BioNordika
YOUR SCIENTIFIC SUPPLIER

Science where you are

Volume 3 Issue 2

IMMUNOFLUORESCENCE

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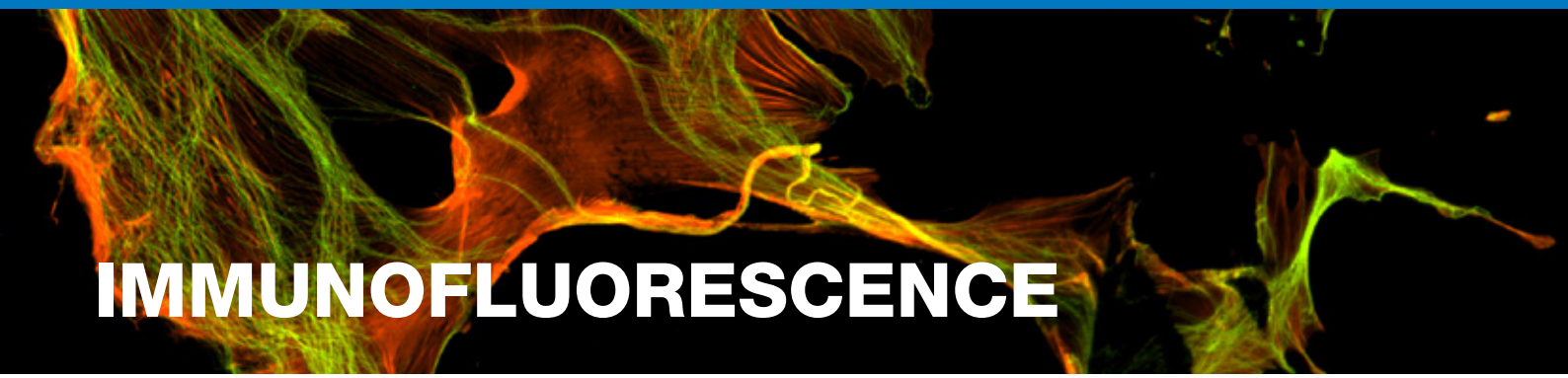


IMMUNOFLUORESCENCE

Studies of protein properties, expression, transport, degradation and their interactions with other cellular systems are integral to biomedical research, drug discovery and developmental biology. Eukaryotic proteins function in signaling pathways, metabolism, structure, adhesion, cell movement, active and passive transport, DNA repair, viral disease mechanisms, the immune system, fertilization, differentiation, epigenetics, cancer and the cell division cycle. It is fundamental to visualize the basic events in the cell to be able to understand the properties and function of certain proteins.

Cell Signaling Technology

Antibodies validated for IF

Cell Signaling Technology (CST) provides antibodies that have undergone rigorous validation to provide researchers with specific antibodies that yield the brightest signal and lowest possible background. CST offers Alexa Fluor® conjugated antibodies that are optimized for IF analyses. CST IF-validated antibodies are powerful tools that can be used to determine the activation status of proteins and characterize signaling pathways relevant to cancer and other diseases.

Direct Conjugated Primary Antibodies and Custom Conjugation Services

Offering primary conjugated antibodies with a variety of conjugation types to meet your needs for Flow Cytometry, IF, Western Blot, and other applications. The conjugated antibodies are tested and validated on the lot level by CST´s in-house team of scientists for specificity, sensitivity, and reproducibility.

Flow Cytometry

CST offers over 600 antibodies recommended for flow cytometry, including a number of Alexa Fluor® and other conjugates optimized for this application.

Fluorescent Multiplex Immunohistochemistry (mIHC)

Fluorescent Multiplex Immunohistochemistry (mIHC) allows for simultaneous detection of multiple proteins of interest on formalin-fixed paraffin-embedded (FFPE) tissue. One approach

CST'S RIGOROUS VALIDATION PROTOCOLS HELPS TO PROVIDE SPECIFIC AND RELIABLE ANTIBODIES, WHICH HELPS YOU GET REPRODUCIBLE AND MEANINGFUL DATA EVERYTIME.

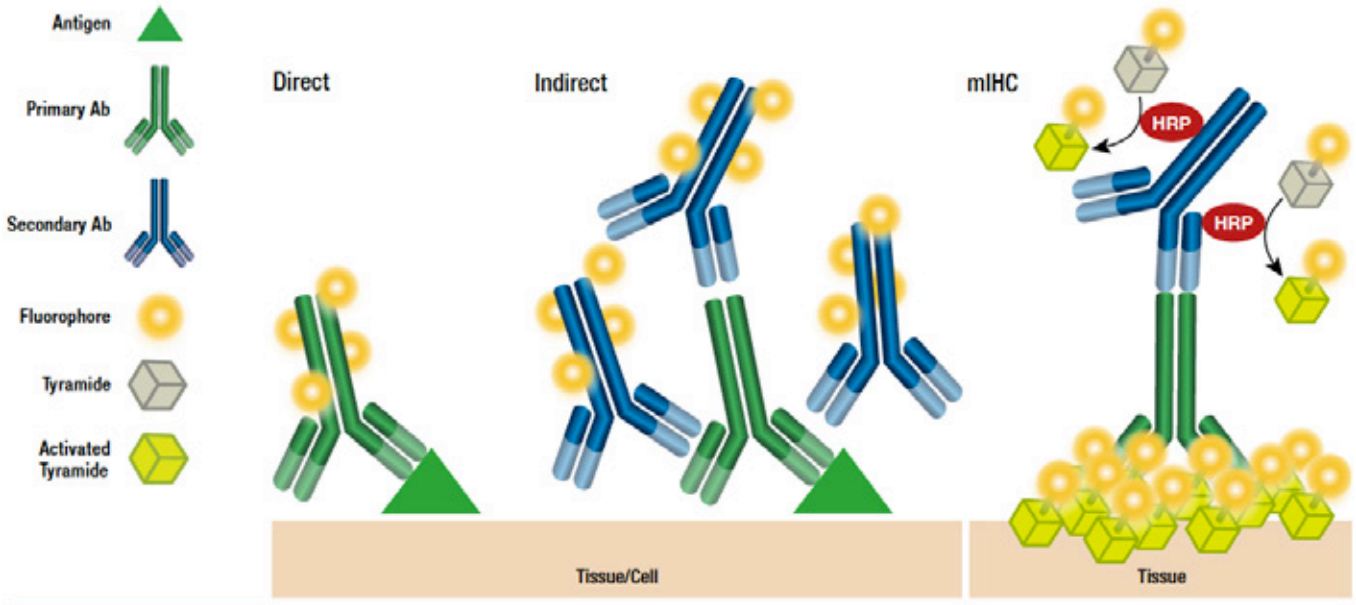
IF Companion Products

- Secondary antibodies
  - » Anti-mouse IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488/555/594/647 Conjugate)
  - » Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488/555/594/647 Conjugate)
  - » Anti-rat IgG (H+L), (Alexa Fluor® 488/555/647 Conjugate)
- Cellular dyes
- Isotype controls
- Blocking reagents and buffers
- Inhibitors & activators
- siRNA

to fluorescent mIHC involves HRP-catalyzed deposition of tyramide-fluorophore conjugates in the vicinity or at the site of the antigen. This approach provides two key advantages: marked signal amplification and simplified panel design, wherein any IHC-P validated antibody regardless of host species or isotype can be used. CST offers thoughtfully pre-selected monoclonal antibody panels relevant to the study of tumor immunology. The panels are stringently validated for multiplex IHC and accompanied by a detailed and highly optimized protocol.

Being able to visualize multiple targets simultaneously is important in research fields like tumor immunology where it is necessary to catalog subsets of immune and cancer cells within the tumor microenvironment. For example, tumors may evade immune detection by manipulating the expression of immune checkpoint proteins (e.g., PD-1, CTLA-4, TIM-3) to "turn off" activated T cells. Alternatively, T cells may become less effective at destroying tumor cells as they succumb to exhaustion, a phenomenon characterized by changes in immune checkpoint protein expression, T cell expression of VISTA, and the appearance of CD68+ co-infiltrating macrophages. Thus, molecular profiling of immune checkpoint proteins together with immune cell phenotyping is key to understanding the complex tumor microenvironment and to enabling the development of tailored, combinatorial therapeutic intervention.

Request the Immunofluorescence Guide!



	Direct	Indirect	mIHC
Antigen Detection	Direct immunoflourescence with primary antibody conjugated to a fluorophore	Indirect immunoflourescence with secondary antibody conjugated to a fluorophore	Indirect immunoflourescence HRP-conjugated secondary antibody with tyramide amplification
Multiplex Protocol	Parallel staining	Parallel staining	Serial staining
Primary Antibody	Same host species can be used for multiple targets	Different host species or isotope for each target	Same host species can be used
Secondary Antibody	No	Yes	Yes
Signal Amplification	None	Moderate	High

Rockland

For over 50 years, Rockland Immunochemicals Inc. has supported global research efforts by developing antibodies and assays. They produce antibodies in their USA animal facilities following processes that control for manufacturing variables to deliver unsurpassed repeatability in antibody production and minimal lot-to-lot variation.

Rockland products are guaranteed to give predictable, repeatable results. They are one of the most referenced companies in the industry and continue to collaborate to develop products such as isoform specific AKT antibodies, GFP and RFP antibodies, and fluorochrome conjugated primary and secondary antibodies validated by the most demanding assays.

Rockland Fluorescent Dye Conjugates

Fluorescent Label	Color	Abs (nm)	Em (nm)	MW (daltons)
DyLight™ 405	Violet	400	420	793
Aminomethylcoumarin (AMCA)	Violet Blue	353	442	410
ATTO 425	Blue	436	484	498
Cy2™	Blue Green	489	505	897
DyLight™ 488	Blue Green	493	518	1,011
ATTO 488	Green	501	523	981
Fluorescein (Fluorescein)	Green	495	528	390
ATTO 532	Yellow Green	532	553	1,081
Cy3™	Yellow Green	552	565	949
DyLight™ 549	Yellow Green	550	568	982
Rhodamine (TRITC)	Orange	550	570	444
R-Phycoerythrin (RPE)	Orange	488	575	240,000
ATTO 550	Orange	554	576	791
Cy3.5™	Orange Red	581	596	1,286
Texas Red®	Red	596	620	625
ATTO 594	Red	601	627	1,389
Allophycocyanin	Far-Red	650	660	100,000
Cy5™	Far-Red	650	667	975
ATTO 647N	Far-Red	644	669	843
DyLight™ 649	Far-Red	646	674	843
ATTO 655	Far-Red	663	684	887
Cy5.5™	Near Infra-Red	678	703	1,312
DyLight™ 680	Near Infra-Red	682	715	950
DyLight™ 800	Infra-Red	770	794	1,050

Fluorescent labeled antibodies can be used to detect target proteins in fluorescent western blots, or dot blots, in FLISA assays, as well as immunofluorescent microscopy, flow cytometry and immunohistochemistry. By using the right combination of fluorescent labeled antibodies multiple targets can be visualized simultaneously in a single assay (fluorescent multiplexing).

DyLight™ Fluorescent Dye Conjugates

DyLight Conjugates exhibit higher fluorescence intensity and photostability than many other dye conjugates. DyLight NIR dyes provide intense fluorescence and sensitivity requiring less conjugated antibody to study your cells. DyLight dyes are exceptional alternatives to Alexa Fluor®, CyDye® and IRDye® fluorescent dyes.

ATTO-TEC Fluorescent Dye Conjugates

Due to their photostability and photophysical properties ATTO-TEC fluorescent labels are ideal for many bio-analytical applications. Absorption and fluorescence of the ATTO NIR dyes are optimized for the red region of the spectrum, with the advantage of efficiently suppressing or by-passing any auto-fluorescence of biological samples.

CyDye® Fluorescent Dye Conjugates

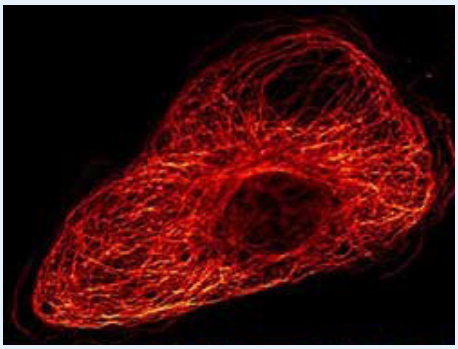
CyDyes are excellent alternatives to most other fluorescent dyes as they are brighter and offer greater photostability. Depending on the specific CyDye, they may also produce less background and may be less sensitive to pH.

Fluorescent TrueBlot

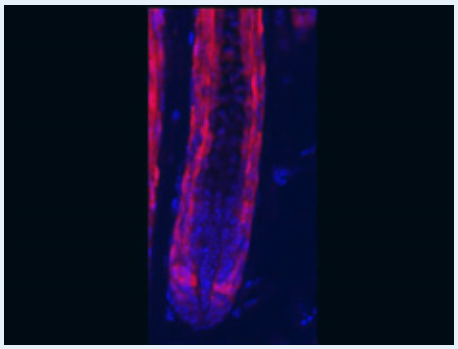
Fluorescent TrueBlot®, a new format of our popular TrueBlot® product line, combines the power and specificity of the original products with the versatility of fluorescent and near infra-red dyes. Conjugating highly optimized TrueBlot® reagents to a full spectrum of fluorescent labels, results in multipurpose reporter molecules that can be used in a variety of immunoassays.

Featured Fluorescent Dye Conjugated Antibodies

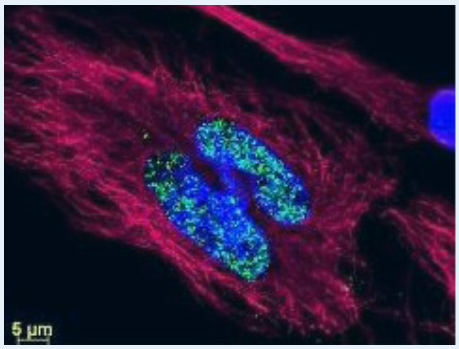
605-453-013	Goat IgG (H&L) Antibody ATTO 532 Conjugated Pre-Adsorbed (Rabbit Polyclonal)
600-401-379	RFP Antibody Pre-adsorbed (Rabbit Polyclonal)
611-641-122	Rabbit IgG (H&L) Antibody DyLight™ 488 Conjugated Pre-Adsorbed (Sheep Polyclonal)
610-142-002	Mouse IgG (H&L) Antibody Dylight™ 549 Conjugated (Goat Polyclonal)



Tubulin in PtK2- male Rat Kangaroo Kidney Epithelial Cells was detected using ATTO 532 labeled secondary antibody.



Immunofluorescence Microscopy of Rabbit Anti-RFP antibody. Primary antibody: RFP antibody. Fluorescein rabbit secondary antibody. Localization: RFP is nuclear and occasionally cytoplasmic. Staining: Hop-derived cells in the hair follicle, labeled in red.



This image shows anti-histone detection using a DyLight™ 488 conjugate (green). Anti-Tubulin was detected using a DyLight™ 549 conjugate (red). Nuclei were counter-stained using DAPI (blue).

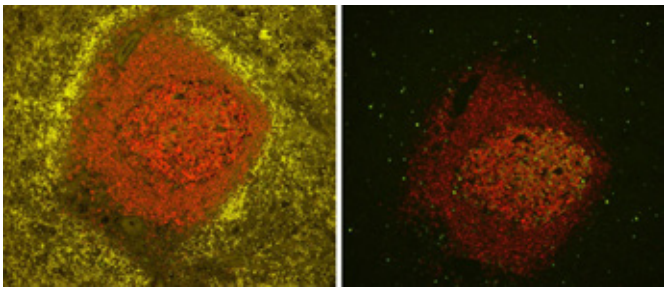
Vector Labs

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

NEW! Autofluorescence Quenching

Reveal true immunofluorescence – even in challenging tissues

Vector® TrueVIEW™ Autofluorescence Quenching Kit (#SP-8400) provides a novel way to remove unwanted fluorescence in tissue sections due to aldehyde fixation, red blood cells, and structural elements such as collagen and elastin. This unique formulation binds and effectively quenches the autofluorescent elements in even the most problematic tissues, such as kidney, spleen and pancreas. The use of Vector® TrueVIEW™ Quenching reagent leads to significant enhancement in overall signal-to-noise in most immunofluorescence assays.



Spleen (FFPE), antigen retrieved with Antigen Unmasking Solution, stained using Mouse Anti-CD20 (red) and Rabbit Anti-Ki67 (green), followed by VectaFluor™ Duet Kit, and mounted in VECTASHIELD® HardSet™ Mounting Medium with DAPI.

Left: No TrueVIEW™ Quencher treatment- note interfereing autofluorescence.

Right: Treated with TrueVIEW™ Autofluorescence Quencher. No TrueVIEW™ Quencher => 20x objective, red channel exposed 200ms, green channel 200ms. With TrueVIEW™ Quencher => 20x objective, red channel exposed 200ms, green channel exposed 800ms.

VECTASHIELD Mounting Media

VECTASHIELD® Antifade Mounting Medium is a unique, stable formula for preserving fluorescence and preventing photobleaching. The 10ml ready-to-use mounting media are stored at 4°C, available in non-hardening and hardening versions, and with and without counterstains.

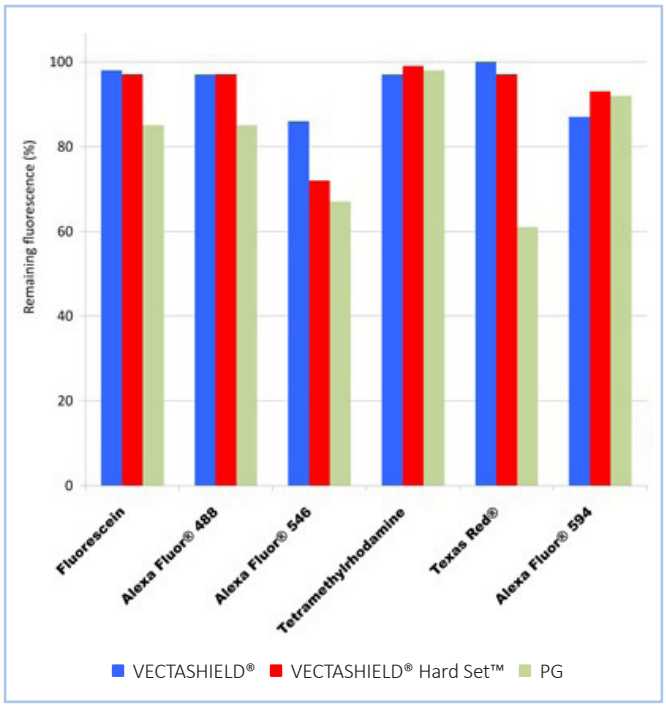
Features:

- Inhibits photobleaching of fluorescent dyes and fluorescent proteins
- Ideal refractive index (1.45)
- Ready-to-use- from the refrigerator to the benchtop
- No warming necessary
- Can be stored without sealing for long term analysis

Counterstain	Non-hardening	Hardening (HardSet)
None	H-1000	H-1400
DAPI	H-1200	H-1500
PI	H-1300	
TRITC - Phalloidin		H-1600

VECTASHIELD HardSet Antifade Mounting Medium with Phalloidin (#H-1600)

This special formulation of VECTASHIELD® HardSet™ Mounting Medium contains TRITC-phalloidin. Phalloidin is a bicyclic heptapeptide that specifically binds at the interface between the F-actin subunits. Fluorescent derivatives of phalloidin are used to stain actin filaments producing an orange-red fluorescence.



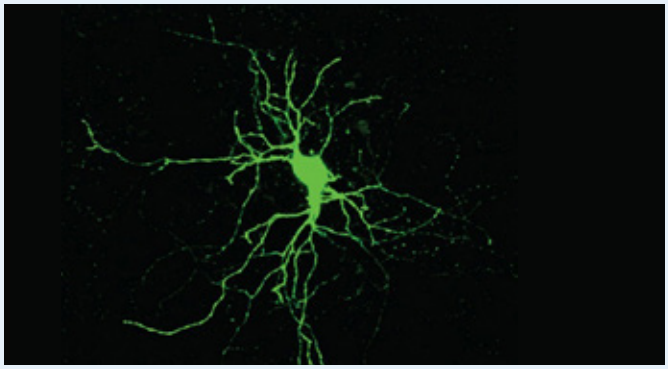
VECTASHIELD Mounting Medium Antifade Comparison
Vector Labs measure antifade properties of VECTASHIELD® mountants using frozen tissue sections immunohistochemically stained with fluorescently labeled secondary antibodies. The intensity after 30 second exposure is expressed as a percentage of the intensity at zero time.

Secondary Antibodies

VectaFluor™ Ready-to-Use (R.T.U.) DyLight® dye-labeled Secondary Antibodies offer maximum convenience for fluorescence staining of cells and tissues. Their affinity purified, extensively cross-adsorbed secondary antibodies are conjugated to DyLight®dyes (488 or 594) in a manner that ensures maximum degree of labeling without compromising antibody affinity or specificity. Species available are anti-rabbit (DI-1788 and DI-1794), anti-mouse (DI-2788 and DI-2794) and anti-goat (DI-3788 and DI-3794).

NEW! VectaFluor™ Duet Kit for double labeling in one step. Apply two colors in **one step** using the VectaFluor™ Duet Immunofluorescence Double Labeling Kit with DyLight®dyes. These kits are designed to save time and effort in double labeling immunofluorescence protocols that can potentially 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. Kits available: DK-8828 (mouse-green/rabbit-red) and DK-8818 (mouse-red/rabbit-green).

Prostate tissue staining (DK-8818)
Rabbit Anti-PSA MAb and Mouse Anti-Smooth Muscle Actin detected simultaneously with VectaFluor Duet Immunofluorescence Double Labeling Kit, DyLight 488 Anti-Rabbit (green)/DyLight 594 Anti-Mouse (red). Mounted in VECTASHIELD HardSet Mounting Media.

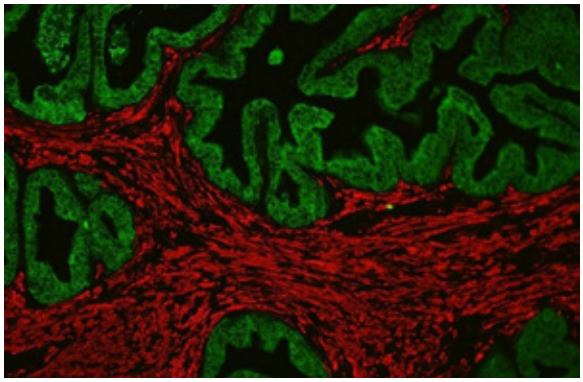


Neuronal Tracers

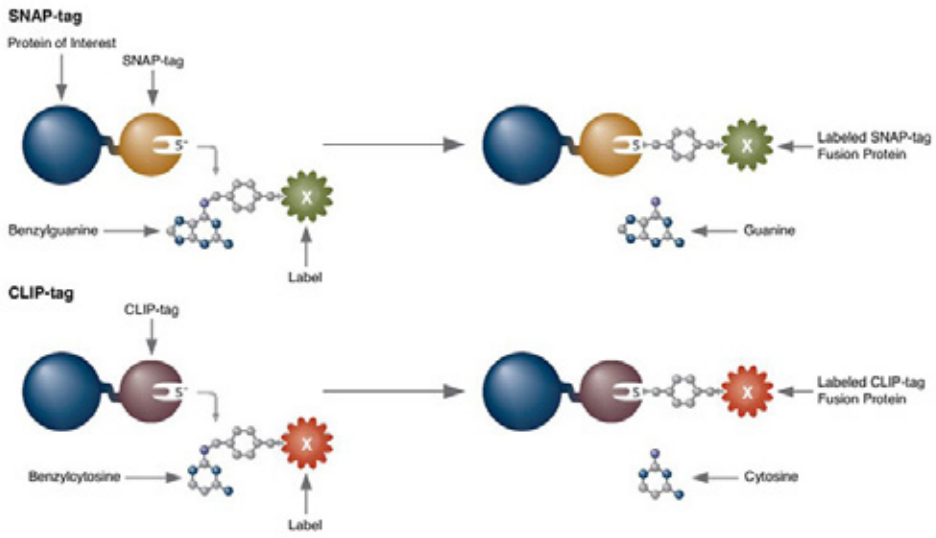
Neuronal tracers are used to elucidate anatomical connections of the nervous system. Tracers are transported retrogradely, from axon to the soma, or anterogradely, from the soma to the axon. These tracers are used to determine cells of origin of axons that innervate a certain structure and to identify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

Neurobiotin

- NEUROBIOTIN-Plus (SP-1150 – 5mg) An amino derivative of biotin that can be used as an intracellular label for cells, particularly neurons. Used for visualizing neural architecture and for the identification of gap junction coupling.
- NEUROBIOTIN 488 (SP-1125 – 2mg) Bright green fluorophore, similar in fluorescence to fluorescein, Cy2 or Alexa Fluor® 488.
- NEUROBIOTIN 350 (SP-1155 – 2mg) Bright blue fluorophore, similar in fluorescence to AMCA or Alexa Fluor® 350.



New England Biolabs



The SNAP- (gold) or CLIP-tag (purple) is fused to the protein of interest (blue). Labeling occurs through covalent attachment to the tag, releasing either a guanine or a cytosine moiety.

Protein labeling with the SNAP/CLIP system

Protein labeling systems offer many advantages. For example, color changes can be easily implemented by using different substrates. Protein labeling systems can be used with non-cell permeable substrates to enable the specific imaging of cell surface targets.

- Clone and express once, then use with a variety of substrates
- Non-toxic to living cells
- Wide selection of fluorescent substrates
- Highly specific covalent labeling
- Simultaneous dual labeling

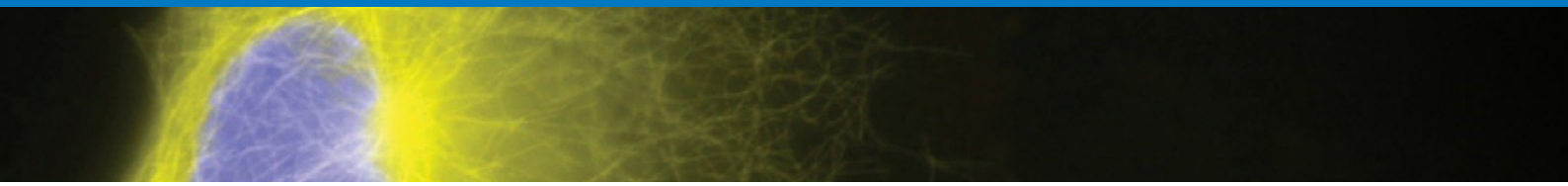
Cellular imaging analysis with recombinant protein labeling systems are among the most sensitive fluorescence methods for imaging expression, transport, co-localization and degradation in either fixed or living cells.

In living cells, protein labeling substrates can be introduced and followed in cells over time. Two separate cellular targets can be imaged simultaneously, using the SNAP- and CLIP-tags from New England Biolabs. Cellular functions and structures can be visually detected using methods such as wide-field fluorescence, confocal, time-resolved and fluorescence resonance energy transfer (FRET) microscopy, or cell based high content assays.

NEB® offers a large selection of fluorescent labels (substrates) for SNAP- and CLIP-tag fusion proteins. SNAP-tag® substrates consist of a fluorophore conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker, while CLIP-tag™ substrates consist of a fluorophore conjugated to a cytosine leaving group via a benzyl linker. These substrates will label their respective tags without no cross reactivity making it suitable for dual-labeling. Cell-permeable substrates (SNAP-Cell® and CLIP-Cell™) are suitable for both intracellular and cell-surface labeling, whereas non-cell-permeable substrates (SNAP-Surface® and CLIP-Surface™) are specific for fusion proteins expressed on the cell surface only.

Applications of SNAP-tag/CLIP-tag

- Simultaneous dual protein labeling inside live cells
- Protein localization and translocation
- Pulse-chase experiments
- Receptor internalization studies
- Selective cell surface labeling
- Protein pull-down assays
- Protein detection in SDS-PAGE
- Flow cytometry
- High throughput binding assays in microtiter plates
- Biosensor interaction experiments
- FRET-based binding assays
- Single molecule labeling
- Super-resolution microscopy



SNAP-tag Cell-permeable

Substrates	Cat#	Excitation*	Emission*†		Size
SNAP-Cell® 505-Star	#S9103	504	532		50 nmol
SNAP-Cell® Oregon Green®	#S9104	490	514		50 nmol
SNAP-Cell® 647-SiR	#S9102	645	661		30 nmol
SNAP-Cell® Fluorescein	#S9107	500	532		50 nmol
SNAP-Cell® 430	#S9109	421	444/484 nm		50 nmol
SNAP-Cell® TMR-Star	#S9105	554	580		30 nmol

SNAP-tag Non-cell-permeable

Substrates	Cat#	Excitation*	Emission*†		Size
SNAP-Surface® 549	#S9112	560	575		50 nmol
SNAP-Surface® 594	#S9134	606	626		50 nmol
SNAP-Surface® 649	#S9159	655	676		50 nmol
SNAP-Surface® 488	#S9124	506	526		50 nmol
SNAP-Surface® Alexa Fluor® 488	#S9129	496	520		50 nmol
SNAP-Surface® Alexa Fluor® 546	#S9132	558	574		50 nmol
SNAP-Surface® Alexa Fluor® 647	#S9136	652	670		50 nmol

CLIP-tag Cell-permeable

Substrates	Cat#	Excitation*	Emission*†		Size
CLIP-Cell™ 505	#S9217	504	532		50 nmol
CLIP-Cell™ TMR-Star	#S9219	554	580		30 nmol

CLIP-tag Non-cell-permeable

Substrates	Cat#	Excitation*	Emission*†		Size
CLIP-Surface™ 647	#S9234	660	673		50 nmol
CLIP-Surface™ 488	#S9232	506	526		50 nmol
CLIP-Surface™ 547	#S9233	554	568		50 nmol

* Excitation and emission values determined experimentally for labeled protein tag.
† Colors are based on the electromagnetic spectrum. Actual color visualization may vary.

Cayman

Fluorescent Probes

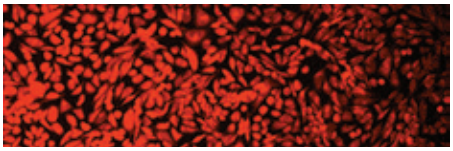
Achieve the versatility, sensitivity, and quantitative capabilities you need in your research with Cayman's diverse library of more than 200 fluorescent probes. We offer a range of probes to detect intracellular events, protein interactions, a host of enzyme substrates, and many other significant targets. Use this at-a-glance guide to select the most appropriate fluorochromes for your experiment

Cell Viability, Cell Cycle, Cell Proliferation

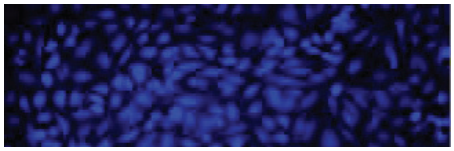
Item No	Product Name	Detects	Excitation Maximum (nm)	Emission Maximum (nm)
14338	Acridine Orange	cell cycle phase; pH	502 (dsDNA) 460 (ssDNA and RNA) 475 (low pH)	525 (dsDNA) 650 (ssDNA and RNA) 590 (low pH)
11397	7-Aminoactinomycin D	DNA; cell viability	488, 546, 578	650
14948	Calcein AM*	cell viability	494	517
20635	Calcein Deep Red™ Acetate*	cell viability	646	659
20641	Calcein Orange™ Diacetate*	cell viability	525	550
20632	Calcein Red™ AM*	cell viability	560	574
20639	Calcein UltraBlue™ AM*	cell viability *(higher photostability and stronger fluorescence intensity at physiological pH than Calcein Blue)*	360	445
16802	CFSE	cell proliferation	494	520
20695	CytoCalcein™ Violet 500	CytoCalcein™ Violet 500	405	500
20698	CytoTrace™ Red CMTPX	cell division; cell trafficking; cell-to-cell interactions	577	602
19774	DRAQ7™	DNA; cell viability	599/644	697
19583	Green CMFDA	cell division; cell trafficking	492	517
15547	Hoechst 33342 (hydrochloride)	DNA (A-T-rich sequences); cell cycle; nuclear morphology	350	461
15003	JC-1	mitochondrial integrity; apoptosis	485 (monomer) 520-570 (aggregate)	535 (monomer) 570-610 (aggregate)
14289	Propidium Iodide	DNA; RNA; cell viability	488-535	617
14488	Pyronin Y	dsRNA; cell cycle	540-550	560–580
14322	Resazurin (sodium salt)	enzyme activity; cell viability	530-540	585-595
21686	Resorufin Acetate	cytosolic ALDH esterase and chymotrypsin activity	571	585

**Also available: membrane-impermeable non-AM forms*

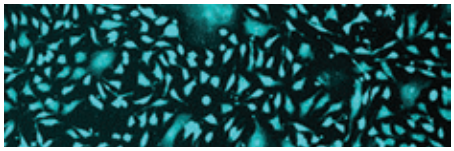
View a complete list of cell health fluorescent probes online at www.caymanchem.com



CPA cells stained with Calcein Orange™ Diacetate



Live HeLa cells stained in medium with Calcein UltraBlue™ AM



CPA cells stained with CytoCalcein™ Violet 500

Genscript

THE™ Elite Antibodies

Genscript's THE™ Elite Antibodies are designed to meet the most demanding research requirements. THE™ Elite Antibodies are divided in three major categories: Tag & Cell Marker Antibodies, Loading Control Antibodies, and Assay Antibodies. These include antibodies towards His-tag, GST-tag, Flag (DYKDDDDK), V5-tag, BrdU, β-Actin, α-Tubulin, cAMP and cGMP. Each product is available in both unconjugated and conjugated (Fluorescent Dyes, FITC, HRP and Biotin) forms.

Origene

TrueMAB™ Primary Antibodies

TrueMAB™ antibodies are generated using recombinant human proteins with preserved conformations as antigens. TrueMAB™ antibodies are therefore valuable tools for immunoassays such as immunofluorescence where high sensitivity and specificity for the recognition of native epitopes on the target proteins' conformational structures is desired.

Antibodies against Fluorescent Proteins

OriGene offers a comprehensive collection of antibodies against fluorescent proteins. The full list is presented below and includes mCherry, tdTomato, eGFP, turbo GFP, ZsGreen1, mKate and DsRed2. These antibodies are also available as conjugates to HRP or magnetic beads for Western blot, immunoprecipitation or protein purification.

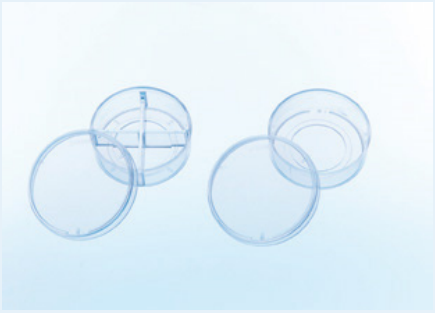
Class	Protein	Excitation (nm)	Emission (nm)
Cyan	AmCyan1	458	489
	eCFP	439	476
	mBFP	402	457
	mCFP	458	480
Green	eGFP	488	507
	mGFP	483	506
	tGFP	*482*	502
	ZsGreen1	493	505
Yellow	mBanana	540	553
	mYFP	508	524
	tYFP	525	538
Orange	mOrange	548	562
	mOrange2	549	565
Red	DsRed-Express2	554	591
	KillerRed	585	610
	mCherry	587	610
	mStrawberry	574	596
	tRFP	553	574
Photo-Switchable	Dendra2	490/553	507/573
	PS-CFP2	400/490	468/511

Greiner

Imaging Consumables

SensoPlate™ – Glass Bottom Plates

The SensoPlate™ product line offers plates with optically clear glass bottom and a black polystyrene frame suitable for microscopic applications where low autofluorescence and optical clarity are required *i.e.* confocal microscopy. SensoPlates™ are available sterile with lid in 24, 96, 384 and 1536 formats.



CELLview™ Cell Culture Dish

The CELLview™ Cell Culture Dish allows researchers to get high resolution microscopic images of their *in vitro* cultures by combining the convenience of a standard size 35 mm disposable plastic dish with a glass bottom of high optical quality. The subdivided version of the CELLview™ Cell Culture Dish significantly minimizes the number of cells and reagents required per individual assay as well as reducing the time needed for four different analyses.

CELLview™ slide

CELLview™ slide is excellently suited for all microscopic applications requiring cell culture with subsequent cell stimulation and/or immunocytochemical analysis. It consists of a transparent slide with an embedded cover glass and a black compartmentalization divided into 10 round wells, which can be detached prior to further analysis, mounting or long time storage. Beside the TC surface CELLview™ slide is also available with the Advanced TC™ surface for sensitive cells or complex applications.