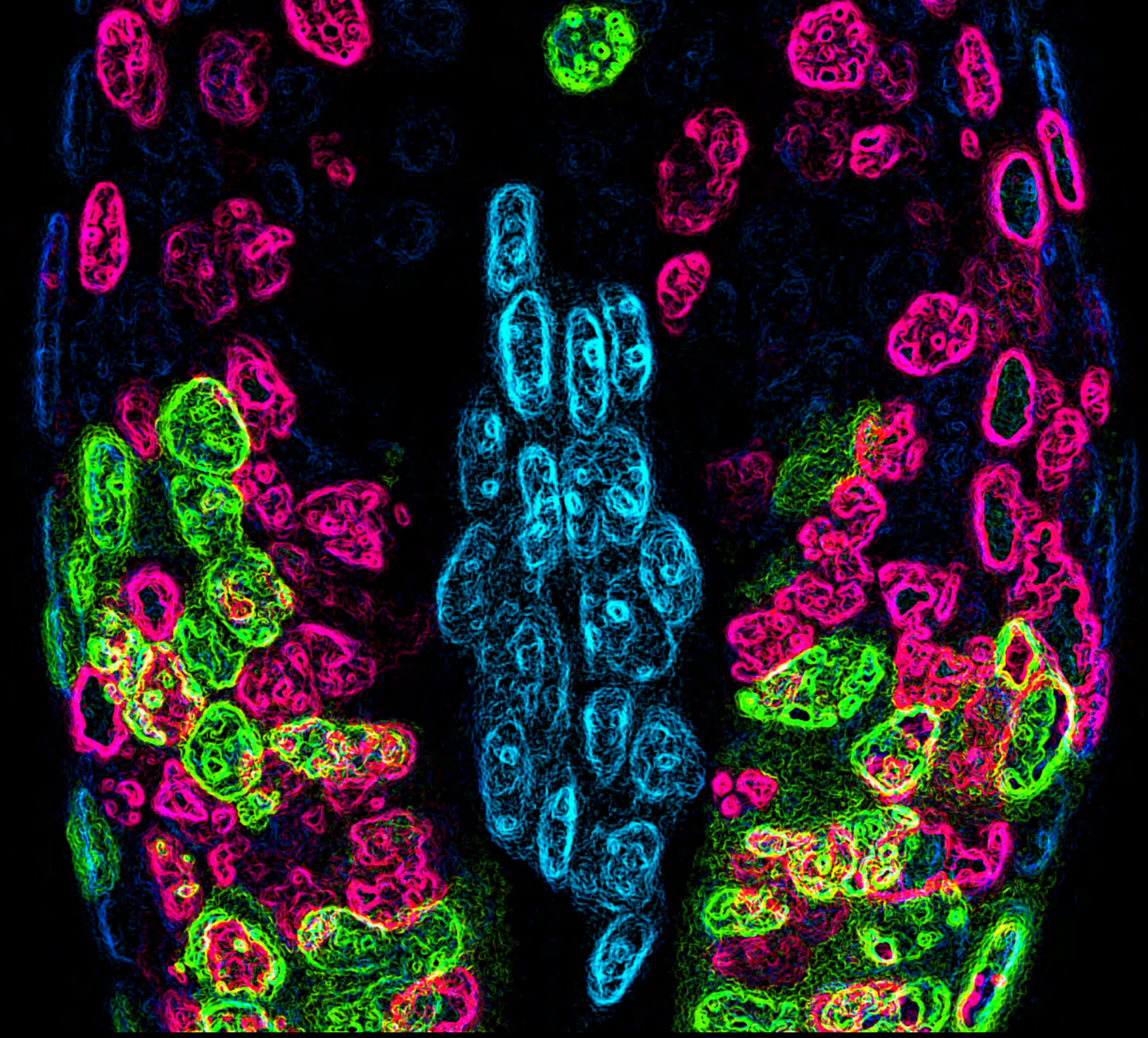


Immunofluorescence Guide

VECTOR LABORATORIES

part of Maravai LifeSciences

Making IF as easy as ABC

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

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

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

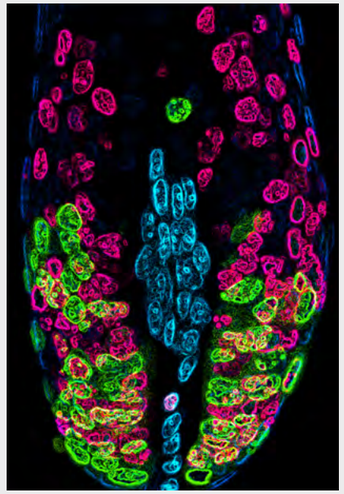
C. A knowledge base of over 100 years of combined IF experience to help you accelerate the pace of discovery.

It's as simple as that.

Dorsal root ganglia cells (neurons and satelite glia): Beta III tubulin (ms), DyLight® 488 Anti-Mouse IgG, • S100 (rb), DyLight® 594 Anti-Rabbit IgG. Mounted in VECTASHIELD® HardSet™ Antifade Mounting Medium with DAPI. Image courtesy of Dr. Emma East, Department of Life Sciences, The Open University, Milton Keynes, UK.

Table of Contents

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



Front cover: Fluorescent images with neon effect showing successive proliferation within the bulb of a hair follicle. Proliferating cells labeled for CldU (red), IdU (green) with cells dividing twice taking up both labels (yellow). Epidermal nuclei (blue) and dermal papilla nuclei (cyan) labeled with DAPI. Image provided by Nigel Hammond (Dixon Lab). Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK.

Immunofluorescence Workflow

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

Tissue Preparation	Antigen Retrieval	Quench/ Block	Primary Antibody/ Lectins*	Secondary Antibody	Tertiary Reagent	Counterstain/ Mount	Visualize
• VECTABOND™ Tissue Section Adhesive • ImmPrint™ Histology Pen • ImmEdge™ Hydrophobic Barrier Pen	• Antigen Unmasking Solutions, Citrate- or Tris-based	• Avidin/Biotin Blocking Kit • Streptavidin/Biotin Blocking Kit • Normal Sera • Animal-Free Blocker™ and Diluent • BSA • Casein Solution • Vector® M.O.M.™ Mouse Ig Blocking Reagent • Carbo-Free™ Block • BLOXALL™ Endogenous HRP/AP Blocking Solution	• Biotinylated lectins • Fluorochrome-conjugated lectins • Unconjugated lectins	• VectaFluor™ DyLight® R.T.U. Secondary Antibodies • VectaFluor™ Duet IF Double Labeling Kits • Mouse on Mouse (M.O.M.™) Immunodetection Kits • Biotinylated secondary antibodies • Fluorochrome-conjugated secondary antibodies • Unconjugated secondary antibodies	• VectaFluor™ Excel DyLight® Amplified Fluorescent Staining Systems • Fluorochrome-conjugated avidin or streptavidin • Biotinylated anti-avidin amplifying reagent • Biotinylated anti-streptavidin amplifying reagent	• VECTASHIELD® Antifade Mounting Medium • VECTASHIELD® Antifade Mounting Medium with DAPI • VECTASHIELD® Antifade Mounting Medium with Propidium Iodide (PI) • VECTASHIELD® HardSet™ Antifade Mounting Medium • VECTASHIELD® HardSet™ Antifade Mounting Medium with DAPI • VECTASHIELD® HardSet™ Antifade Mounting Medium with TRITC-Phalloidin	

* For more information visit: vectorlabs.com/lectins

Immunofluorescence Selection Guide

Follow the simple steps below to choose the most appropriate labeling and detection solution for your experiment.

1 Choose Primary Antibody

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

VECTABOND™ Reagent
(Tissue Section Adhesive)

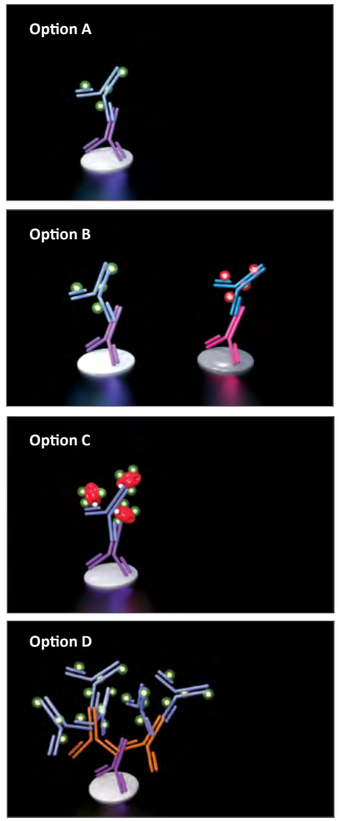
Blocking Reagents

- Choices determined by the options selected in Steps 1-2
- Streptavidin/Biotin Blocking Kit (if using biotin/streptavidin system)
- Avidin/Biotin Blocking Kit
- Normal Sera (from the species of secondary antibody)
- M.O.M.™ Mouse Ig Blocking Reagent
- R.T.U. Animal-Free Blocker™ and Diluent
- BSA
- Casein Solution

SECONDARY DETECTION SYSTEMS

2 Choose Secondary Antibody and Tertiary Detection System

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



One Step
Single-label.
Fast. Convenient.

- Fluorophore-conjugated secondary antibodies
- VectaFluor™ R.T.U. DyLight® Labeled Secondary Antibodies

or

One Step
Dual-label two-antigen detection.
Fast. Convenient.

- VectaFluor™ Duet IF Double Labeling Kits

or

Two Step
Biotin-based.

- Biotinylated secondary antibody and fluorophore conjugated avidin or streptavidin.
- See Step 3 for additional amplification

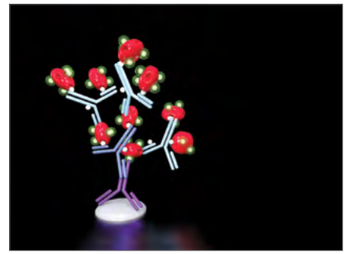
or

Two Step
Highest sensitivity. Non-biotin based.

- VectaFluor™ Excel Amplified Fluorescent Staining System (Amplifier Antibody + fluorescent tertiary antibody)

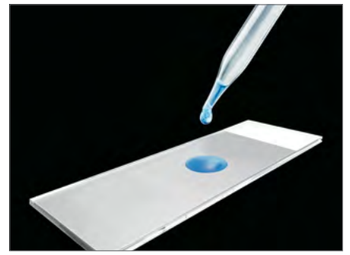
3 Choose Signal Amplification with Biotin-based Systems (Step 2, Option C)

- Multiple rounds of amplification possible (with biotin-based systems)



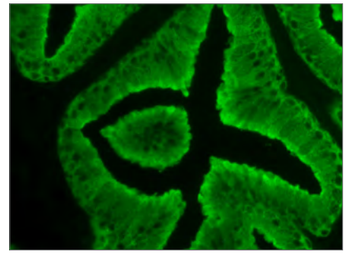
4 Choose Mounting Media with or without a Counterstain

- VECTASHIELD® Antifade Mounting Media, with or without counterstain



5 Visualize

- Fluorescence microscope
- View using appropriate excitation/emission filters



Prostate: Anti-PSA (goat), VectaFluor™ DyLight® 488 Anti-Goat IgG. Mounted in VECTASHIELD® HardSet™ Mounting Medium.

Legend


Primary antibody


Fluorophore-conjugated secondary antibody


Amplifier antibody


Biotinylated anti-avidin / streptavidin


Fluorophore


Biotin


Fluorophore-conjugated avidin / streptavidin

Pioneering in IHC/IF Technology

Observation is one of the fundamental steps in the scientific method. However, for centuries the scientific study of tissues was limited to observations of dissections with the unaided eye (gross anatomy).

This all changed in the 17th century when Anton Van Leeuwenhoek fabricated a microscope that allowed observations of tissues at the cellular level, thus establishing the science of histology. While early researchers found it relatively simple to distinguish between the cell boundaries and subcellular compartments in plants, doing so in animal tissue presented a much greater challenge. It wasn't until the late 19th century with the introduction of dyes, such as hematoxylin that Paul Mayer used to successfully stain nuclei, that the subcellular structure of tissues became visible and the science of histochemistry emerged.

The number of available tissue dyes and stains increased during the early 20th century, as did the number of molecular families they identified. However, the ability to identify individual cellular- or tissue-specific proteins remained elusive. This changed in the mid-20th century when Dr. Albert Coons demonstrated that fluorescently labeled antibodies could be used to localize bacteria inside macrophages, thus helping to pioneer the science of immunohistochemistry (IHC). Over the next two decades our understanding of antibodies, antigens and immunology grew rapidly. However, IHC remained largely a specialized research tool used primarily in university settings. Then in the late 1960's, Dr. Stratis Avrameas and Dr. Paul Nakane independently developed methods to covalently couple the enzyme horseradish peroxidase (HRP) to antibodies. HRP in the presence of diaminobenzidine and hydrogen peroxide creates a brown precipitate at the site of the HRP-conjugated antibody. The precipitate can be visualized using an ordinary light microscope. This allowed for the IHC results to be viewed in any lab having a light microscope, with no need for expensive, complicated fluorescence instrumentation.

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

Up to this time, visualization using fluorescence microscopy was challenging due to the rapid photobleaching of fluorophores when exposed to the light of the microscope. This significantly limited the time over which a sample could be observed. In the early 1990's, VECTASHIELD® Antifade Mounting Medium was introduced by Vector Laboratories as the first commercially available mountant for fluorescence. Not only did it have no autofluorescence (in the popular visualization channels), it was also effective in preventing the photobleaching, or fading of the fluorophores. This advancement in microscopy not only made image acquisition and analysis much more convenient, it provided researchers tools to challenge the limits of fluorescence detection.

In the last decade, immunofluorescence applications have been further improved by the adaptation of new super-resolution methods. Super-resolution microscopy allows imaging at a scale smaller than 200 nm. Due to its characteristics and convenience, VECTASHIELD® Mounting Medium has been found to be quite suitable for super-resolution imaging methods like stochastic optical reconstruction microscopy (STORM) and structured illumination microscopy (3D-SIM). Olivier, et al., describes VECTASHIELD® Mounting Medium as a "simple yet powerful buffer for 3D-STORM".

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Nakane P and Pierce GB Jr "Enzyme-labeled antibodies for the light and electron microscopic localization of tissue antigens" *J. Cell. Biol.* 33, 307-318 (1967)

Leduc E, Avrameas S, and Bouteille M "Ultrastructural localization of antibody in differentiating plasma cells" *J. Exp. Med.* 127, 109-118. (1968)

Hsu S-M, Raine L, and Fanger H "Use of Avidin-Biotin-Peroxidase Complex (ABC) in Immunoperoxidase Techniques: A Comparison between ABC and Unlabeled Antibody (PAP) Procedures" *J. Histochem. Cytochem.* 29(4), 577-580 (1981)

Shi SR, Key ME, and Kalra KL "Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections" *J Histochem Cytochem.* Jun, 39(6), 741-8 (1991)

Bretschneider S, Eggeling C, and Hell SW "Breaking the Diffraction Barrier in Fluorescence Microscopy by Optical Shelving," *PRL* 98, 218103 (2007)

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Olivier N, Keller D, Rajan VS, Gönczy P, and Manley S "Simple buffers for 3D STORM microscopy," *Biochemical Optics Express* 4, 885-899 (2013)

Wegel, E, et al. "Imaging cellular structures in super-resolution with SIM, STED and Localisation Microscopy: A practical comparison", *Scientific Reports*, 6, 27290 (2016)

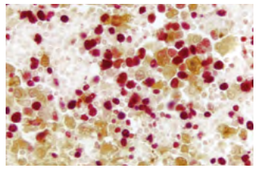
Childs GV "History of Immunohistochemistry" *Pathobiology of Human Disease* 3775-3796 (2014)

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

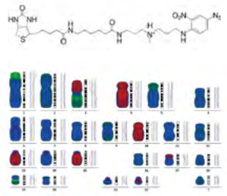
1966
Stratis Avrameas and Paul Nakane independently developed methods to covalently couple the enzyme horseradish peroxidase (HRP) to antibodies

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

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



1986
Vector Labs launched PHOTOPROBE® Biotin

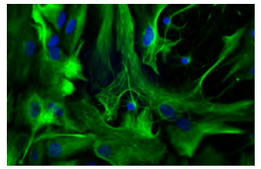


1991
S.R. Shi developed the antigen retrieval process for formaldehyde fixed tissues

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

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

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

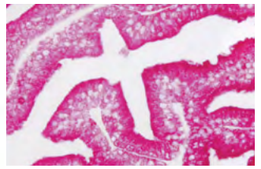


2005
Vector Labs developed detection reagents enabling greater access to antigens within tissues and improving multiple antigen labeling (ImmPRESS™ enzyme polymer)

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

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

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



2016
Strategic investment by Maravai LifeSciences

maravai LifeSciences

Choosing a Detection System

Immunofluorescence Overview

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

Vector Laboratories develops and manufactures a wide selection of reagents for IF, including traditional fluorophore-conjugated antibodies and an extensive range of avidin/biotin products. Recent additions include conjugates with contemporary fluorophores such as DyLight® dyes as well as kits that offer a significant increase in sensitivity or help streamline workflows. The VECTASHIELD® and VECTASHIELD® HardSet™ Antifade Mounting Media are market-leading products on which researchers consistently rely to complete workflows and achieve signal retention for image acquisition and specimen archiving.

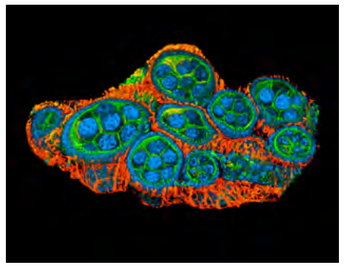
Colon (FFPE): Antigen retrieved with Antigen Unmasking Solution (citrate-based, pH 6.0) and stained with Cy®5 Sambucus Nigra Lectin (SNA; fuchsia). VECTASHIELD® Antifade Mounting Medium with DAPI counterstain (blue).

Comparison of Detection Systems

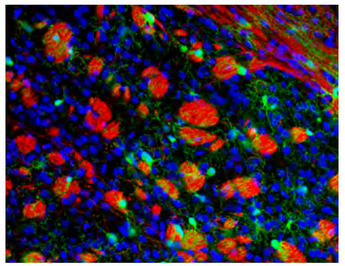
Choose the appropriate detection system for your experiment based on fluorophore (color), sensitivity, formats, flexibility, time and cost.

Detection System	Fluorophore	Color	Sensitivity	Concentrate	Ready-to-Use (R.T.U.) Format	Biotin-Free	Modular	Cost/ Assay
One Step								
VectaFluor™ R.T.U. Secondary Antibodies	DyLight®	Green Red	••		•	•		••
VectaFluor™ Duet IF Double Labeling Kits	DyLight®	Green Red	••		•	•		•••
Fluorophore-Conjugated Secondary Antibodies	Traditional† DyLight® Cyanine®	Blue Green Orange Red Orange Red Far Red	••	•		•		•
Two Step								
VectaFluor™ Excel Amplified kits	DyLight®	Green Red	••••		•	•		•••
Streptavidin / Avidin Fluorophore Conjugates*	Traditional† DyLight® Cyanine®	Blue Green Red Far Red	•••	•			•	•
Mouse on Mouse (M.O.M.™) Kits								
Mouse on Mouse (M.O.M.™) System	Traditional† DyLight® Cyanine®	Blue Green Red Far Red	•••	•			•	••

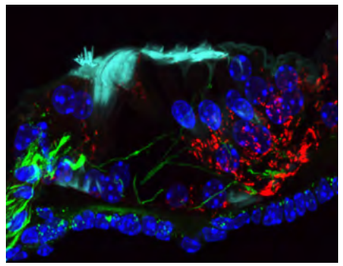
† Traditional fluorophores include: Fluorescein (FITC), Rhodamine (TRITC), Texas Red®, AMCA, Phycoerythrin (PE)
** Sensitivity can be increased with multiple rounds of biotinylated anti-(strept)avidin and strept(avidin) fluorophore conjugates.*



Fruit fly ovarian nurse cells. Cutaway three-dimensional reconstruction from a confocal stack of a Drosophila melanogaster ovary. E-cadherin (GFP, green), f-actin (red) and DAPI (blue). This image is a collaborative effort by Dr. Ian Newton and Dr. Paul Appleton, School of Life Sciences, University of Dundee, Dundee, UK.



Coronal section of a Pdgfra/Rosa26 transgenic mouse brain at postnatal day (P15). The image depicts myelin basic protein (red) and oligodendrocyte precursor cells identified by the expression of GFP in the striatum. Cell nuclei are shown in blue. Image provided by Dr. Andrea Domenico Rivera, Institute of Biological and Biomedical Sciences, University of Portsmouth, Portsmouth, UK.



Adult organ of Corti labeled with anti-beta3-tubulin (green), phalloidin (cyan), anti-connexin 30 (red) and DAPI (blue). Image provided by Dr. Dan Jagger, Ear Institute, University College London, London, UK.

Considerations for IF Detection

Immunofluorescence detection reagents are used to localize and visualize target antigens expressed in tissue sections or cultured cells. When applied optimally, these highly specific reagents provide a defined contrast between their fluorescence, which demarcates the antigen, and the non-fluorescent region of the preparation. There are several options to achieve labeling for single and multiple antigen detection.

Direct Detection

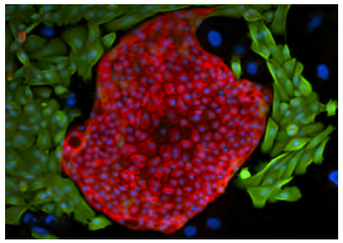
One common IF method uses fluorophore-conjugated primary antibodies. This direct approach enables fast and easy IF visualization once the antibody has been conjugated; however, there are some disadvantages to this traditional method. For example, binding affinity and avidity could be compromised by the conjugation process, which would reduce signal and prevent moderately or weakly expressed antigens from being detected. Furthermore, expensive primary antibodies used at high concentrations could be cost prohibitive, and the visualization options would be limited to only one fluorophore.

Indirect Detection (One Step)

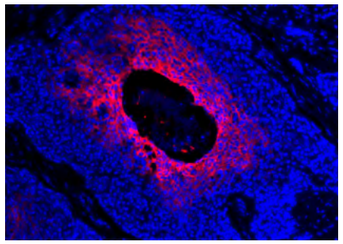
The indirect method, which uses labeled secondary antibodies, produces reliable, reproducible and economical IF results. This method avoids the disadvantages of directly conjugated primary antibodies and provides signal amplification that is necessary for most cell- and tissue-section labeling. Additionally, this one-step detection method is modular and allows simple substitution of the secondary with different fluorophore conjugates. Please refer to Table 2, page 13 for our range of concentrated reagents. Fluorophore-conjugated secondary antibodies would be recommended where a moderate to high expression of target antigen is expected.

Indirect Detection (Two Step)

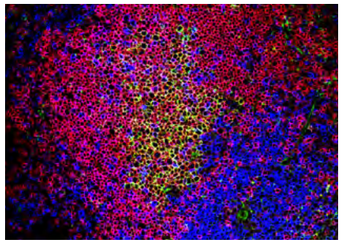
Further signal amplification is introduced by using biotinylated secondary antibodies with avidin or streptavidin fluorophore conjugates. This well established and widely published methodology exploits the very high affinity between avidin or streptavidin and the small vitamin biotin. This two-step detection method enables the detection of weakly expressed antigens and provides a flexible and modular system with easy fluorophore substitution using different avidin or streptavidin conjugates (see pages 18-20). Additional amplification can be achieved by using biotinylated anti-avidin/streptavidin. For applications where use of biotin-based reagents for signal amplification would be problematic, we offer a non-biotin based two-step fluorescence approach with our VectaFluor™ Excel Amplified DyLight® Antibody kits (see page 16).



Staining of human breast cancer colony-forming culture for basal (Cytokeratin 14, green) and luminal markers (Cytokeratin 18, red). Image provided by Wendy Greenwood, method by Dr. Michael Prater, The Cancer Research UK Cambridge Institute, Cambridge, UK.



Hypoxia within hyperplastic breast tissue. A section of human breast tissue labeled for CAIX using immunofluorescence, counterstained and mounted using VECTASHIELD® HardSet™ Antifade Mounting Medium with DAPI. Image provided by Dr. Carl Daly and Dr. Sarah Dean, Healthcare Science, University of the West of England, Bristol, UK.



Germinal center (GC) reaction in the spleen after acute viral infection. After recognition of viral antigens, T cells (blue) migrate from the T cell zone into the follicle where they interact with B cells (purple). The T cells 'help' B cells, instructing the formation of GCs (green) in which virus-specific B cells undergo selection, class switching and somatic hypermutation to secrete anti-viral antibodies to clear the infection. This work was conducted by Miriam Eckstein and Dr. Martin Vaeth, Department of Pathology, New York University, NY, USA.

Species Cross-Reactivity

Beyond the choices provided in the Selection Guide (pages 4-5), consideration should be given to the species of the tissue under examination and the species of the primary antibody. In cases of closely related species, it is recommended to use a secondary antibody that has been specifically adsorbed to remove cross-reacting antibodies. In instances where a mouse primary antibody is being applied to mouse tissue sections, it is recommended to use the M.O.M.™ Immunodetection System (see pages 22-23).

Multiple Antigen Labeling

The visualization of two or more antigens on the same tissue section requires careful planning and specific reagent selection to generate unequivocal and reproducible staining results. We have recently introduced our VectaFluor™ Duet IF Double Labeling Kits that provide convenience and a straightforward approach to this often difficult and time-consuming application (see page 15).

Choosing fluorophores

Immunofluorescence detection reagents are labeled with fluorophores that absorb (excitation) and emit (emission) light at specific wavelengths. Fluorophores suitable for immunofluorescence are available across the complete visible light spectrum. The light source and filter cubes in a particular microscope must match the excitation and emission requirements of the specific fluorophore to achieve the optimal signal-to-noise ratios. For example, the absorption and emission peak wavelengths of fluorescein are 495 nm and 515 nm, respectively. Therefore, an excitation light source that is near 495 nm will yield the greatest emission signal. An emission filter that spans 515 nm will capture the emitted signal. These wavelengths are fixed properties of the fluorophores and the filter, and when properly paired, the system will yield the strongest signal and lowest background.

Table 1. Excitation and emission wavelengths and visual colors for immunofluorescence fluorophores.

Fluorophore	Color		Excitation Max (nm)	Emission Max (nm)
AMCA		Blue	350	450
DyLight® 488		Green	493	518
Fluorescein		Green	495	515
Cy®3		Orange	550	570
Rhodamine		Orange	550	575
DyLight® 549		Orange	562	576
Phycoerythrin		Red-Orange	565	574
DyLight® 594		Red	593	618
Texas Red®		Red	595	615
Cy®5		Far Red	649	670
DyLight® 649		Far Red	652	672

Fluorophore-Conjugated Secondary Antibodies

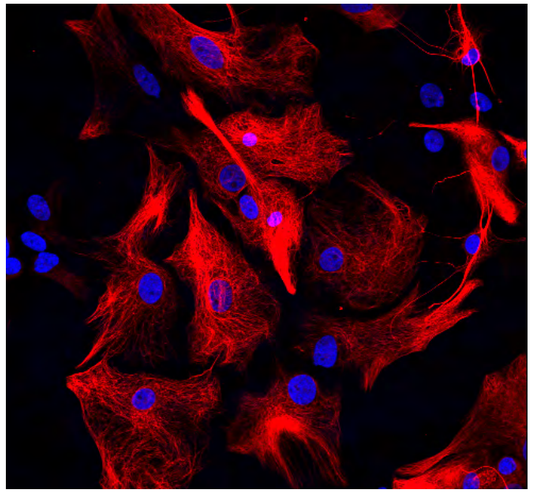
All antibodies available from Vector Laboratories for immunological applications are prepared using optimized, proprietary immunization schedules that produce high-quality antibodies. The antibodies are affinity-purified, and solid-phase adsorption techniques are used to remove cross-reactivities that are likely to interfere with specific detection. The conjugated antibodies have the optimal degree of labeling to maximize signal output without compromising antibody specificity or affinity.

We offer researchers a range of traditional and contemporary conjugated fluorophores, including fluorescein, rhodamine, Texas Red®, AMCA and phycoerythrin. DyLight® dyes offer greater photostability, pH independence and brighter fluorescence than conventional fluorophores. DyLight® dye-conjugated antibodies are ideal for cell- and tissue-based immunofluorescence and a variety of other applications. The DyLight® dye conjugates are stable at pH 4-9 and compatible with many buffers and diluents.

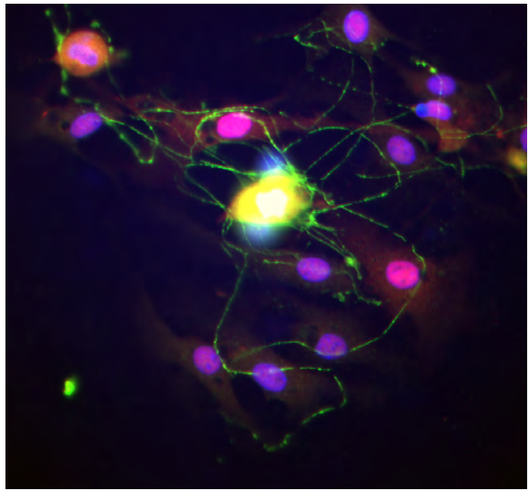
The Cy®3 and Cy®5 Dyes offer bright and stable fluorescence and are used in a variety of applications. Cy®3 dye is bright orange with an excitation/emission of 550 nm/570 nm. Cy®5 is a far-red dye with an excitation/emission of 649 nm/670 nm. Cy®5 is often used as an additional label in multiplexing protocols or in super resolution imaging because of its photo switchable properties.

Fluorophore-Conjugated Secondary (target species) Antibodies

- Rabbit IgG
- Mouse IgG
- Mouse IgM
- Human IgG
- Rat IgG
- Goat IgG
- Sheep IgG



Astrocytes: Stained for GFAP and detected with DyLight® 594-conjugated secondary antibody. Mounted in VECTASHIELD® HardSet™ Mounting Medium with DAPI. Image courtesy of Dr. Emma East, Department of Life Sciences, The Open University, Milton Keynes, UK.



Dorsal root ganglia cells (neurons and satellite glia): Beta III tubulin(ms), DyLight® 549 Anti-Mouse IgG • s100(rb), 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, Milton Keynes, UK.

We offer a comprehensive range of fluorophore-conjugated secondary antibodies. These affinity-purified, highly specific antibodies, directed against the most commonly used primary antibody target species, are available with a wide choice of fluorophores and are presented in a concentrated format.

Table 2. Fluorophore-conjugated secondary antibodies.

Product	AMCA	Fluorescein	Texas Red®	Kits: AMCA, Fluorescein, Texas Red®	DyLight® 488	DyLight® 549	DyLight® 594	DyLight® 649	R-Phycoerythrin	Cy®3	Cy®5
Anti-Mouse IgG (H+L), made in horse	CI-2000	FI-2000	TI-2000	FI-2100	DI-2488	DI-2549	DI-2594	DI-2649	EI-2007	CY-2300	CY-2500
Anti-Mouse IgG (H+L), rat-adsorbed, made in horse		FI-2001									
Anti-Mouse IgM, mu-chain specific, made in goat		FI-2020									
Anti-Rabbit IgG (H+L), made in horse					DI-1088		DI-1094				
Anti-Rabbit IgG (H+L), made in goat	CI-1000	FI-1000	TI-1000	FI-1200	DI-1488	DI-1549	DI-1594	DI-1649		CY-1300	CY-1500
Anti-Rat IgG (H+L), made in rabbit		FI-4000									
Anti-Rat IgG (H+L), mouse-adsorbed, made in rabbit		FI-4001									
Anti-Rat IgG, made in goat										CY-4300	CY-4500
Anti-Goat IgG (H+L), made in horse					DI-3088		DI-3094				
Anti-Goat IgG (H+L), made in rabbit	CI-5000	FI-5000									
Anti-Sheep IgG (H+L), made in rabbit		FI-6000									
Anti-Human Fluorophore-Conjugated Secondary Antibodies											
Anti-Human IgG (H+L), made in goat		FI-3000									
Anti-Human IgE, ε (Epsilon) chain specific, made in goat		FI-3040									
Anti-Human IgG, γ (Gamma) chain specific, made in goat		FI-3080									
Anti-Human IgM, μ (Mu) chain specific, made in goat		FI-3020									
Anti-Human κ (Kappa) Chain, made in goat	CI-3060	FI-3060									
Anti-Human Lambda Chain, made in goat	CI-3070	FI-3070									

VectaFluor™ Ready-To-Use Antibody Reagents

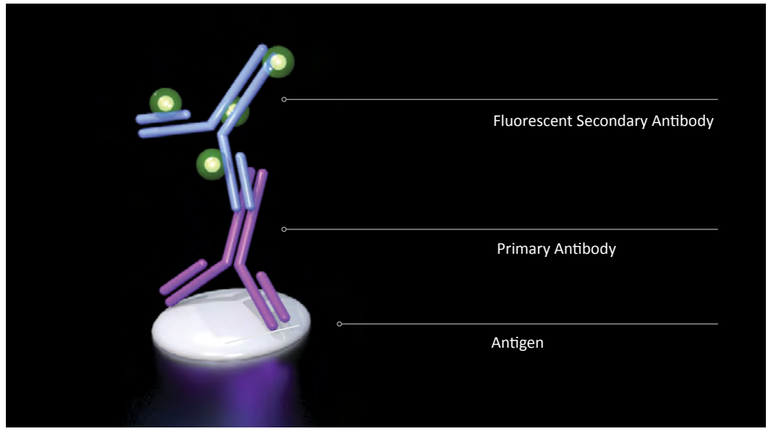
As investigators push research boundaries and require more sensitive, photo-stable fluorescent products, we have met this demand by developing a range of DyLight® dye-conjugated secondary antibodies and novel detection kits that we have named VectaFluor™ reagents. The VectaFluor™ products are presented as pre-diluted, ready-to-use (R.T.U.) solutions that reduce optimization requirements at the researchers' end, thereby saving time and minimizing potential dilution errors which assists with greater consistency in collaborative efforts across lab environments.

Maximum performance is achieved when these VectaFluor™ reagents are used in combination with our VECTASHIELD® Antifade Mounting Media (see pages 24-27).

VectaFluor™ R.T.U. Antibody Kits

The VectaFluor™ Ready-to-Use (R.T.U.) DyLight® dye-conjugated secondary antibodies offer maximum convenience for fluorescence staining of cells and tissues. These affinity-purified, highly cross-adsorbed secondary antibodies are conjugated to DyLight® dyes in a manner that ensures the maximum degree of labeling without compromising antibody affinity or specificity. DyLight® dyes offer advantages such as bright fluorescence, excellent photostability and pH independence.

VectaFluor™ R.T.U. Antibody Reagents are suitable for use with rabbit, mouse, goat, sheep, and bovine IgG primary antibodies and are supplied as ready-to-use, pre-diluted, stabilized solutions (15 ml) with ready-to-use 2.5% normal horse serum (15 ml) for blocking.

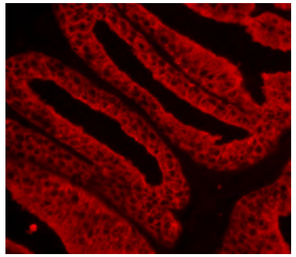


Product	DyLight® 488 (Green)	DyLight® 594 (Red)
VectaFluor™ Anti-Rabbit IgG, made in horse	DI-1788	DI-1794
VectaFluor™ Anti-Mouse IgG, made in horse	DI-2788	DI-2794
VectaFluor™ Anti-Goat IgG, made in horse	DI-3788	DI-3794

VectaFluor™ R.T.U Antibody Kits

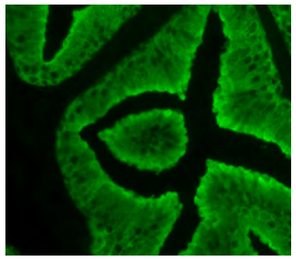
- Rabbit IgG
- Mouse IgG
- Goat IgG

DyLight® 594 Kits
Excitation: 593 nm
Emission: 618 nm
Color: Red



Prostate: Anti-PSA (goat), VectaFluor™ DyLight® 594 Anti-Goat IgG. Mounted in VECTASHIELD® HardSet™ Mounting Medium.

DyLight® 488 Kits
Excitation: 493 nm
Emission: 518 nm
Color: Green



Prostate: Anti-PSA (goat), VectaFluor™ DyLight® 488 Anti-Goat IgG. Mounted in VECTASHIELD® HardSet™ Mounting Medium.

VectaFluor™ Duet Immunofluorescence Double Labeling Kits

Apply two colors in one step using the VectaFluor™ Duet IF Double Labeling Kits. These kits save time and effort in double-labeling immunofluorescence (IF) protocols, which can be long and tedious. The kits are configured to detect a mouse and a rabbit primary antibody with green and red fluorescence in one step.

- Two colors, one step
- Ready-to-use (R.T.U.)
- Robust cocktail formulation of DyLight® anti-mouse IgG and DyLight® anti-rabbit IgG

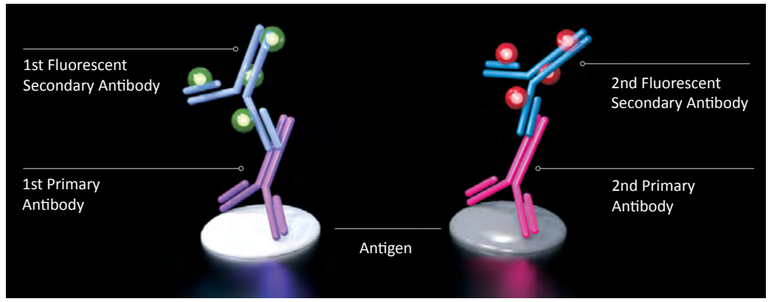
Two kit configurations are available:

Selection of a VectaFluor™ Duet IF Double Labeling Kit format is based on individual preference; however, certain parameters should be considered. For example, prevalence of the respective target antigens within a tissue section, and whether the more abundant antigen will be viewed by a green or red signal are important factors. Also consider possible overlap or co-localization of the antigens and which antibody combination would produce optimal results.

VectaFluor™ Duet Immunofluorescence Double Labeling Kit Contents:

– 15 ml 2.5% Normal Horse Serum for blocking, R.T.U.
– 15 ml VectaFluor™ Duet Reagent, R.T.U.

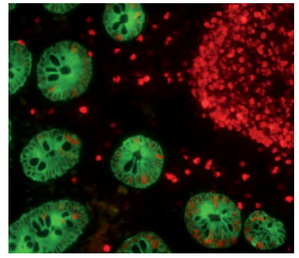
The affinity-purified, highly cross-adsorbed secondary antibodies are conjugated to DyLight® dyes in a manner that maximizes the degree of labeling without compromising antibody affinity or specificity. The red and green DyLight® dye-conjugated anti-mouse and anti-rabbit antibodies are then combined into a robust, stable cocktail formulation that yields sensitive and consistent dual staining. The VectaFluor™ Duet IF Double Labeling Kit is compatible with fluorescence staining of cells and tissues.



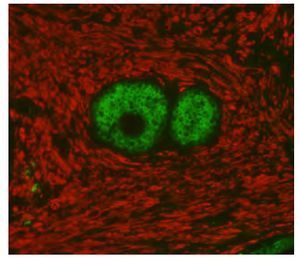
Product	Catalog Number
VectaFluor™ Duet IF Double Labeling Kit - DyLight® 488 Anti-Rabbit (green) - DyLight® 594 Anti-Mouse (red)	DK-8818
VectaFluor™ Duet IF Double Labeling Kit - DyLight® 594 Anti-Rabbit (red) - DyLight® 488 Anti-Mouse (green)	DK-8828

VectaFluor™ Duet Kits

- Rabbit IgG (green)/Mouse IgG (red)
- Mouse IgG (green)/Rabbit IgG (red)



Colon: Mouse Anti-Cytokeratin (AE1/AE3) and Rabbit Anti-Ki67 detected simultaneously with VectaFluor™ Duet IF Double Labeling Kit, DyLight® 488 Anti-Mouse (green)/ DyLight® 594 Anti-Rabbit (red). Mounted in VECTASHIELD® HardSet™ Mounting Medium.



Prostate: Rabbit Anti-PSA mAb and Mouse Anti-Smooth Muscle Actin detected simultaneously with VectaFluor™ Duet IF Double Labeling Kit, DyLight® 488 Anti-Rabbit (green)/DyLight® 594 Anti-Mouse (red). Mounted in VECTASHIELD® HardSet™ Mounting Medium.

VectaFluor™ Excel Amplified Staining System

The VectaFluor™ Excel Amplified Staining System offers a convenient, non-biotin amplification method for fluorescence applications. This system uses an Amplifier Antibody – a specially prepared, high-affinity, unconjugated anti-mouse IgG or anti-rabbit IgG antibody produced in goat – followed by VectaFluor™ DyLight® dye-conjugated anti-goat IgG antibody.

The affinity-purified, highly cross-adsorbed anti-goat IgG antibody is conjugated to DyLight® dyes in a manner that ensures maximum degree of labeling without compromising antibody affinity or specificity. DyLight® dyes offer advantages such as bright fluorescence, excellent photostability and pH independence.

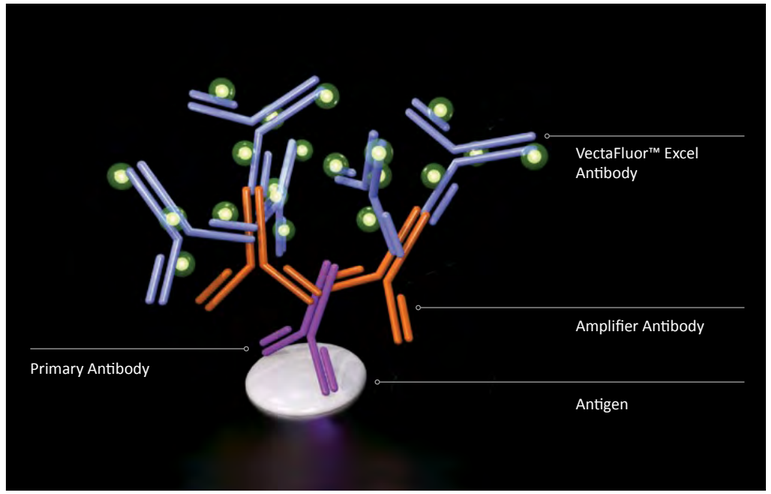
- Stabilized, ready-to-use solutions
- Non-biotin signal amplification
- High sensitivity
- Low background

VectaFluor™ Excel Kit Contents:

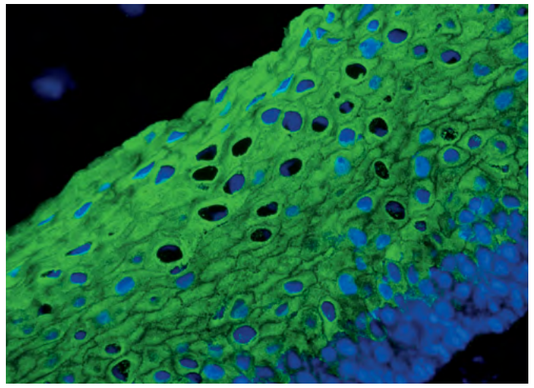
– 15 ml 2.5% Normal Horse Serum for blocking, R.T.U.
– 15 ml Amplifier Antibody, R.T.U. (goat anti-mouse IgG or goat anti-rabbit IgG)
– 15 ml VectaFluor™ DyLight® dye-conjugated Horse Anti-Goat IgG, R.T.U.

VectaFluor™ R.T.U. Antibody Kits

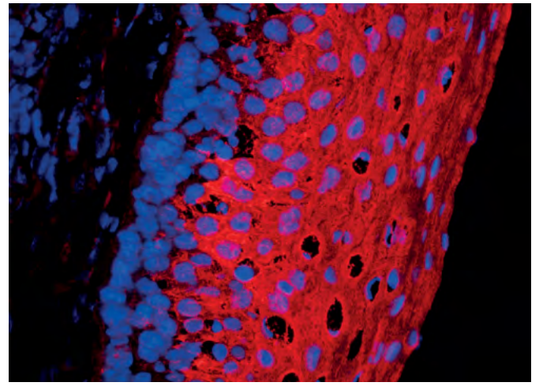
- Rabbit IgG (green or red)
- Mouse IgG (green or red)



Product	DyLight® 488 (Green)	DyLight® 594 (Red)
VectaFluor™ Excel Amplified Anti-Rabbit IgG Kit	DK-1488	DK-1594
VectaFluor™ Excel Amplified Anti-Mouse IgG Kit	DK-2488	DK-2594



Tonsil: Anti-Multi-Cytokeratin, VectaFluor™ Excel Amplified DyLight® 488 Anti-Mouse IgG Kit. Mounted in VECTASHIELD® HardSet™ Mounting Medium with DAPI.



Tonsil: Anti-Multi-Cytokeratin, VectaFluor™ Excel Amplified DyLight® 594 Anti-Mouse IgG Kit. Mounted in VECTASHIELD® HardSet™ Mounting Medium with DAPI.

Frequently Asked Questions:

1) Can the VectaFluor™ Excel kits be applied to fixed cultured cells?

Yes, investigators have successfully applied these kits on fixed cultured cells directly, and cultured cells that have been formalin-fixed and paraffin-embedded. Please see references 1 and 2 below, respectively.

2) Are the VectaFluor™ Excel Kits compatible with other fluorescent secondary antibodies for double staining applications?

Yes, as indicated in reference 3 below. For this application to be successful however, investigators must use detection reagents raised in species that will not cross-react with the detection reagents of the VectaFluor™ Excel kit.

3) What are the advantages of using the VectaFluor™ Excel kits compared with secondary antibodies directly conjugated with fluorophores?

The main advantage of using the VectaFluor™ Excel kits is the increase in sensitivity the Amplifier Antibody generates. In most staining applications, investigators would see an increase of at least three- to four-fold over that of a secondary antibody directly conjugated with a fluorophore. This increase in sensitivity enables unambiguous visualization of weakly expressed antigens, as well as further dilution of potentially expensive primary antibodies.

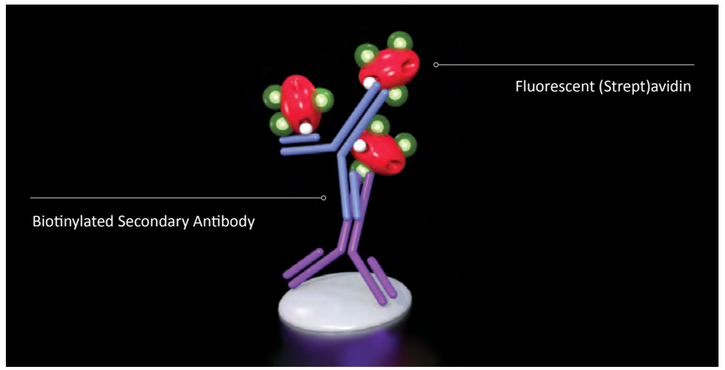
4) Can the VectaFluor™ Excel kits be applied to any species of tissue?

The VectaFluor™ Excel kits were developed and optimized on human tissue sections. As with any secondary detection system, investigators should note potential cross-reactivity between the reagents being applied to a tissue section and inherent proteins. The VectaFluor™ Excel Anti-Rabbit IgG kits can be applied to rodent and primate species. The VectaFluor™ Excel Anti-Mouse IgG kits are recommended for non-rodent tissues. Note however, that due to the VectaFluor™ Excel Anti-Goat IgG Reagent supplied in all VectaFluor™ Excel kits, recognition of proteins in goat, sheep, and bovine species may occur.

References:
1) Azumi E, et al. (2016) *Orthodontic Waves* 75:97-104
2) Rengstl B, et al. (2017) *PLoS ONE* 12(5): e0177378
3) Baillie R, et al. (2016) *J. Clin. Pathol.* 69(8):742-744

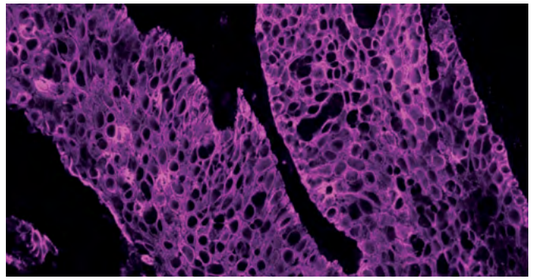
Fluorophore-Conjugated Streptavidin/Avidin Reagents

The fluorophore-conjugated streptavidin and avidin reagents are highly purified and have low non-specific binding. These fluorescent conjugates can be used to detect biotinylated secondary antibodies and other macromolecules in various applications, including immunofluorescence, *in situ* hybridization and flow cytometry. The fluorescent signal can be amplified using biotinylated secondary antibodies and fluorophore-conjugated streptavidin or avidin.

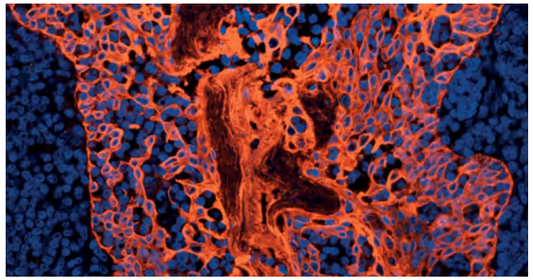


Streptavidin/Avidin Fluorophores

- Blue (AMCA)
- Green (DyLight® 488 and Fluorescein)
- Orange (DyLight® 549, Cy®3 and Phycoerythrin)
- Red (DyLight® 594 and Texas Red®)
- Far Red (DyLight® 649, and Cy®5)



Tonsil (FFPE) was antigen-retrieved with Antigen Unmasking Solution and stained with Anti-Pan Cytokeratin (mouse; clone AE1/AE3), Biotinylated Horse Anti-Mouse IgG, and Cy®5 Streptavidin.



Tonsil (FFPE) was antigen retrieved with Antigen Unmasking Solution and stained with Anti-Pan Cytokeratin (mouse; clone AE1/AE3), Biotinylated Horse Anti-Mouse IgG and Cy®3 Streptavidin. Mounted in VECTASHIELD® HardSet™ Mounting Medium with DAPI.

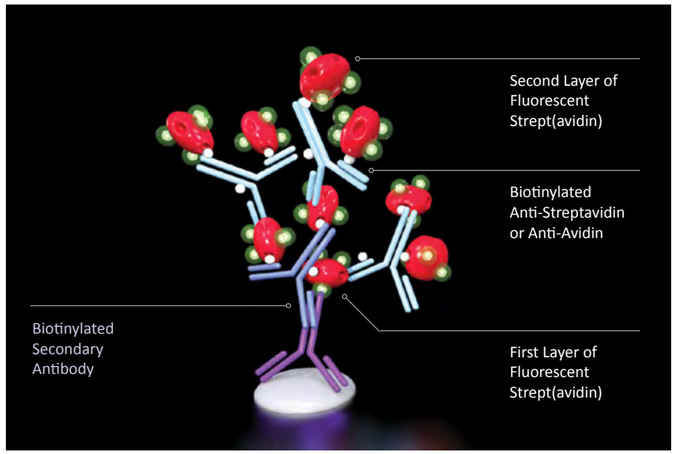
Product	AMCA	Fluorescein	Rhodamine	Texas Red®	Kits: AMCA, Fluorescein, Texas Red®	DyLight® 488	DyLight® 549	DyLight® 594	DyLight® 649	Phycoerythrin	Cy®3	Cy®5
Streptavidin	SA-5008	SA-5001		SA-5006	SA-1200	SA-5488	SA-5549	SA-5594	SA-5649	SA-5207	SA-1300	SA-1500
Avidin	A-2008	A-2001	A-2002	A-2006	A-1100							
Avidin DCS		A-2011	A-2012	A-2016								
Avidin DN		A-3101										

Anti-Streptavidin and Anti-Avidin Antibody Reagents

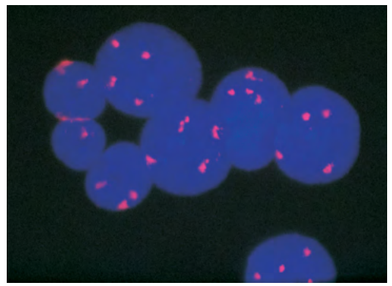
Use of the Biotinylated Anti-Streptavidin or Biotinylated Anti-Avidin antibodies is an ideal approach to increase sensitivity in (strept)avidin/biotin detection systems. These antibodies bind to streptavidin or avidin through both of their antigen-binding sites and the covalently-attached biotin residues. After the first application of a fluorophore-conjugated streptavidin or avidin, the signal is amplified by incubation with a Biotinylated Anti-Streptavidin or a Biotinylated Anti-Avidin antibody. That incubation is followed by a second incubation with fluorophore-conjugated streptavidin or avidin. This multi-layered approach accumulates more fluorophores at the target site and can provide a multi-fold amplification.

Biotinylated Anti-Avidin and Biotinylated Anti-Streptavidin amplification is ideal for the following applications:

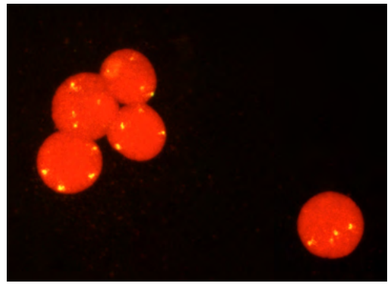
- Immunofluorescence / Immunohistochemistry
- *In situ* hybridization
- Microarray assays
- ELISAs
- Blotting



Product	Biotin	Unconjugated	DyLight® 488	DyLight® 549
Anti-Streptavidin	BA-0500	SP-4000	SP-4488	SP-4549
Anti-Avidin	BA-0300	SP-2000		



FastTag® Biotin-conjugated 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-conjugated 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).

Secondary and Tertiary Detection Reagents

Our secondary antibodies are prepared by hyper-immunizing animals in a manner that produces high affinity antibodies. These are then purified by an affinity chromatography procedure designed to remove any low-affinity antibodies. Cross-reactivities that can interfere with specific labeling are removed by solid-phase adsorption techniques. The final product is then subjected to rigorous quality-control assays including immunodiffusion, solid-phase enzyme immunoassays, gel electrophoresis, solid-phase binding assays and IHC tissue staining. These unconjugated antibodies are used to generate our enzyme conjugated and biotinylated secondary antibodies.

Biotinylated and Unconjugated Secondary Antibodies

Our high-affinity, purified, biotinylated and unconjugated secondary antibodies are manufactured under controlled conditions to retain maximum specificity and affinity. Our secondary antibodies are subjected to rigorous quality control assays and can be used for tissue and cell staining, ELISAs, and blotting.

	Biotinylated					Unconjugated		
	Host Species (Concentrate)			Host Species (R.T.U.)†		Host Species (Concentrate)		
Secondary Antibodies	Goat	Rabbit	Horse	Goat	Horse	Goat	Rabbit	Horse
Anti-Cat IgG (H+L)	BA-9000							
Anti-Chicken IgG (H+L)	BA-9010							
Anti-Goat IgG (H+L)		BA-5000	BA-9500		BP-9500		AI-5000	
Anti-Guinea Pig IgG (H+L)	BA-7000							
Anti-Hamster IgG (H+L)	BA-9100					AI-9100		
Anti-Horse IgG (H+L)	BA-8000							
Anti-Mouse IgG (H+L)	BA-9200		BA-2000	BP-9200	BP-2000	AI-9200		AI-2000
Anti-Mouse IgG (H+L), rat adsorbed			BA-2001					
Anti-Mouse IgM (H+L), Mu chain specific	BA-2020							
Anti-Rabbit IgG (H+L)	BA-1000		BA-1100	BP-9100	BP-1100	AI-1000		
Anti-Rat IgG (H+L)	BA-9400	BA-4000		BP-9400			AI-4000	
Anti-Rat IgG (H+L), mouse adsorbed	BA-9401	BA-4001					AI-4001	
Anti-Sheep IgG (H+L)		BA-6000						
Anti-Swine IgG (H+L)	BA-9020							
Universal Anti-Mouse/Rabbit IgG (H+L)			BA-1400		BP-1400			
Universal Pan-Specific Anti-Mouse/Rabbit/Goat IgG (H+L)			BA-1300					

† Ready-to-use, prediluted stabilized solutions

	Biotinylated	Unconjugated
	Host Species (Concentrate)	Host Species (Concentrate)
Anti-Human Secondary Antibodies	Goat	Goat
Anti-Human IgG (H+L)	BA-3000	AI-3000
Anti-Human IgE, ε (Epsilon) chain specific	BA-3040	AI-3040
Anti-Human IgG, γ (Gamma) chain specific	BA-3080	AI-3080
Anti-Human IgM, μ (Mu) chain specific	BA-3020	AI-3020
Anti-Human κ (Kappa) Chain, kappa chain specific	BA-3060	AI-3060

Enzyme-Conjugated Secondary Antibodies

Our high-affinity, purified antibodies are cross-linked with alkaline phosphatase (AP) or horseradish peroxidase (HRP) of the highest specificity. Our conjugation method ensures the maximum preservation of enzyme activity and antibody specificity. Recommended applications include tissue staining, ELISAs, and blotting.

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

Avidin and Streptavidin Enzyme Conjugates

Our enzyme-conjugated avidin and streptavidin are suitable for use in solid-phase assays, tissue- or cell-staining systems, and blotting. The conjugates are produced in optimized ratios with enzymes of the highest specific activity. Covalent linkages are specifically chosen to provide stable, highly active conjugates.

Product	Catalog Number
Alkaline Phosphatase	
Alkaline Phosphatase Streptavidin	SA-5100
Alkaline Phosphatase Avidin D	A-2100
Peroxidase	
Horseradish Peroxidase Streptavidin, concentrate	SA-5004
Horseradish Peroxidase Streptavidin, R.T.U.†	SA-5704
Horseradish Peroxidase Avidin D, concentrate	A-2004
Horseradish Peroxidase Avidin D, R.T.U.†	A-2704

† Ready-to-use, prediluted stabilized solutions

Species on Species Detection (Mouse)

Solutions when your primary antibody is the same species as your specimen.

When a primary antibody is the same species as the specimen, the secondary antibody cannot distinguish between the endogenous immunoglobulins and the primary antibody. This can result in high background staining that obscures antigen-specific staining. Mouse on Mouse detection is especially important because of the vast number of primary antibodies made in mouse and the wide use of mice in model systems, xenografts, and other applications.

Skeletal muscle: Alpha-sarcoglycan (m), M.O.M.™ Fluorescein Kit (green) • Muscle-specific actin (m), M.O.M.™ Basic Kit, Texas Red® Avidin DCS (red).

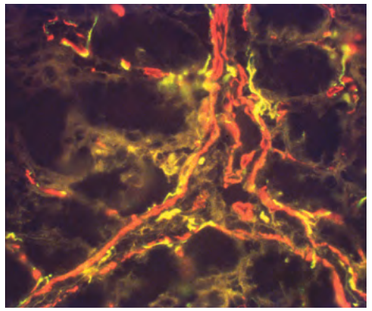
Mouse on Mouse (M.O.M.™) Immunodetection Kits

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

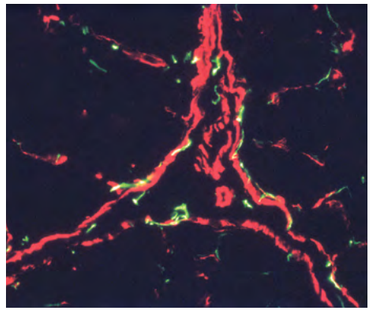
- Significantly reduces endogenous mouse Ig staining when using mouse primary antibodies on mouse tissue
- Simple protocols
- Eliminates tedious calculations
- Eliminates primary antibody prebinding steps
- Clear, crisp, specific staining of antigens of interest
- Compatible with fluorescent or enzyme-based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

Recommended applications:

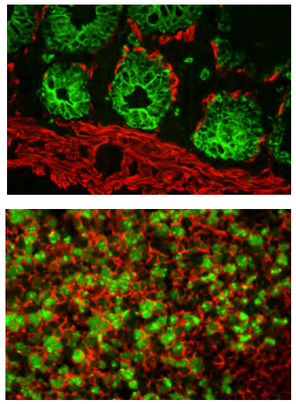
- Studies in genetically engineered mice
- Transgenic and knock-out models
- Mouse xenograft tissue
- Normal mouse tissue



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



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



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

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

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

Mounting Media

Choosing an effective mounting medium is especially important for immunofluorescence imaging. Fluorophores are susceptible to photobleaching and fading from both the imaging excitation light and during storage. The right mounting medium will protect your samples for short- and long-term use and archiving.

Rat muscle (FFPE): GFAP (red) and NF200 (green). Counterstained and coverslipped with VECTASHIELD® Mounting Medium with DAPI (blue). The double IF was performed by Dr. Lynn Dong, Dept of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA.

VECTASHIELD® Antifade Mounting Media

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

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

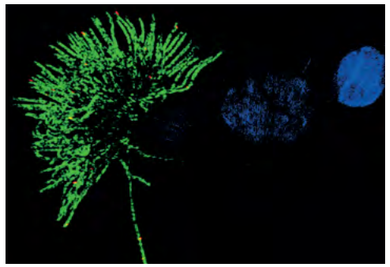
VECTASHIELD® Antifade Mounting Medium

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

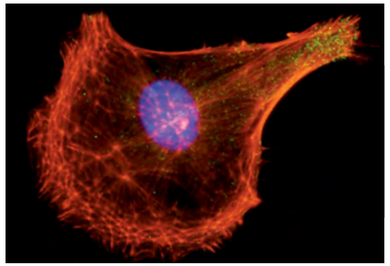
VECTASHIELD® HardSet™ Antifade Mounting Medium

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

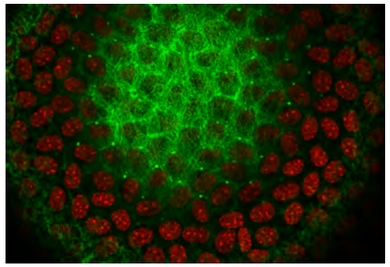
Product	No Counterstain	DAPI	PI	TRITC-Phalloidin
VECTASHIELD® Mounting Medium (non-hardening)	H-1000	H-1200	H-1300	
VECTASHIELD® HardSet™ Mounting Medium (hardening)	H-1400	H-1500		H-1600



Structured illumination super resolution photomicrograph of a ciliated bovine airway epithelial cell labeled for acetylated alpha tubulin (cilia marker; green), phosphodiesterase 5 (red) and nuclei (blue). Sample prepared and image taken by Michael E. Price, University of Nebraska Medical Center. With assistance of Janice A. Taylor and James R. Talaska, Advanced Microscopy Core Facility, University of Nebraska Medical Center, NE, USA.



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

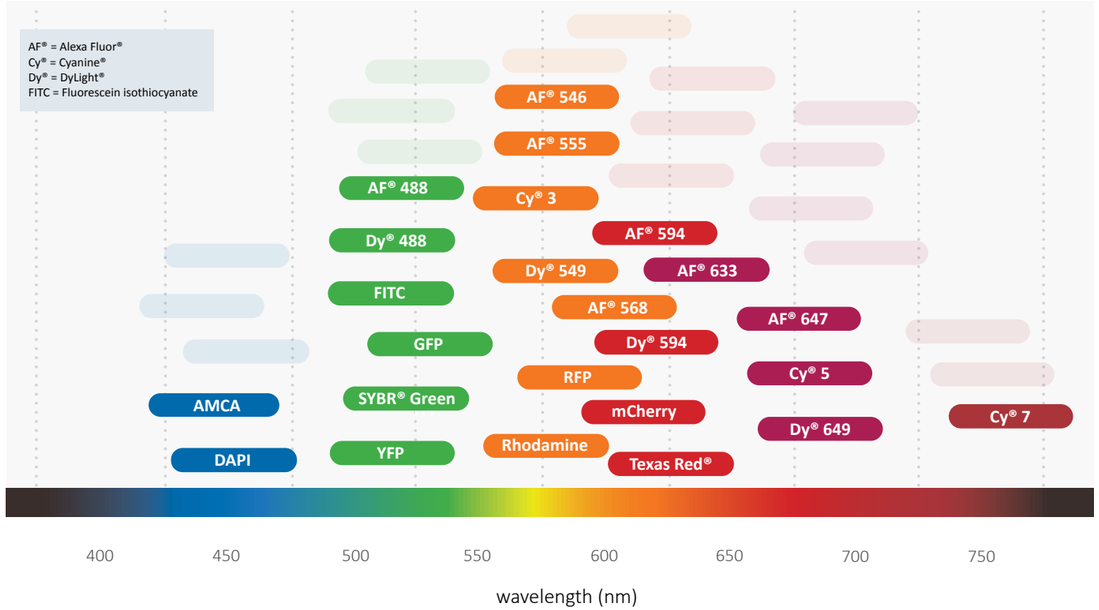


One optical section of a whole mouse lens stained with phalloidin (F-actin, green) and DAPI (nuclei, red). This image was captured by Dr. Catherine Cheng, Department of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA, USA.

VECTASHIELD® Mounting Media and Fluorophore Compatibility

VECTASHIELD® Mounting Media are the most widely referenced antifade mounting media for immunofluorescence applications. Currently over 60,000 published references cite using VECTASHIELD® Mounting Media and describe compatibility with over 130 fluorophores and fluorescent markers. This data underscores the prominence of VECTASHIELD® reagents in this application.

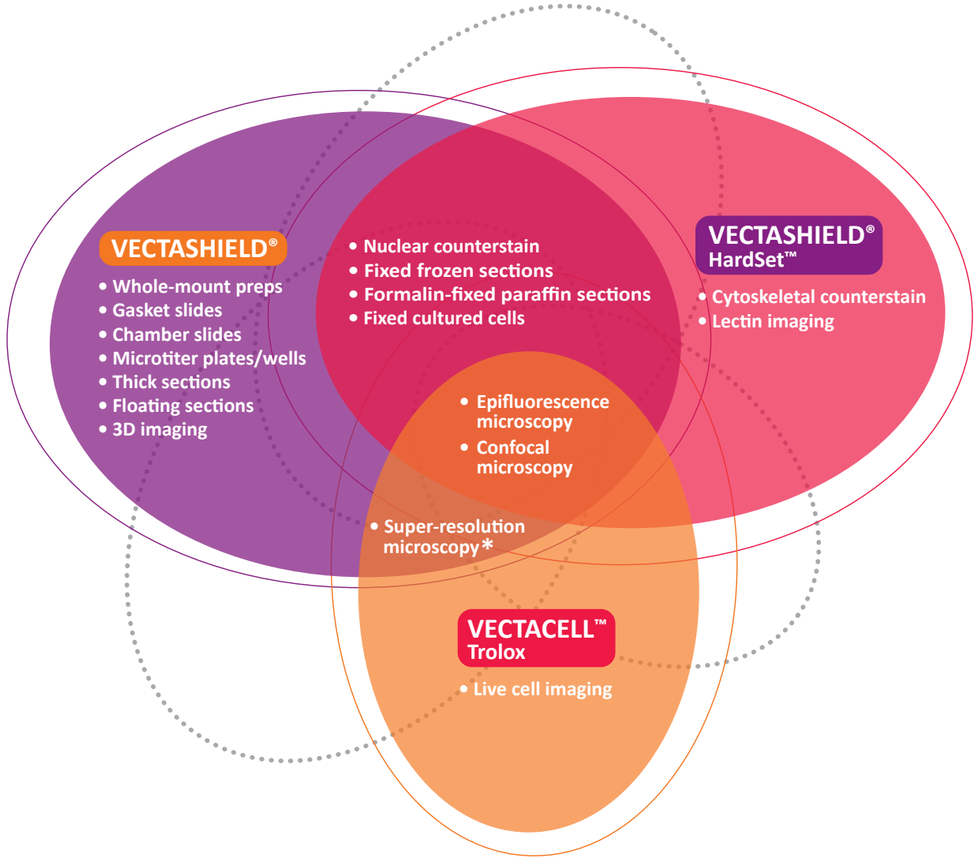
The graphic below highlights the most commonly referenced fluorophores used in combination with VECTASHIELD® Antifade Mounting Media.



The fluorescent compounds listed in the table below are select reagents that are also cited as being successfully used in combination with VECTASHIELD® Antifade Mounting Media. The range of these compounds, from traditional to contemporary, across a broad spectral range, and used in an array of applications, showcase the versatility of VECTASHIELD® reagents. For a comprehensive list of the >130 fluorophores and fluorescent markers that have been used with VECTASHIELD® products please visit our website at: **vectorlabs.com/vslist**

Fluorophore				
acridine orange	coumarin	Fluoro-Jade®	NeuroTrace®	Quantum dot/Qdot
Alexa Fluor® 350	dihydroethidium	Lucifer yellow	Nile red	SYTOX® Green
Alexa Fluor® 680	DRAQ5™	LysoTracker®	Oil red O	TAMRA
Atto® dyes	Evans blue	LysoTracker® Red	Pacific Blue™	thioflavin s
BODIPY®	fast blue	MitoTracker® Red	PicoGreen®	TOTO®-3

VECTASHIELD® Mounting Media Formats and Applications



The illustration above features established applications for our antifade mounting media formats. VECTASHIELD® Antifade Mounting Media are widely utilized to protect the inherent fluorescent properties of traditional and contemporary fluorophores in many applications using epifluorescence and confocal microscopy.

The versatility of the original VECTASHIELD® format solves the demands of labs and core facilities using multiple platforms and fluorescent markers. Furthermore, VECTASHIELD® reagents are also recognized as leading media in emerging techniques such as super resolution microscopy (SRM).

Of the SRM techniques currently being performed, the properties of VECTASHIELD® Antifade Mounting Media have been found to be advantageous in stochastic optical reconstruction microscopy (STORM) and structured illumination microscopy (SIM).

*Super Resolution (STORM and SIM) select references:

Olivier N, Keller D, Rajan VS, Gönczy P, and Manley S "Simple buffers for 3D STORM microscopy," *Biochemical Optics Express* 4, 885-899 (2013)

Wegel, E., et al. "Imaging cellular structures in super-resolution with SIM, STED and Localisation Microscopy: A practical comparison", *Scientific Reports*, 6, 27290. (2016)

VectaCell™ Products for Live Cell Imaging

Whereas immunofluorescence staining gives a snapshot of a cell or tissue at a specific time point, live cell imaging allows the observation of biological processes over a period of time. This is important for studying biological functions, interactions, and structures in various applications (e.g., the effects of drugs and other biomolecules).

VectaCell™ reagents enable and enhance live cell imaging studies. VectaCell™ Trolox Antifade Reagent reduces phototoxicity and photobleaching of reagents to increase cell viability and prolong signal. VectaCell™ Acridine Orange and VectaCell™ Rhodamine 123 reagents offer convenience and ease of use for visualizing different cellular components.

VectaCell™ Trolox Antifade Reagent

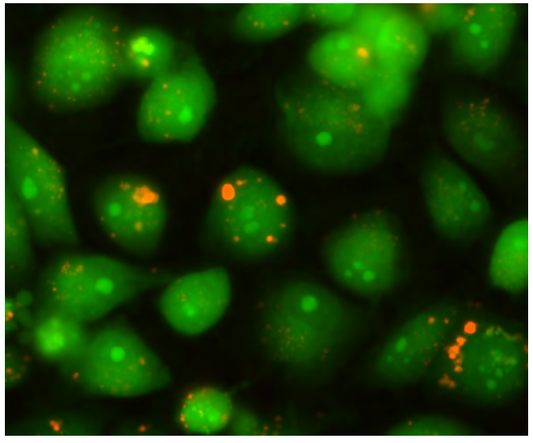
VectaCell™ Trolox Antifade Reagent is an antifading additive for live cell imaging. VectaCell™ Trolox Antifade Reagent contains both Trolox and its oxidized form Trolox-quinone. This redox system reduces photo-bleaching and blinking during live cell imaging.

Trolox is a water-soluble and cell-permeable analog of vitamin E that efficiently prevents formation of different reactive oxygen species, such as singlet oxygen ($^1O_2$), superoxide anion (O_2-) or hydrogen peroxide (H_2O_2). Photo-excitation of a fluorophore generates reactive oxygen species that can lead to photo-bleaching and oxidative damage in cells. Trolox has a cytoprotective effect and low cytotoxicity for different cell lines.

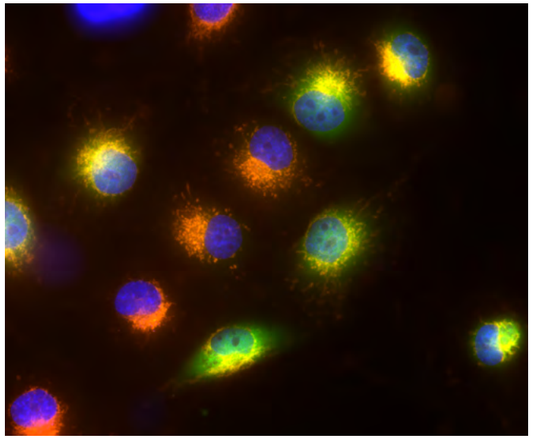
Live Cell Imaging of Organelles

VectaCell™ Acridine Orange is a fluorescent dye that stains acidic organelles, such as lysosomes, autosomes or yeast vacuoles. At low pH inside organelles, the dye will emit an orange fluorescence (peak at 590 nm). For optimal endosome visualization, use a blue light excitation (475 nm).

VectaCell™ Rhodamine 123 is a fluorescent dye for staining active mitochondria. This dye accumulates in the mitochondrial membrane based on membrane polarization. Excitation peak at 505 nm, emission peak at 534 nm.



Acidic endosomes stained with VectaCell™ Acridine Orange in MCF-7 cells expressing GFP.



Mitochondria stained with VectaCell™ Rhodamine 123 (orange) in MCF-7 cells expressing GFP. Nuclei stained with DAPI (blue).

Product	Catalog Number
VectaCell™ Trolox	CB-1000
VectaCell™ Acridine Orange	CB-2000
VectaCell™ Rhodamine 123	CB-2100

Accessory Reagents

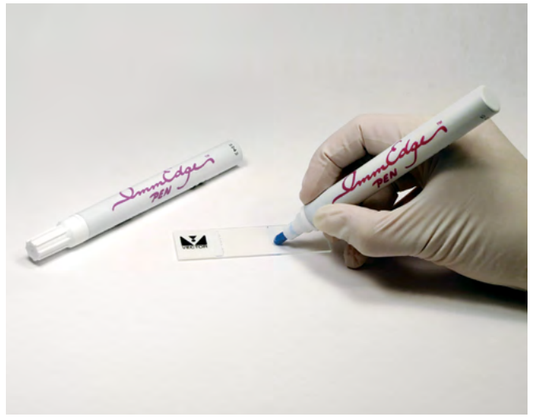
VECTABOND™ Reagent Tissue Section Adhesive

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

ImmEdge™ Hydrophobic Barrier Pen

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

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



ImmPrint™ Histology Pen

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

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

Control Antibodies

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

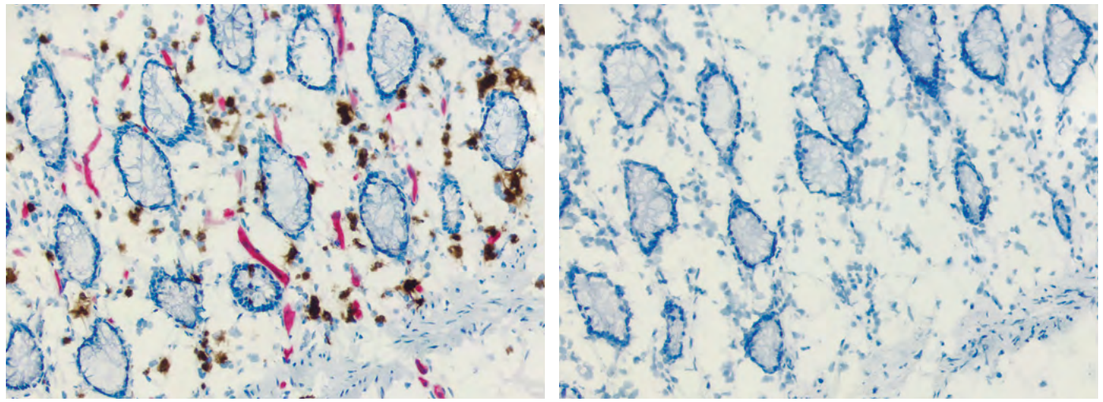
Antigen Unmasking Solutions

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

Product	Catalog Number
VECTABOND™ Reagent (Tissue Section Adhesive)	SP-1800
ImmEdge™ Hydrophobic Barrier Pen	H-4000
ImmPrint™ Histology Pen	H-6100
Control Antibodies	
Rabbit IgG	I-1000
Mouse IgG	I-2000
Rat IgG	I-4000
Goat IgG	I-5000
Antigen Unmasking Solutions	
Citrate-based (100X) (pH 6.0)	H-3300
Tris-based (100X) (pH 9.0)	H-3301

Blocking Background Signal

Blocking agents minimize background signal from endogenous enzyme activity, biotin, and non-specific binding of tissue elements (charged particles, macromolecules, Fc receptors) with detection reagents.



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

BLOXALL™ Endogenous Peroxidase and Alkaline Phosphatase Blocking Solution

Tissues may contain endogenous peroxidase, pseudo-peroxidase, and/or alkaline phosphatase activity that will produce background staining. BLOXALL™ Endogenous HRP/AP Blocking Solution inactivates each of these enzymes in one step.

- Compatible with formalin-fixed, paraffin-embedded tissue sections, frozen tissue sections, and cell preparations
- Ready-to-use in a convenient dropper bottle
- More effective than conventional blocking methods
- Brief 10-minute incubation

Levamisole Solution

Levamisole Solution specifically inhibits endogenous alkaline phosphatase activity.

- Can be added to the alkaline phosphatase substrate solution
- Does not inhibit the isoenzyme used for the VECTASTAIN® ABC-AP reagents, ImmPRESS™- AP Reagents and other alkaline phosphatase conjugates
- Ready-to-use in a convenient dropper bottle

Avidin/Biotin Blocking Kit

The Avidin/Biotin Blocking Kit blocks all endogenous biotin, biotin receptors, and avidin binding sites present in tissues to prevent non-specific binding of avidin or biotinylated reagents with avidin-biotin detection systems. Ready-to-use in a convenient dropper bottle.

Streptavidin/Biotin Blocking Kit

Streptavidin/Biotin Blocking Kit blocks all endogenous biotin, biotin receptors, and streptavidin binding sites present in tissues to prevent non-specific binding of streptavidin or biotinylated reagents with biotin/streptavidin detection systems. Ready-to-use in a convenient dropper bottle.

Normal Sera

Our Normal Sera are pooled samples collected from healthy adult animals. The serum is heat-treated and centrifuged to remove precipitates and then filtered. Each serum is tested with the appropriate antibody to check for possible cross-reactivities. The sera can be used to block non-specific binding or as an antibody diluent.

2.5% Normal Sera

Our 2.5% Normal Sera are pooled samples collected from healthy adult animals.

- Heat-treated and centrifuged to remove precipitates and then filtered
- Tested for cross-reactivities
- Can be used for blocking non-specific binding or as an antibody diluent
- Ready-to-use, prediluted, stabilized solution

Bovine Serum Albumin (BSA)

Immunohistochemical Grade

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

10x Casein Solution

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

Carbo-Free™ Blocking Solution

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

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

R.T.U. Animal-Free Blocker™ and Diluent

This plant protein-derived product is a universal antibody diluent and blocking reagent intended for cell- and tissue-based IHC and IF applications. This ready-to-use solution can be used as an alternative to normal sera, BSA, casein and non-fat dry milk.

R.T.U. Animal-Free Blocker™ and Diluent is supplied without any sodium azide. It can therefore be used with both peroxidase and alkaline phosphatase antibody conjugates and all secondary detection reagents including polymer systems and avidin/biotin reagents that incorporate these enzymes. This makes the blocking solution especially convenient in multiple antigen labeling IHC applications in which antibodies from different species and a variety of detection systems are used on the same tissue section.

R.T.U. Animal-Free Blocker™ and Diluent is a unique formulation different from our concentrated (5x) animal-free blocker. It has been designed with optimized conditions and neutral pH specifically for IHC and IF methods.

Animal-Free Blocker™ (5x concentrate solution)

Animal-Free Blocker™ is a plant-derived blocking agent and diluent for nucleic acid blotting, protein blotting, IHC, and other applications. This reagent contains no animal-derived protein and can be used as an alternative to sera, BSA, casein, or non-fat dry milk.

Product	Catalog Number
BLOXALL™ Endogenous HRP/AP Blocking Solution	SP-6000
Levamisole Solution	SP-5000
Avidin/Biotin Blocking Kit	SP-2001
Streptavidin/Biotin Blocking Kit	SP-2002
Normal Goat Serum	S-1000
Normal Horse Serum	S-2000
Normal Chicken Serum	S-3000
Normal Swine Serum	S-4000
Normal Rabbit Serum	S-5000
2.5% Normal Goat Serum	S-1012
2.5% Normal Horse Serum	S-2012
Bovine Serum Albumin (BSA)	SP-5050
10x Casein Solution	SP-5020
Carbo-Free™ Blocking Solution	SP-5040
R.T.U. Animal-Free Blocker™ and Diluent	SP-5035
Animal-Free Blocker™	SP-5030

Contact Details

US Office

Vector Laboratories, Inc.
30 Ingold Road
Burlingame, CA 94010, USA
Tel: +1 (650) 697-3600
Tel (Ordering and Technical Support): +1 (800) 227-6666
Fax: +1 (650) 697-0339
Customer Service: vector@vectorlabs.com
Technical Support: technical@vectorlabs.com
International Inquiries: intl@vectorlabs.com

UK Office

Vector Laboratories Ltd.,
3 Accent Park, Bakewell Road, Orton Southgate,
Peterborough, PE2 6XS, United Kingdom
Tel: +44 (0) 1733 237999
Customer Service: vector@vectorlabs.co.uk
Ordering: sales@vectorlabs.co.uk
Technical Support: technical@vectorlabs.co.uk

Ordering Information

Order online at: www.vectorlabs.com

Orders may also be placed by email, telephone, fax, or mail. Please include the following with each order:

- Product name and catalog number
- Unit size and quantity
- Billing and shipping addresses
- Purchase order number
- Name, phone number, address and email address of person placing order

Orders using VISA, Mastercard, or American Express are accepted and processed immediately. Telephone orders over $2000 may require written confirmation. A confirmation should be boldly marked “Confirming Order. Do Not Duplicate.” Duplicate shipments due to incorrectly marked confirming orders cannot be returned for credit.
No returned product will be accepted or credited without prior authorization from Vector Laboratories.

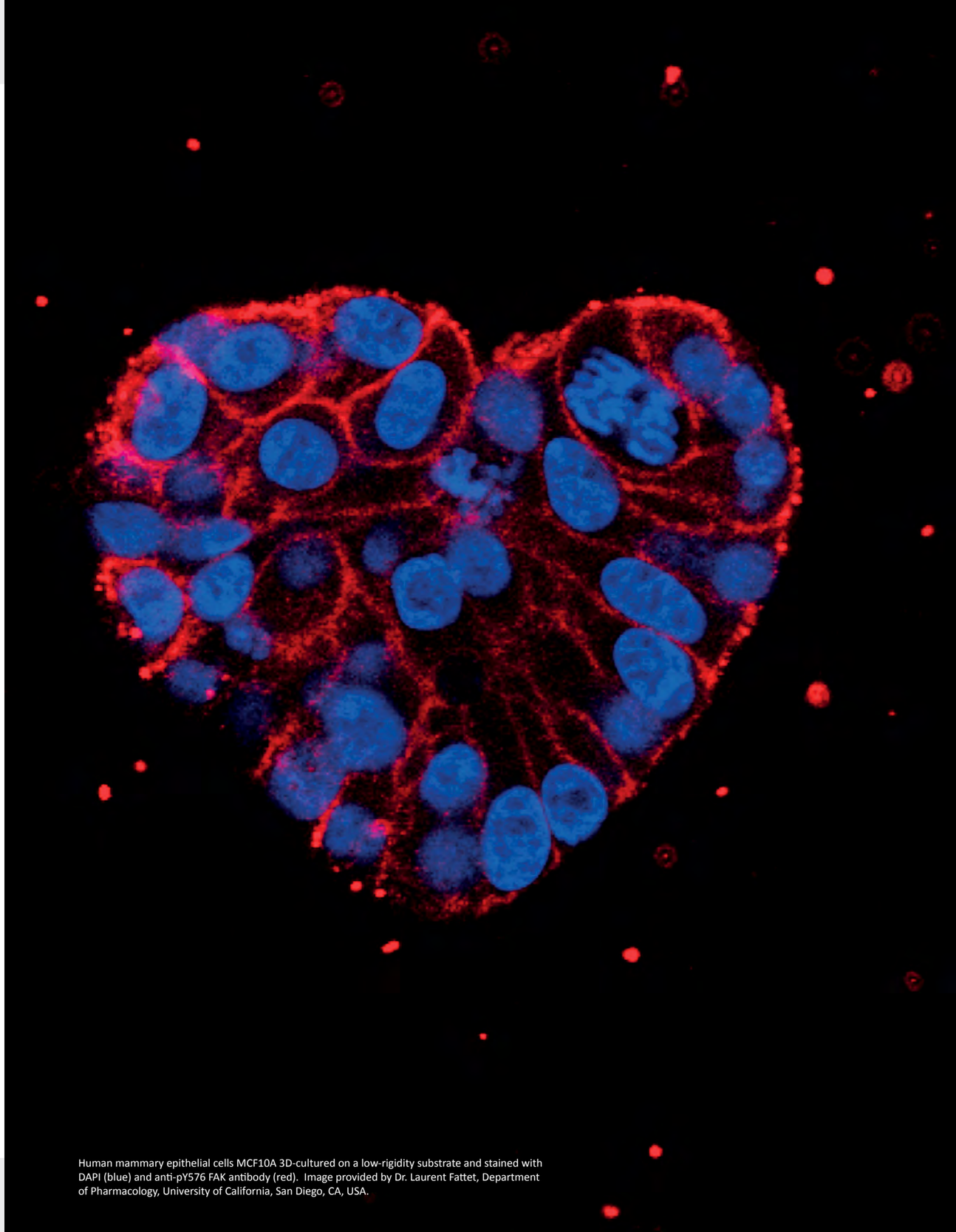
Please contact us to discuss discounts for custom or large orders.

Payment / shipping terms:

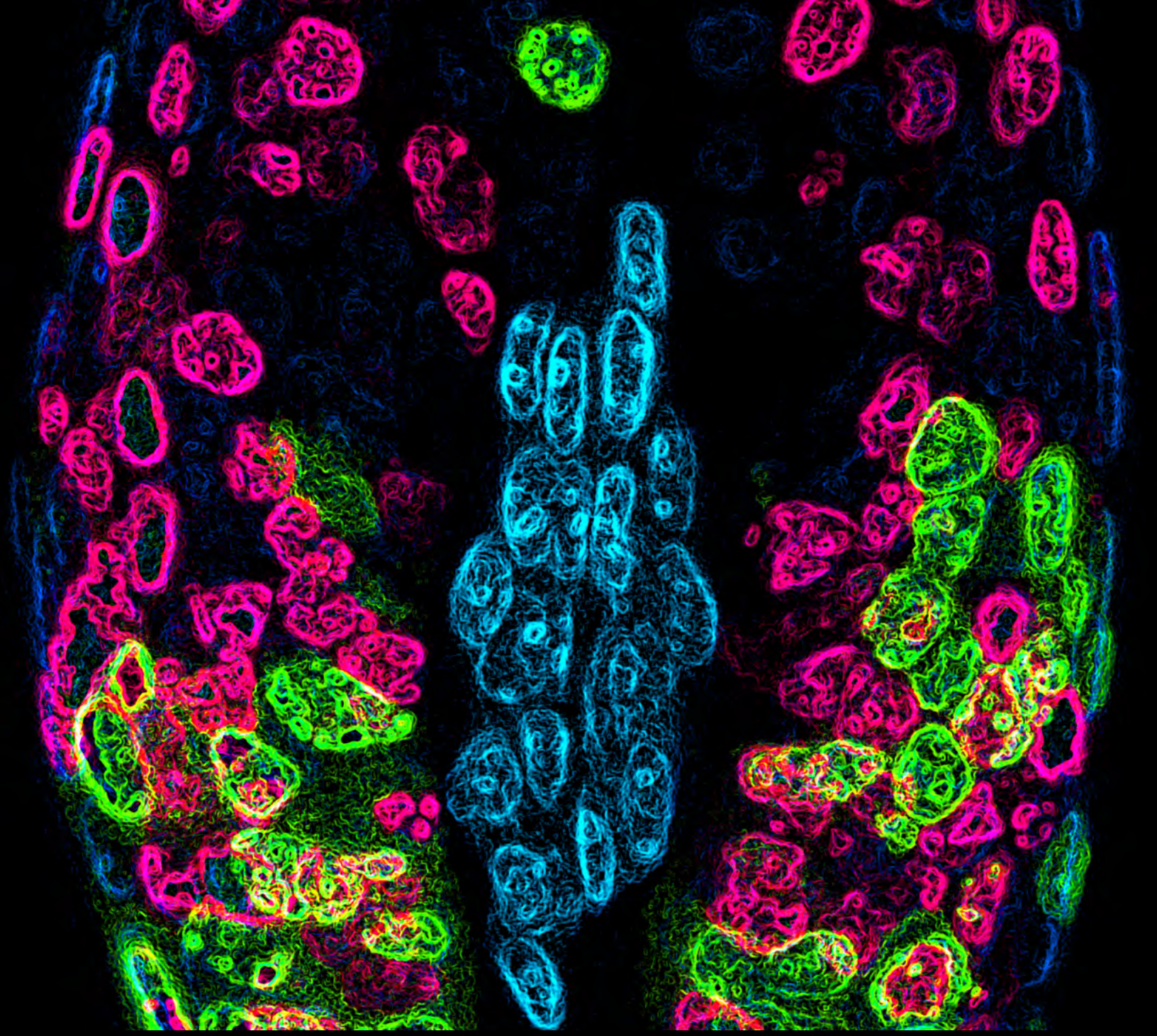
Payment terms: net 30 days. Prices are FCA Burlingame, California. Shipping charges will be prepaid and added to the invoice. Orders are usually shipped the same day they are received. Unless requested otherwise, all products are shipped 2nd-day air. RCA_{60} products are shipped in the USA only according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

International Orders

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



Human mammary epithelial cells MCF10A 3D-cultured on a low-rigidity substrate and stained with DAPI (blue) and anti-pY576 FAK antibody (red). Image provided by Dr. Laurent Fattet, Department of Pharmacology, University of California, San Diego, CA, USA.



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