mg
Fluorescein	FL-1241	2 mg
Agarose (3 mg lectin/ml gel)	AL-1243	2 ml
		5 ml
Biotin	B-1245	2 mg

Griffonia (Bandeiraea) Simplicifolia Lectin I (GSL I, BSL I)

GSL I is a family of glycoproteins with molecular weights of approximately 114 kDa. There are two types of subunits, termed "A" and "B", with slightly different molecular weights. These subunits combine to form tetrameric structures, resulting in five isolectins. The "A"-rich lectin preferentially agglutinates blood group A erythrocytes and thus appears to be specific for α-*N*-acetylgalactosamine residues, while the "B"-rich lectin preferentially agglutinates blood group B cells and is specific for α-galactose residues. Our GSL I is a mixture of the five isolectins. GSL I has been reported to bind several glycoproteins including laminin.

Inhibiting/Eluting Sugar: mixture of 200 mM galactose/200 mM N-acetylgalactosamine

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1100	5 mg
Fluorescein	FL-1101	2 mg
		5 mg
Rhodamine	RL-1102	2 mg
Agarose (4 mg lectin/ml gel)	AL-1103	2 ml
Biotin	B-1105	2 mg

GSL I – isolectin B_4

GSL I-B_4 isolectin contains only the B subunits. It is a useful marker for endothelial cells from nonprimates such as mouse, rat, rabbit, and goat as well as a marker for non-peptidergic unmyelinated primary afferent neurons.

Inhibiting Sugar: 500 mM galactose or 100 mM raffinose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1104	1 mg
Fluorescein	FL-1201	0.5 mg
Biotin	B-1205	0.5 mg
DyLight® 594	DL-1207	0.5 mg

Griffonia (Bandeiraea) Simplicifolia Lectin II (GSL II, BSL II)

This lectin is a dimeric glycoprotein composed of two subunits of nearly identical size with each subunit having disulfide-linked chains and a binding site for α- or β-linked *N*-acetylglucosamine residues. Unlike other *N*-acetylglucosamine specific lectins, increasing the number of *N*-acetylglucosamine residues beyond two does not improve affinity. GSL II has been reported to be unique in its ability to recognize exclusively α- or β-linked *N*-acetylglucosamine residues on the nonreducing terminal of oligosaccharides.

Inhibiting/Eluting Sugar: Chitin Hydrolysate or 200 mM N-acetylglucosamine

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1210	5 mg
Fluorescein	FL-1211	2 mg
Agarose (3 mg lectin/ml gel)	AL-1213	2 ml
Biotin	B-1215	2 mg

Hippeastrum Hybrid (Amaryllis) Lectin (HHL, AL)

This lectin is composed of four subunits of identical size and is immunologically related to GNL and NPL. Amaryllis lectin binds only α-mannose residues, unlike Con A, LCA and PSA that also bind α-glucosyl structures. Like NPL there appears to be an extended binding site for polymannose structures, not requiring mannose to be at the non-reducing terminus. HHL binds both (α-1,3) and (α-1,6) linked mannose structures, as well as some yeast galactomannans.

Inhibiting Sugar: 100 mM α-methylmannoside

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1380	5 mg
Biotin	B-1385	2 mg

Jacalin

Jacalin is a lectin composed of four subunits of approximately 16 kDa each. This lectin appears to bind only *O*-glycosidically linked oligosaccharides, preferring the structure galactosyl (β-1,3) *N*-acetylgalactosamine. This structure (the T-antigen) is the oligosaccharide to which peanut agglutinin (PNA) binds. However, unlike PNA, Jacalin will bind a mono- or disialylated form of this structure. This lectin has been used to purify human IgA. The specificity of this lectin also affords the opportunity to localize or isolate glycoproteins with *O*-glycosidically linked oligosaccharide side chains.

Inhibiting/Eluting Sugar: 800 mM galactose or 100 mM melibiose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1150	10 mg
		25 mg
Fluorescein	FL-1151	5 mg
Agarose (4 mg lectin/ml gel)	AL-1153	2 ml
		5 ml
		10 ml
Biotin	B-1155	5 mg

Lens Culinaris Agglutinin (LCA, LcH)

Lens culinaris agglutinin is composed of four subunits - two of about 17 kDa and two of 8 kDa. LCA recognizes sequences containing α-linked mannose residues but recognizes additional sugars as part of the receptor structure, giving it a narrower specificity than Con A. An α-linked fucose residue attached to the *N*-acetylchitobiose portion of the core oligosaccharide markedly enhances affinity. By exploiting this narrower specificity, glycoproteins and glycopeptides can be subfractionated with LCA after initial isolation with Con A.

LCA has been found to be one of the most effective agents in preventing skin allograft rejection in model systems. LCA has been used to purify numerous glycoproteins, including immunoglobulins, histocompatibility antigens, and α_2-macroglobulin.

Inhibiting/Eluting Sugar: mixture of 200 mM α-methylmannoside/200 mM α-methylglucoside

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1040	10 mg
		25 mg
Fluorescein	FL-1041	5 mg
Rhodamine	RL-1042	5 mg
Agarose (3 mg lectin/ml gel)	AL-1043	2 ml
		5 ml
		10 ml
Biotin	B-1045	5 mg

Lotus Tetragonolobus Lectin (LTL)

Lotus tetragonolobus lectin is a family of closely related glycoproteins that appear to have similar specificities toward α-linked L-fucose containing oligosaccharides. Although many of the binding properties of *Lotus* lectin are similar to those of *Ulex europaeus* lectin I, the binding affinities and some specificities for oligosaccharides are markedly different between these fucose-specific lectins.

Inhibiting/Eluting Sugar: 50 mM - 100 mM L-fucose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1320	5 mg
Fluorescein	FL-1321	2 mg
Agarose (3 mg lectin/ml gel)	AL-1323	2 ml
Biotin	B-1325	2 mg

Lycopersicon Esculentum (Tomato) Lectin (LEL, TL)

Tomato lectin is a very stable single subunit glycoprotein containing about 50 percent arabinose and galactose and may form multimeric aggregates in solution. Tomato lectin, although sharing some specificities with potato lectin, *Datura* lectin, and wheat germ agglutinin, has been reported to be dissimilar in many respects. LEL binds well to glycophorin and Tamm-Horsfall glycoprotein and has been used effectively to label vascular endothelium in rodents.

Inhibiting/Eluting Sugar: Chitin Hydrolysate

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1170	2 mg
Fluorescein	FL-1171	1 mg
Agarose (2 mg lectin/ml gel)	AL-1173	2 ml
Biotin	B-1175	1 mg
Texas Red®	TL-1176	1 mg
DyLight® 594	DL-1177	1 mg

Maackia Amurensis Lectin I (MAL I, MAL)

This lectin is the leucoagglutinin or mitogenic isolectin from *Maackia* seeds. *Maackia amurensis* lectin I binds gal (β-1,4) glcNAc but tolerates substitution of *N*-acetyllactosamine with sialic acid at the 3 position of galactose. However, MAL I does not appear to bind this structure when substitution with sialic acid is on the 6 position of galactose.

Inhibiting Sugar: 200 mM lactose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1310	5 mg
Fluorescein	FL-1311	2 mg
Biotin	B-1315	2 mg

Lectins (continued)

Maackia Amurensis Lectin II (MAL II, MAH)

Maackia amurensis lectin II is a glycoprotein consisting of two subunits each of which is composed of disulfide-linked chains.

Although the specificity of this lectin is not well defined, MAL II appears to bind only particular carbohydrate structures that contain sialic acid. Unlike *Sambucus nigra* lectin (SNA) which seems to prefer structures with (α-2,6) linked sialic acid, MAL II appears to bind sialic acid in an (α-2,3) linkage. Tissue staining patterns are also very different among MAL I, SNA and MAL II.

Inhibitor: Human glycophorin

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1260	2 mg
Biotin	B-1265	1 mg

Maclura Pomifera Lectin (MPL)

There appear to be two different chains in combinations giving rise to five isolectins. The carbohydrate specificity of either type of subunit appears to be the same, preferring α-linked *N*-acetylgalactosamine structures.

Inhibiting Sugar: 500 mM galactose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1340	5 mg
Fluorescein	FL-1341	2 mg
Biotin	B-1345	2 mg

Narcissus Pseudonarcissus (Daffodil) Lectin (NPL, NPA, DL)

This lectin exists as a tetramer at neutral pH but below pH 5.0 and above pH 9.0, NPL dissociates into monomers. NPL has a specificity toward α-linked mannose, preferring polymannose structures containing (α-1,6) linkages. Binding to mannose polymers can occur via internal mannose residues and is not dependent on structural integrity of a non-reducing end sugar. NPL also binds some galactomannans, and differs in other binding characteristics from a related lectin, *Galanthus nivalis* lectin. Unlike Con A, LCA, or PSA, NPL does not bind glucose.

Inhibiting Sugar: 400 mM α-methylmannoside

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1370	5 mg
Biotin	B-1375	2 mg

Peanut Agglutinin (PNA)

PNA binds preferentially to the T-antigen, a galactosyl (β-1,3) *N*-acetylgalactosamine structure present in many glycoconjugates such as M and N blood groups, gangliosides, and many other soluble and membrane-associated glycoproteins and glycolipids. With certain exceptions, the receptor sequence for PNA is normally sialylated which prevents the lectin from binding to its receptor oligosaccharide (see Jacalin). Even sialic acid which is not bound directly to the receptor sugars may inhibit binding. The presence of calcium ions in diluents can enhance the binding of PNA to receptors, possibly by neutralizing the negative charges on sialic acid residues adjacent to the receptor sequence.

PNA is useful in distinguishing between normal and tumor tissues and in assessing malignancy in transitional mucosa. In addition, PNA binding can be used to measure cellular maturity in lymphoid tissues, to distinguish a variety of lymphocyte subpopulations in man and experimental animals, and to measure the levels of lymphoid cell populations in many diseases. PNA can be employed in the fractionation of stem cells in mice for use in bone marrow transplantation across histocompatibility barriers.

A major cell surface receptor for PNA may be asialo GM_1 ganglioside. Since PNA shares specificity with the antibody to this glycolipid, PNA and the antibody can be used interchangeably in some applications.

Inhibiting/Eluting Sugar: 200 mM galactose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1070	5 mg
		10 mg
		25 mg
Fluorescein	FL-1071	5 mg
		10 mg
Rhodamine	RL-1072	5 mg
Agarose (5 mg lectin/ml gel)	AL-1073	2 ml
		5 ml
Biotin	B-1075	5 mg

Phaseolus Vulgaris Agglutinin (PHA)

Phaseolus vulgaris agglutinin is the name ascribed to a family of lectins, each of which consists of four subunits. There are two different types of subunits. One appears to be involved primarily in red cell agglutination and has been designated the "E" subunit (for erythroagglutinin). The other type is involved in lymphocyte agglutination and mitogenic activity and has been termed the "L" subunit (for leucoagglutinin). These subunits combine to produce five isolectins.

One of these isolectins has four "E" subunits and is designated PHA-E. PHA-E possesses strong hemagglutinating activity but is a poor mitogen. PHA-L, with four "L" type subunits, does not agglutinate red cells but is a potent mitogen. The other three isolectins, designated E_3L_1, E_2L_2, and E_1L_3, have erythroagglutinating and mitogenic activities proportional to the number of respective "E" or "L" subunits. We have termed the mixture of the five isolectins PHA (E+L).

PHA-L has been found to be an excellent, specific marker for use in anterograde neuronal tracing. (See page D4.)

Phaseolus vulgaris Erythroagglutinin (PHA-E)

Elution: 100 mM acetic acid

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1120	5 mg
Fluorescein	FL-1121	2 mg
Agarose (3 mg lectin/ml gel)	AL-1123	2 ml
Biotin	B-1125	2 mg

Phaseolus vulgaris Agglutinin (PHA-E+L)

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1220	25 mg

Phaseolus vulgaris Leucoagglutinin (PHA-L)

Elution: 100 mM acetic acid

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1110	5 mg
Fluorescein	FL-1111	2 mg
Rhodamine	RL-1112	2 mg
Agarose (3 mg lectin/ml gel)	AL-1113	2 ml
Biotin	B-1115	2 mg

Pisum Sativum Agglutinin (PSA)

Pisum sativum agglutinin is nearly identical in structure and carbohydrate specificity to *Lens culinaris* agglutinin. The lectin has specificity toward α-linked mannose-containing oligosaccharides, with an *N*-acetylchitobiose-linked α-fucose residue included in the receptor sequence. Calcium and manganese ions are required for activity. PSA has been used to fractionate cells, to isolate glycoproteins and glycopeptides, to distinguish between normal and virally transformed cells, as a T-cell mitogen, and as an inhibitor of allograft rejection.

Inhibiting/Eluting Sugar: mixture of 200 mM α-methylmannoside/200 mM α-methylglucoside

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1050	10 mg
Fluorescein	FL-1051	5 mg
Rhodamine	RL-1052	5 mg
Agarose (3 mg lectin/ml gel)	AL-1053	2 ml
Biotin	B-1055	5 mg

Psophocarpus Tetragonolobus Lectin I (PTL I, WBA I)

PTL I binds with carbohydrate structures containing α-linked *N*-acetylgalactosamine. Lactose and other β-linked galactosides are poor inhibitors of binding. Tissue staining patterns using this lectin are clearly defined. Stratified squamous epithelium, endothelial cells, and underlying basement membranes of blood vessels are intensely stained with PTL I. Pancreatic zymogen granules are also strongly labeled with the lectin.

Inhibiting Sugar: 100 mM N-acetylgalactosamine

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1360	5 mg
Biotin	B-1365	2 mg

Psophocarpus Tetragonolobus Lectin II (PTL II, WBA II)

Like PTL I, this lectin binds preferentially to *N*-acetylgalactosamine, with this sugar also being the most inhibitory monosaccharide. However, in contrast to PTL I, this lectin prefers the β anomeric configuration. PTL II shows a high affinity toward blood group H structures and the T-antigen. PTL I and PTL II also differ in their erythrocyte agglutinating properties.

Inhibiting Sugar: 100 mM N-acetylgalactosamine

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1400	5 mg
Biotin	B-1405	2 mg

Lectins (continued)

Ricinus Communis Agglutinin I (RCA I, RCA_{120})

This lectin consists of two subunits of 60 kDa which can be dissociated by reducing agents into closely related chains between 27 kDa and 33 kDa. One of the chains appears to be common to the "B" chain of another castor bean lectin, ricin, while the other chain is unique to RCA I.

Inhibiting/Eluting Sugar: 200 mM galactose or lactose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1080	10 mg
Fluorescein	FL-1081	5 mg
Rhodamine	RL-1082	5 mg
Agarose (4 mg lectin/ml gel)	AL-1083	2 ml
		5 ml
		10 ml
Biotin	B-1085	5 mg

Ricinus Communis Agglutinin II (RCA II, RCA_{60} , ricin)

Ricinus communis agglutinin II is an extremely toxic glycoprotein consisting of two disulfide-linked chains of about 28 kDa and 32 kDa, termed A and B chain, respectively. The B chain can bind to cell surfaces via galactose or *N*-acetylgalactosamine residues of membrane glycoconjugates and facilitates the transport of the lectin into the cell. The A chain has an enzyme activity which can catalytically block protein synthesis and is so toxic that only a single molecule of A chain is required to kill a cell. Alone, the A chain is incapable of entering the cell and is thus not toxic.

RCA II appears to be a family of related lectins with different toxicities. Our RCA II is the most toxic lectin of this family, probably ricin D, having an I.P. $L.D_{50}$ in 20 g mice of less than 50 ng.

Inhibiting/Eluting Sugar: 200 mM galactose or lactose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1090	10 mg
Fluorescein	FL-1091	5 mg
Agarose (4 mg lectin/ml gel)	AL-1093	2 ml
Biotin	B-1095	5 mg

Please note: This product is highly toxic. *Due to its toxicity special packaging is required. This product is sold in the USA only.*

Ricin A Chain

Ricin A chain has been isolated from extremely toxic RCA II. Our ricin A chain has less than 0.01% of the toxicity of the native lectin in a cell culture test system, yet is as potent as native ricin in a cell-free protein synthesis assay. The A chain can be linked to other proteins such as antibodies. These antibody-toxin hybrid molecules can be used to kill specific cells with the appropriate antigenic determinant exposed, such as tumor markers on surfaces of malignant cells. The A chain is supplied in buffered saline with sodium azide as a preservative.

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1190	1 mg

Please note: *Ricin A chain is shipped on wet ice and is sold in the USA only.*

Ricin B Chain

Ricin B chain has been isolated from highly purified RCA II. B chain is the component of ricin which binds surface carbohydrate receptors and facilitates the transport of ricin into the cell. Although our ricin B chain has a substantially lower toxicity than native ricin in both cell culture test systems and cell-free protein synthesis assays, essentially all of the galactose/*N*-acetylgalactosamine binding activity is retained.

Inhibiting Sugar: 200 mM galactose or lactose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1290	1 mg

Sambucus Nigra Lectin (SNA, EBL)

Sambucus nigra lectin binds preferentially to sialic acid attached to terminal galactose in α-2,6 and to a lesser degree, α-2,3 linkage. Binding is also inhibited to some extent by lactose or galactose. This lectin does not appear to bind sialic acid linked to *N*-acetylgalactosamine. SNA has been reported to inhibit cell-free protein synthesis.

Elution: 500 mM lactose in buffered saline followed by 500 mM lactose in acetic acid

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1300	5 mg
Fluorescein	FL-1301	2 mg
Agarose (3 mg lectin/ml gel)	AL-1303	2 ml
Biotin	B-1305	2 mg

Solanum Tuberosum (Potato) Lectin (STL, PL)

Solanum tuberosum lectin consists of two identical 50 kDa subunits. The subunits can dissociate in solution to produce a monomeric form of the lectin which does not agglutinate cells. This lectin binds oligomers of *N*-acetylglucosamine and some bacterial cell wall oligosaccharides containing *N*-acetylglucosamine and *N*-acetylmuramic acid. Although the carbohydrate binding specificity is similar to wheat germ agglutinin and *Datura stramonium* lectin, several differences have been reported for potato lectin.

Inhibiting/Eluting Sugar: Chitin Hydrolysate

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1160	5 mg
Fluorescein	FL-1161	2 mg
Biotin	B-1165	2 mg

Sophora Japonica Agglutinin (SJA)

Sophora japonica agglutinin consists of two subunits, each of which is composed of two chains dissociable by disulfide-reducing agents.

SJA has a specificity toward carbohydrate structures terminating in *N*-acetylgalactosamine and galactose residues, with preferential binding to β anomers. Binding activity of SJA seems to be enhanced at alkaline pH values.

Inhibiting/Eluting Sugar: 200 mM N-acetylgalactosamine

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1130	5 mg
Biotin	B-1135	2 mg

Soybean Agglutinin (SBA)

Composed of four subunits of approximately equal size, soybean agglutinin is a family of closely related isolectins. This glycoprotein has a molecular weight of about 120 kDa and an isoelectric point near pH 6.0. SBA preferentially binds to oligosaccharide structures with terminal α- or β-linked *N*-acetylgalactosamine, and to a lesser extent, galactose residues. Binding can be blocked by substitutions on penultimate sugars, such as fucose attached to the penultimate galactose in blood group B substance.

An important application for SBA is the separation of pluripotent stem cells from human bone marrow. Cells fractionated by SBA do not produce graft *vs* host disease and can be used in bone marrow transplantation across histocompatibility barriers.

Inhibiting/Eluting Sugar: 200 mM N-acetylgalactosamine

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1010	10 mg
		25 mg
Fluorescein	FL-1011	2 mg
Rhodamine	RL-1012	2 mg
Agarose (4 mg lectin/ml gel)	AL-1013	2 ml
Biotin	B-1015	5 mg

Ulex Europaeus Agglutinin I (UEA I)

Ulex europaeus agglutinin I binds to many glycoproteins and glycolipids containing α-linked fucose residues, such as ABO blood group glycoconjugates. This lectin preferentially binds blood group O cells and has been used to determine secretor status. It has been established as an excellent marker for human endothelial cells.

Inhibiting/Eluting Sugar: 50 mM - 100 mM L-fucose

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1060	2 mg
		5 mg
Fluorescein	FL-1061	2 mg
		5 mg
Rhodamine	RL-1062	2 mg
Agarose (2 mg lectin/ml gel)	AL-1063	2 ml
Biotin	B-1065	2 mg

Lectins (continued)

Vicia Villosa Lectin (VVL, VVA)

This lectin is a family of tetrameric glycoproteins consisting of combinations of A and B subunits similar in structure to PHA and GSL I. The dominant isolectins in our preparations appear to be B subunit-rich. VVL recognizes preferentially α- or β-linked terminal *N*-acetylgalactosamine, especially a single α-*N*-acetylgalactosamine residue linked to serine or threonine in a polypeptide (the Tn antigen). Evidence suggests that this lectin also may require specific amino acid sequences at the receptor site of glycosylation. The disaccharide galactosyl (α-1,3) *N*-acetylgalactosamine is also a potent inhibitor of this lectin.

Inhibiting/Eluting Sugar: 200 mM N-acetylgalactosamine

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1230	5 mg
Fluorescein	FL-1231	2 mg
Agarose (3 mg lectin/ml gel)	AL-1233	2 ml
Biotin	B-1235	2 mg

Wheat Germ Agglutinin (WGA)

WGA contains a group of closely related isolectins, with an isoelectric point about pH 9. The receptor sugar for WGA is *N*-acetylglucosamine, with preferential binding to dimers and trimers of this sugar. WGA can bind oligosaccharides containing terminal *N*-acetylglucosamine or chitobiose, structures which are common to many serum and membrane glycoproteins. Bacterial cell wall peptidoglycans, chitin, cartilage glycosaminoglycans, and glycolipids can also bind WGA. Native WGA has also been reported to interact with some glycoproteins via sialic acid residues (see succinylated WGA). This lectin is used for the purification of insulin receptors and for neuronal tracing. See also page D3.

Inhibiting/Eluting Sugar: Chitin Hydrolysate or 500 mM N-acetylglucosamine with salt and/or acid elution generally required

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1020	10 mg
		25 mg
Fluorescein	FL-1021	5 mg
		10 mg
Rhodamine	RL-1022	5 mg
		10 mg
Agarose (7 mg lectin/ml gel)	AL-1023	2 ml
		5 ml
		10 ml
Biotin	B-1025	5 mg
Peroxidase	PL-1026	2 mg

Succinylated Wheat Germ Agglutinin

This derivative has been reported to have properties distinct from the native lectin. Evidence suggests that Succinylated Wheat Germ agglutinin does not bind to sialic acid residues, unlike the native form, but retains its specificity toward *N*-acetylglucosamine. Using conjugates of the native lectin and the succinylated form can provide a system to distinguish between sialylated glycoconjugates and those containing only *N*-acetylglucosamine structures.

Inhibiting/Eluting Sugar: Chitin Hydrolysate or 500 mM N-acetylglucosamine with salt and/or acid elution generally required

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1020S	10 mg
Fluorescein	FL-1021S	5 mg
Rhodamine	RL-1022S	5 mg
Agarose (3 mg lectin/ml gel)	AL-1023S	2 ml
		5 ml
Biotin	B-1025S	5 mg

Wisteria Floribunda Lectin (WFA, WFL)

The binding specificity of WFL is not completely clear but this lectin appears to preferentially bind carbohydrate structures terminating in *N*-acetylgalactosamine linked α or β to the 3 or 6 position of galactose. This lectin has been used to fractionate lymphocyte populations, and although not mitogenic, elicits the production of lymphokines from murine splenocytes.

Inhibiting/Eluting Sugar: 200 mM N-acetylgalactosamine

Conjugate	Catalog Number	Unit Size
Unconjugated	L-1350	5 mg
Fluorescein	FL-1351	2 mg
Agarose (3 mg lectin/ml gel)	AL-1353	2 ml
Biotin	B-1355	2 mg

Lectin Screening Kits

Our lectin screening kits are designed to provide the investigator with a panel of lectins or lectin conjugates. The lectins have been selected to offer a variety of sugar specificities and are of the same high quality as the reagents offered individually. Primary sugar specificities for each kit are shown in the table.

Kit I (LK-2000, BK-1000, FLK-2100, RLK-2200) consists of 1 mg of the following lectins or lectin conjugates: Con A, DBA, PNA, RCA I, SBA, UEA I, WGA.

Kit II (LK-3000, BK-2000, FLK-3100, RLK-3200) consists of 1 mg of the following lectins or lectin conjugates: GSL I, LCA, PHA-E, PHA-L, PSA, Succinylated WGA, SJA*.

**SJA is not included in FLK-3100*

Kit III (BK-3000, FLK-4100) consists of 0.5 mg of the following lectin conjugates: DSL, ECL, GSL II, Jacalin, LEL, STL, VVL.

Lectin Screening Kits I

Product	Catalog Number	Unit Size
Unconjugated Lectin Kit I	LK-2000	1 kit
Biotinylated Lectin Kit I	BK-1000	1 kit
Fluorescein Lectin Kit I	FLK-2100	1 kit
Rhodamine Lectin Kit I	RLK-2200	1 kit

Lectin Screening Kits II

Product	Catalog Number	Unit Size
Lectin Kit II	LK-3000	1 kit
Biotinylated Lectin Kit II	BK-2000	1 kit
Fluorescein Lectin Kit II	FLK-3100	1 kit
Rhodamine Lectin Kit II	RLK-3200	1 kit

Lectin Screening Kits III

Product	Catalog Number	Unit Size
Biotinylated Lectin Kit III	BK-3000	1 kit
Fluorescein Lectin Kit III	FLK-4100	1 kit

Lectin Screening Kits

Lectin	Common Abbreviation	Primary Sugar Specificity
Lectin Kit I		
Concanavalin A	Con A	Mannose
Dolichos biflorus agglutinin	DBA	*N*-Acetylgalactosamine
Peanut Agglutinin	PNA	Galactose
Ricinus communis agglutinin I	RCA I	Galactose, *N*-Acetylgalactosamine
Soybean agglutinin	SBA	*N*-Acetylgalactosamine
Ulex Europaeus agglutinin I	UEA I	Fucose
Wheat Germ agglutinin	WGA	*N*-Acetylglucosamine
Lectin Kit II		
Griffonia simplicifolia lectin I	GSL I	Galactose
Len culinaris lectin	LCA	Mannose
Phaseolus vulgaris Erythroagglutinin	PHA-E	Complex structures
Phaseolus vulgaris Leucoagglutinin	PHA-L	Complex structures
Pisum sativum agglutinin	PSA	Mannose
Wheat Germ agglutinin, succinylated	Succinylated WGA	*N*-Acetylglucosamine
Sophora japonica agglutinin	SJA	*N*-Acetylgalactosamine
Lectin Kit III		
Datura stramonium lectin	DSL	*N*-Acetylglucosamine
Erythrina cristagalli lectin	ECL	Galactose
Griffonia simplicifolia lectin II	GSL II	*N*-Acetylglucosamine
Jacalin	Jacalin	Galactose
Lycopersicon esculentum lectin	LEL	*N*-Acetylglucosamine
Solanum tuberosum lectin	STL	*N*-Acetylglucosamine
Vicia villosa lectin	VVL	*N*-Acetylgalactosamine

Inhibiting Sugars

Chitin Hydrolysate

Chitin Hydrolysate (SP-0090) is supplied as a highly concentrated solution of *N*-acetylglucosamine (glcNAc) and glcNAc oligomers in nearly saturated sodium chloride.

Chitin Hydrolysate can be used as an inhibitor of lectin-conjugate binding or for eluting glycoproteins bound to some agarose lectins. This mixture is an inhibitor for the following lectins: DSL, GSL II, LEL, STL, and WGA.

Sugars

A variety of sugars are provided for use as inhibitors of lectin-conjugate binding or for eluting glycoproteins or other glycoconjugates from columns of agarose lectins. These sugars are supplied as dry powders. If reconstituted in 5 ml of water or buffer the resulting sugar solutions are 400 mM, except for L-fucose and N-acetylgalactosamine, which are each 100 mM.

Product	Catalog Number	Unit Size	Stock Concentration*	Weight of Sugar
Chitin Hydrolysate	SP-0090	10 ml	N.A.	N.A.
Sugars				
N-acetylgalactosamine	S-9001	5 ml	100 mM	111 mg
N-acetylglucosamine	S-9002	5 ml	400 mM	442 mg
galactose	S-9003	5 ml	400 mM	360 mg
lactose	S-9004	5 ml	400 mM	721 mg
α-methylmannoside	S-9005	5 ml	400 mM	388 mg
α-methylglucoside	S-9006	5 ml	400 mM	388 mg
L-fucose	S-9007	5 ml	100 mM	82 mg
N-acetylneuraminic acid (sialic acid)	S-9008	5 ml	400 mM	619 mg

** Stock concentration if reconstituted in 5 ml*

Antibodies to Lectins

These antibodies to lectins are produced by immunizing goats with pure lectins. Following conventional purification steps, specific antibody is isolated by affinity chromatography on lectin-agarose columns. Each anti-lectin is supplied lyophilized in buffered saline.

These highly purified specific antibodies provide excellent intermediate reagents for localizing lectin receptors in tissues and on cells or following lectin transport in neuronal tracing.

Biotinylated anti-lectins are optimally labeled with biotin. They can be used as intermediates to localize lectin receptors in tissues or on cells, or to follow lectin migration in neuronal transport. In some applications, biotinylated anti-lectins used in conjunction with unlabeled lectins may provide greater sensitivity than using biotinylated lectins alone.

Product	Conjugate	Catalog Number	Unit Size
Anti-Concanavalin A	Unconjugated	AS-2004	1 mg
Anti-*Dolichos biflorus* agglutinin	Unconjugated	AS-2034	1 mg
Anti-*Galanthus nivalis* lectin	Unconjugated	AS-2240	1 mg
Anti-*Griffonia (Bandeiraea) simplicifolia* lectin I	Unconjugated	AS-2104	1 mg
Anti-*Lens culinaris* agglutinin/*Pisum sativum* agglutinin	Unconjugated	AS-2044	1 mg
Anti-Peanut agglutinin	Unconjugated	AS-2074	1 mg
	Biotinylated	BA-0074	0.5 mg
Anti-*Phaseolus vulgaris* agglutinin (E+L)	Unconjugated	AS-2224	1 mg
	Biotinylated	BA-0224	0.5 mg
Anti-*Phaseolus vulgaris* agglutinin (E+L)*	Unconjugated	AS-2300	1 mg
Anti-*Ricinus communis* agglutinin I & II	Unconjugated	AS-2084	1 mg
	Biotinylated	BA-0084	0.5 mg
Anti-Soybean agglutinin	Unconjugated	AS-2014	1 mg
Anti-*Ulex europaeus* agglutinin I	Unconjugated	AS-2064	1 mg
	Biotinylated	BA-0064	0.5 mg
Anti-Wheat Germ agglutinin	Unconjugated	AS-2024	1 mg
	Biotinylated	BA-0024	0.5 mg

**made in rabbit*

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