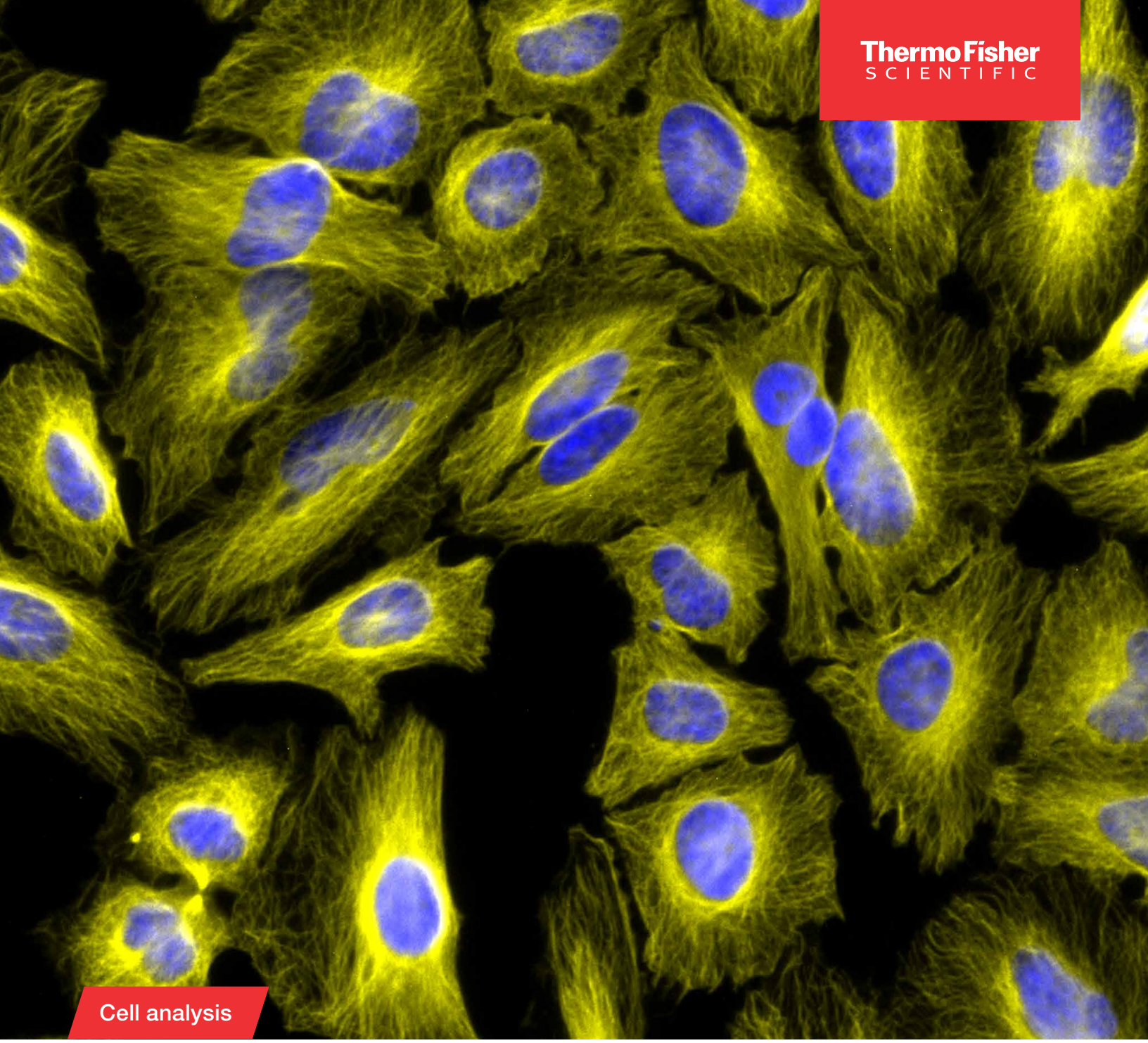


ThermoFisher SCIENTIFIC

Fixed-cell imaging: five steps to publication-quality images

Follow this proven guide to capture the best possible fixed-cell images

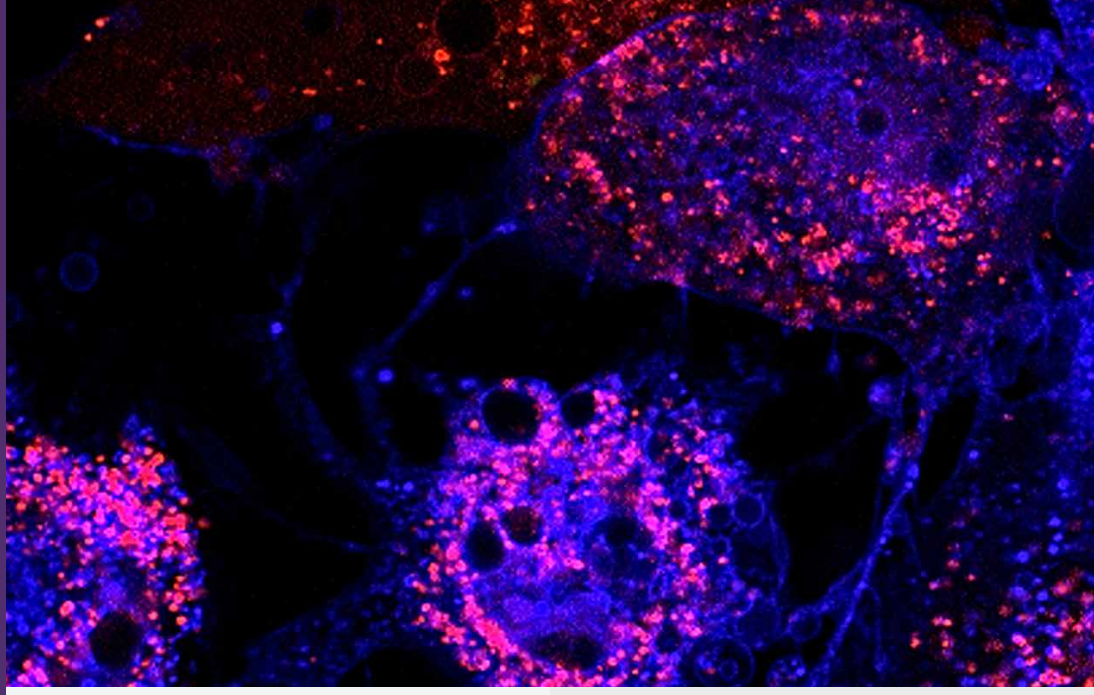
invitrogen

Introduction

We are all driven by great scientific innovation and believe that the journey to discovery is as important as the discovery itself. Choosing the right path can hasten your success, while the wrong path can lead to wrong turns that extend the journey unnecessarily at the expense of time, money, and frustration.

With 40 years dedicated to cell imaging research, we offer long-proven tools and protocols to create quality cell images confidently the first time. In fact, Invitrogen™ imaging reagents and antibodies are cited more frequently in published research than any others. Leverage our experience to enable your success and avoid costly wrong turns.

Whether you are new to cell imaging or an experienced researcher wanting to confirm your knowledge, consider these five proven steps to help ensure that your fixed-cell images are publication-ready the first time.



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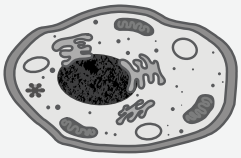
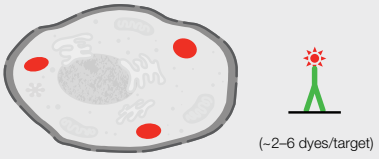


Fix, permeabilize, and block—prepare your cells for fluorescent labeling

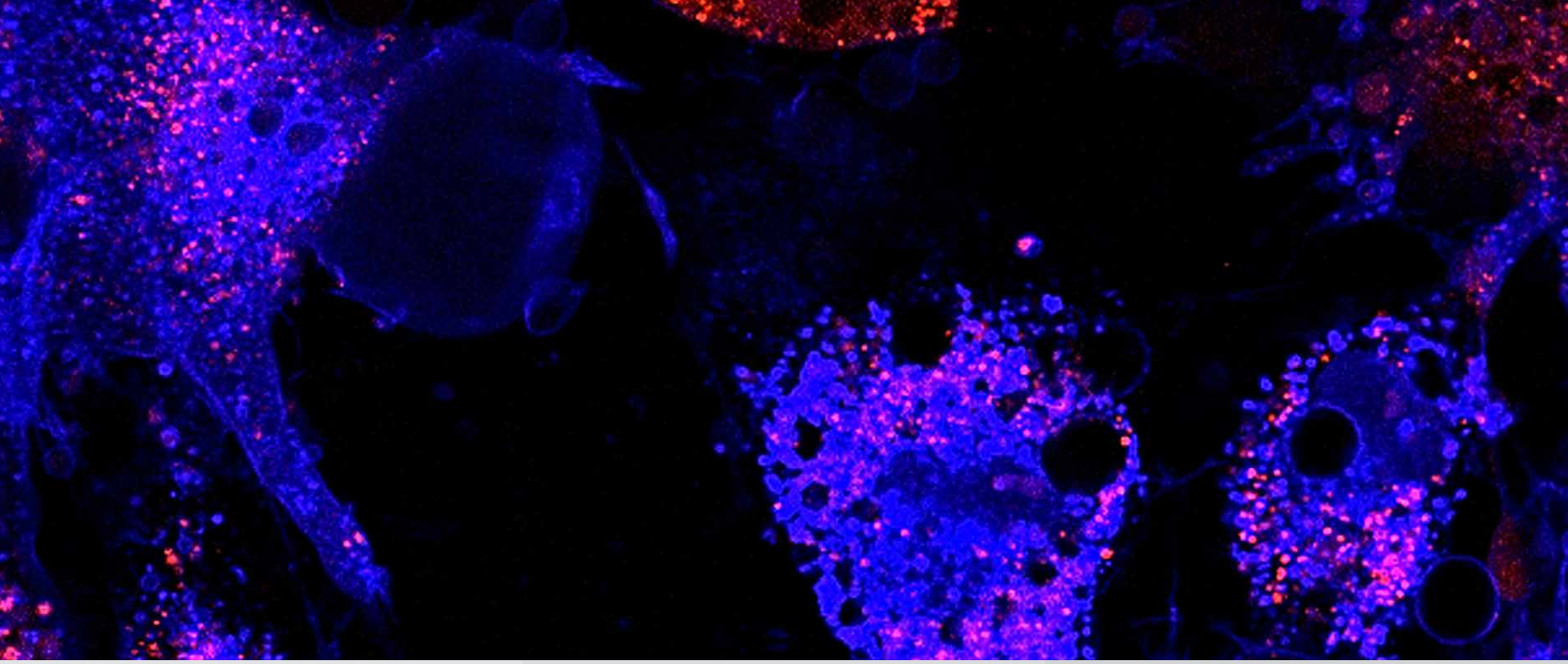
- Invitrogen™ Image-iT™ Fixation/ Permeabilization Kit
- Invitrogen™ BlockAid™ Blocking Solution

2



Label—target cell structures and proteins of interest with organelle-selective dyes, stains, and primary antibodies

- Invitrogen™ NucBlue™, NucRed™, ActinGreen™ 488, and ActinRed™ 555 ReadyProbes™ reagents
- Antibody labeling kits



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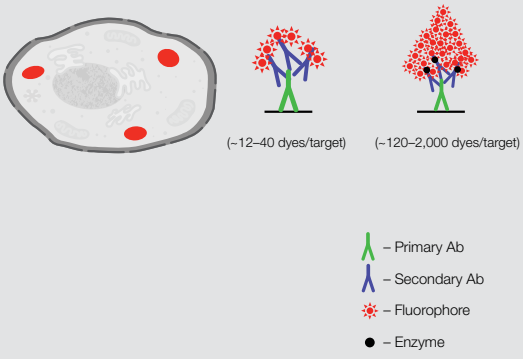
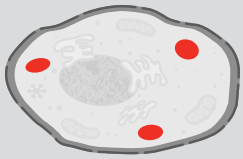


Detect—fine-tune the fluorescence signal for detailed observation

- Invitrogen™ Alexa Fluor™ family of secondary antibodies
- Invitrogen™ Tyramide SuperBoost™ kits with Alexa Fluor™ tyramides using Tyramide Signal Amplification (TSA™) technology
- Invitrogen™ Image-iT™ FX Signal Enhancer ReadyProbes™ Reagent
- Streptavidin conjugates

4



Protect and enhance—protect the signal from photobleaching and enhance the image quality with the best resolution

- Invitrogen™ ProLong™ Glass Antifade Mountant

5

Image—capture imaging discoveries with maximum clarity and definition

- Invitrogen™ EVOS™ Imaging Systems
- Thermo Scientific™ CellInsight™ CX5 High Content Analysis Platform

1 Step 1. Fix, permeabilize, and block

Prepare your cells for labeling

To achieve optimal imaging quality, begin by setting up your study to spotlight proteins and cell structures of interest while keeping everything else out of the picture. Fixation and permeabilization prepare the cell samples for labeling—first, by locking cellular structures, proteins, and nucleic acids in place, and then by making it possible for antibodies and fluorescent stains to permeate the interior of cells and label the targets of interest. Blocking prevents the fluorescent labels from nonspecifically binding to proteins that are not relevant to your research, thereby maximizing the signal-to-noise ratio.

Product highlight

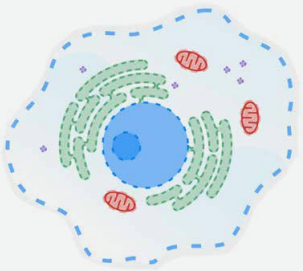
Image-iT Fixation/Permeabilization Kit

- **Convenient**—single-use vials, everything you need in one box
- **Reliable**—methanol-free, which protects fluorescent protein signal
- **Easy to use**—simple protocol, no optimization needed

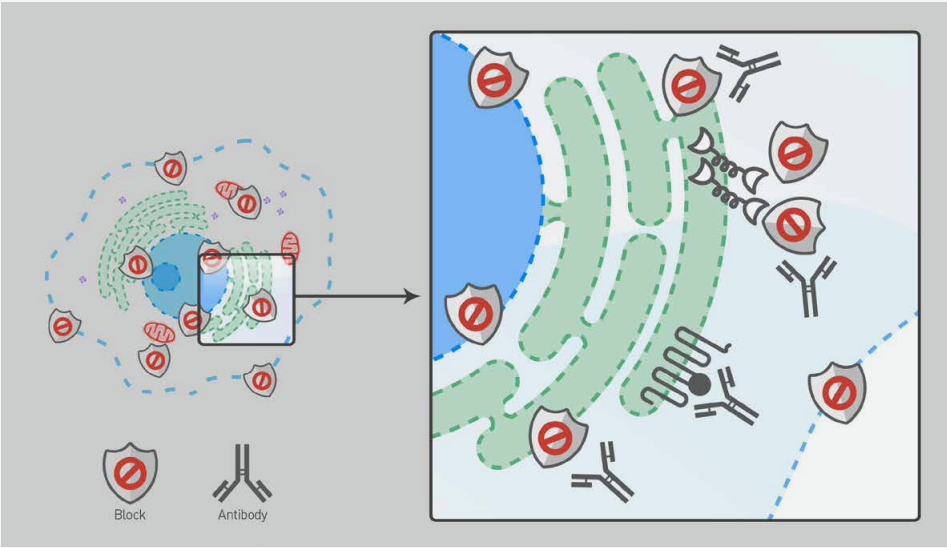
BlockAid Blocking Solution

- **Excellent background reduction**—superior to conventional blocking solutions
- **Ready to use**—no dilution or stock preparation required
- **Versatile**—use with any primary or secondary antibody, regardless of host species

Fixation locks cellular structures in place.



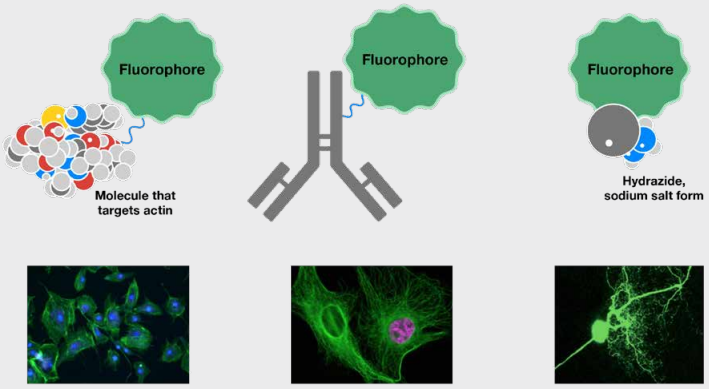
Permeabilization removes membrane lipids, enabling labeling and detection reagents to reach the interior of the cell.



Protein-based blocking agents help reduce nonspecific staining. Antibodies are able to displace the blocking proteins to form high-affinity interactions with their targets, while the remaining blocking proteins prevent low-affinity antibody interactions elsewhere in the sample.

2 Step 2. Label

Labeling various targets with separate fluorescent colors allows you to visualize different structures or proteins within a cell in the same sample. Methods to label your target include fluorescent dyes, immunolabeling, and fluorescent fusion proteins—all of which can provide a means to selectively mark structures and molecules within the cell, allowing you to see them more easily when you image.



A single fluorophore can be modified to carry out any number of labeling jobs, including functionalized forms for labeling cell structure components such as (A) actin and (B) tubulin, or (C) salt forms for whole-cell staining.

Product highlight

Primary antibodies

The Invitrogen™ portfolio offers thousands of high-quality primary antibodies, with specificity to over 85% of the proteome. Some of these antibodies are attached directly to a broad range of intensely fluorescent markers and labels, including Invitrogen™ Alexa Fluor™ dyes.

Explore our extensive portfolio of antibodies at **thermofisher.com/antibodies**

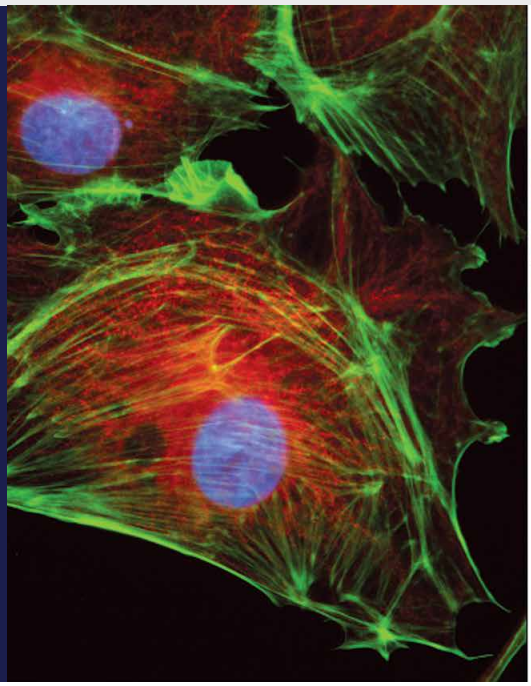
Many fluorescence tools for cell biology are essentially fluorophores that have been modified in different ways or conjugated to various molecules to give them a certain function or allow them to bind to specific organelles or proteins.

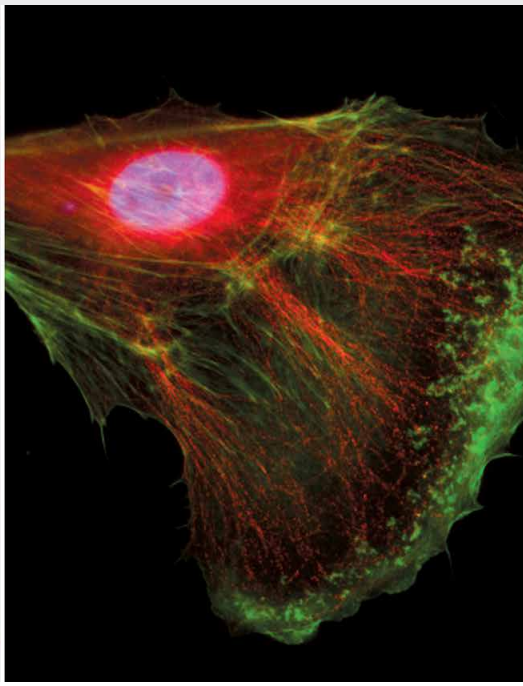
Through chemical modifications, a single fluorophore can be produced in various forms, each with a different specificity. For example, the green-fluorescent Invitrogen™ Alexa Fluor™ 488 dye can be modified to target actin filaments, can be attached to an IgG for use in immunolabeling using our labeling kits, or can act as a whole-cell stain.

Direct labeling

If an antibody is not available with the desired fluorophore, direct labeling can be utilized. A primary antibody directly labeled with a fluorophore often produces lower background fluorescence and less nonspecific binding than labeled secondary antibodies. Further, multiple primary antibodies of the same isotype or derived from the same species can easily be used in the same experiment if they are directly labeled with compatible fluorophores.

Learn more at **thermofisher.com/antibodylabeling**





Step 3. Detect

Fine-tune the fluorescence signal for observation

Detecting complex biological assemblies requires maximum clarity of fluorescence signals and separation of signals from background noise. Standard immunofluorescent labeling rarely provides the highest-quality signal-to-noise visibility. The difference between producing a good and a great publication-quality image requires fine-tuning your sample's signal for peak specificity, definition, and amplification.

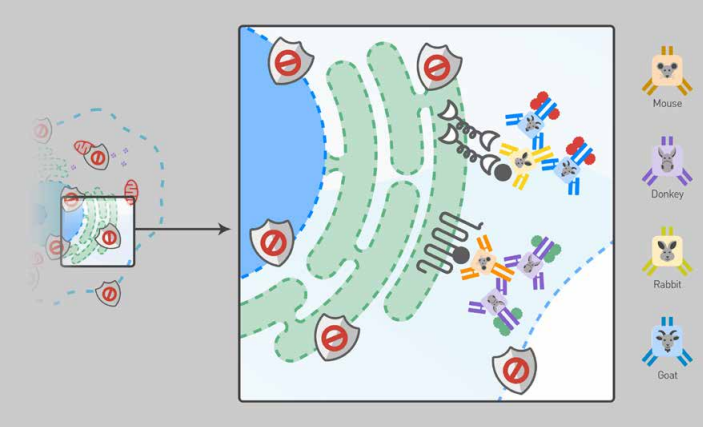
- For high- to medium-abundance protein targets, achieve modest amplification with Alexa Fluor secondary antibody conjugates for high- to medium-abundance protein targets
- For medium- to low-abundance protein targets, achieve signal elevation with either streptavidin conjugates or Invitrogen™ Alexa Fluor™ Plus secondary antibodies
- For low-abundance protein targets, achieve maximum signal enhancement with TSA technology

Quickly and easily choose the labeling solution you need with our immunofluorescence selection guide at **thermofisher.com/immunofluorescence**

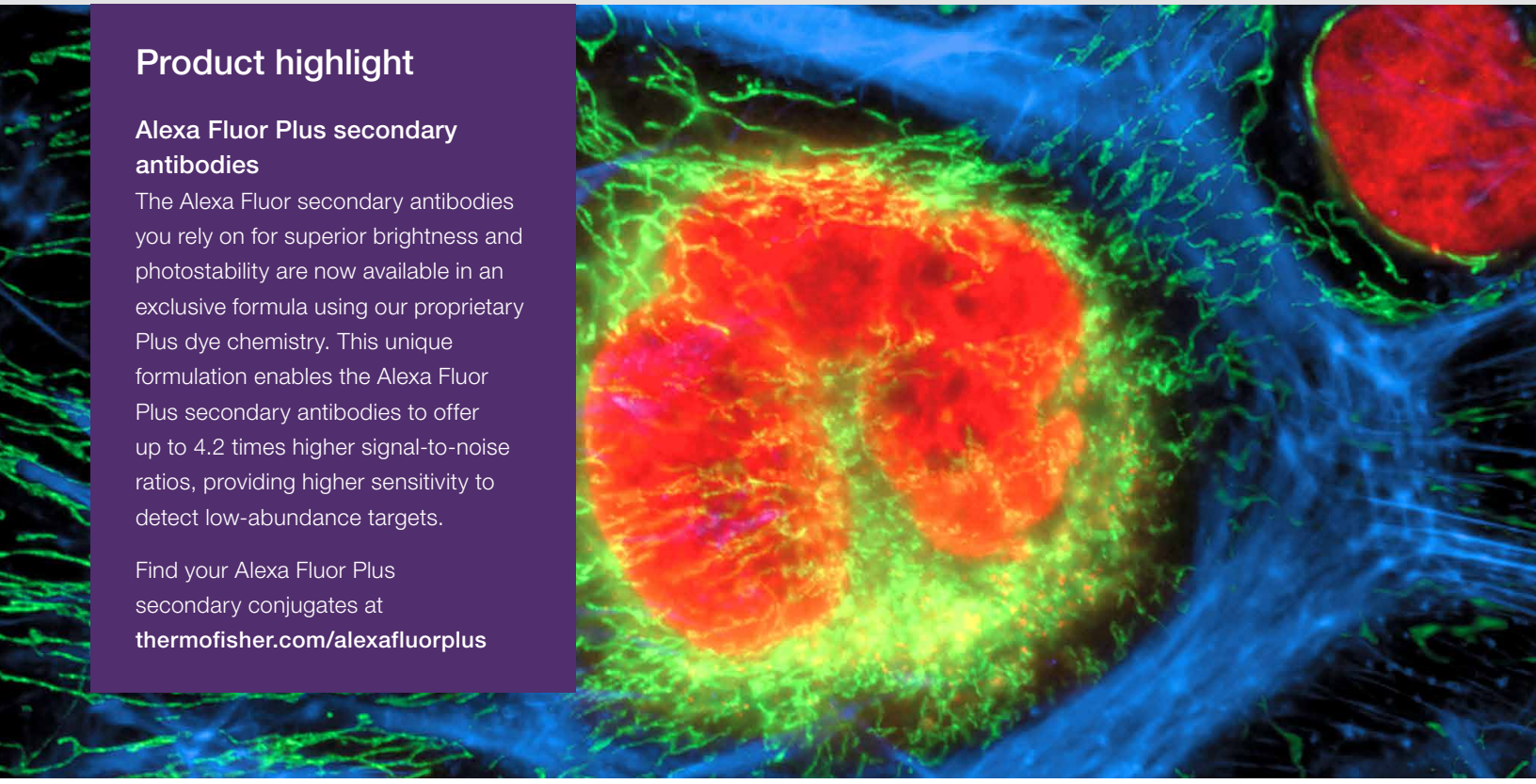
High- to medium-abundance protein targets

Secondary antibodies are used for the indirect detection of targets. While primary antibodies bind directly to the target, secondary antibodies bind indirectly by using the primary antibody as a bridge to the targeted biomolecule. Because multiple secondary antibody molecules can bind to a single primary antibody molecule, this methodology serves to amplify the signal and increase sensitivity to maximize detection.

Find out more at **thermofisher.com/secondaryantibodies**



For secondary detection, the primary antibodies (orange and yellow) bind to their respective epitopes, and fluorophore-labeled secondary antibodies (purple and blue) have specificity for and bind to their respective primary antibodies.



Product highlight

Alexa Fluor Plus secondary antibodies

The Alexa Fluor secondary antibodies you rely on for superior brightness and photostability are now available in an exclusive formula using our proprietary Plus dye chemistry. This unique formulation enables the Alexa Fluor Plus secondary antibodies to offer up to 4.2 times higher signal-to-noise ratios, providing higher sensitivity to detect low-abundance targets.

Find your Alexa Fluor Plus secondary conjugates at **thermofisher.com/alexafluorplus**

Validated primary and secondary antibodies for reproducible results

Thermo Fisher Scientific is committed to adopting higher validation standards for the Invitrogen™ antibody portfolio. We have broadened the range of additional specificity tests utilized to ensure the highest confidence in our products. You can easily identify the products that have already undergone this additional testing, with the Advanced Verification badge.

Learn more at **thermofisher.com/antibodyvalidation**

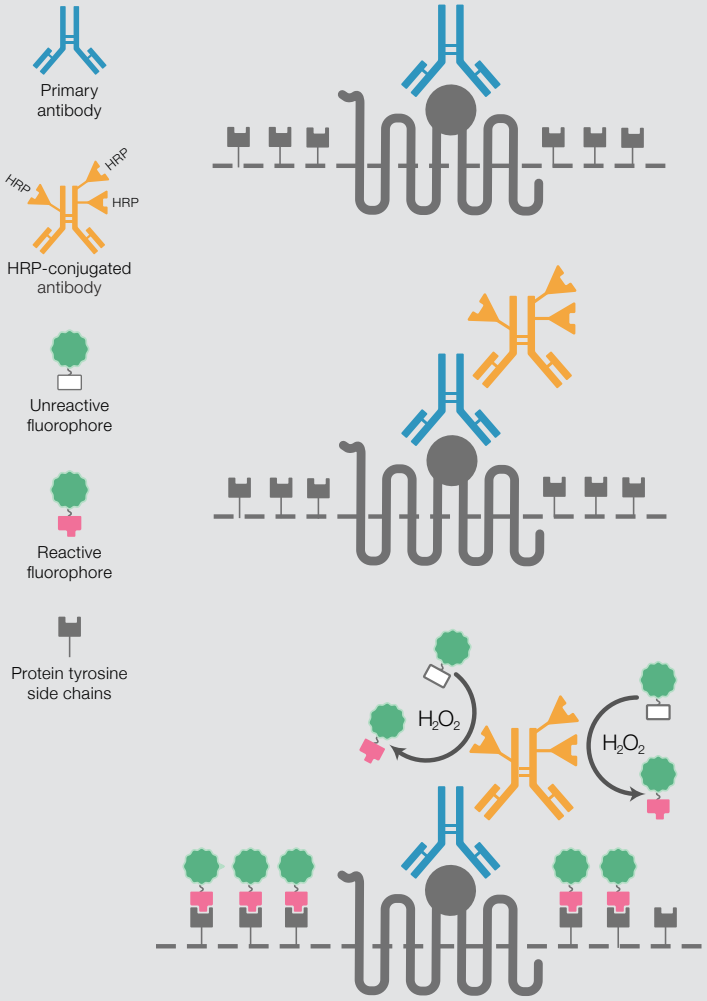
Medium- to low-abundance protein targets

Streptavidin conjugates can provide an increase in the number of fluorophores that label your target, to boost their signals. For improved detection sensitivity, streptavidin-based amplification techniques are widely used in fluorescence imaging to detect primary and secondary antibodies.

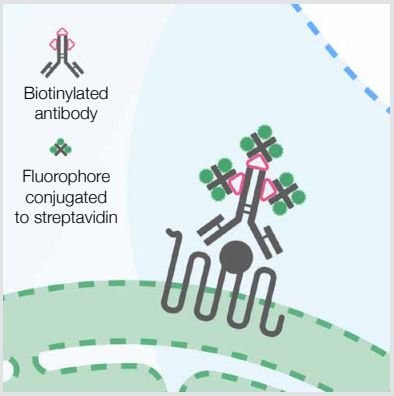
Find out more about imaging with streptavidin at **thermofisher.com/streptavidin**

Low-abundance protein targets

For detection of low-abundance protein targets that are not detectable by conventional means, tyramide signal amplification provides sensitive detection without compromising resolution. Tyramide signal amplification employs an enzyme that releases reactive dyes in the presence of hydrogen peroxide to bring targets out of the background with definition and clarity.



Tyramide signal amplification provides sensitive detection by causing localized high-density deposition of fluorescent labels around epitopes, resulting in superior sensitivity without compromising resolution.



Biotinylated antibodies can bind multiple complexes of a streptavidin-conjugated fluorophore, increasing the number of fluorophores that can be attached to each antibody molecule, thus amplifying the signal compared to traditional secondary labeling.

Learn more at **thermofisher.com/superboost**

Product highlight

Tyramide SuperBoost kits

The Invitrogen™ SuperBoost™ technology is the most sensitive fluorescence imaging detection method for low-abundance protein targets. Offering 10–200 times the sensitivity of standard immunocytochemistry (ICC), immunohistochemistry (IHC), and *in situ* hybridization (ISH) methods, SuperBoost kits are designed for superior signal amplification, definition, and clarity needed for high-resolution imaging. Combining the brightness of Alexa Fluor dyes with trusted poly-HRP–mediated tyramide signal amplification, the SuperBoost reagent generates sensitivity typically 2–10 times above that of standard treatments, including reagents from other suppliers.

Step 4. Protect and enhance

Protect the signal from photobleaching, and enhance the image quality with the best resolution

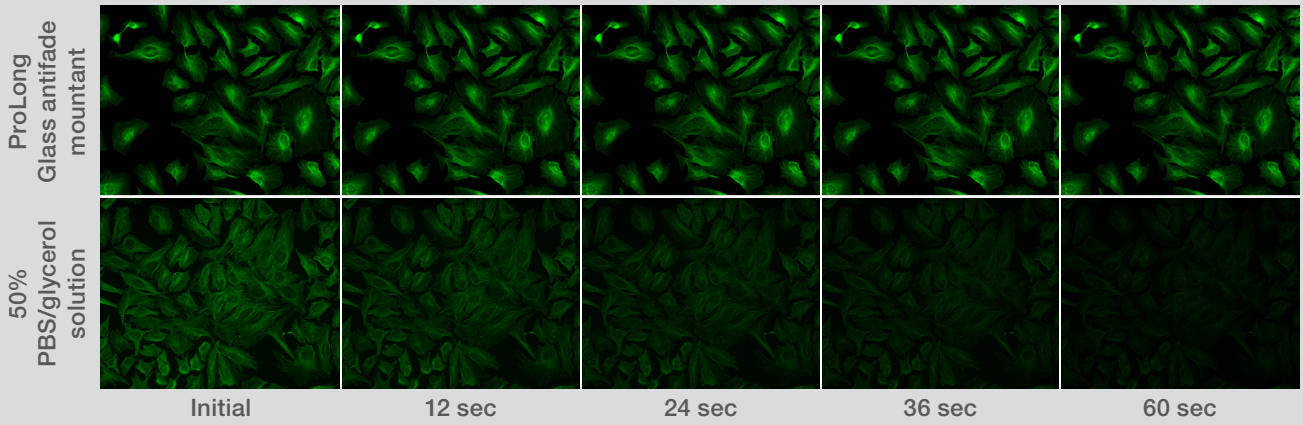
Fluorophores are ideal for high-quality cell imaging but are inevitably prone to photobleaching, which is photochemical degradation and fading of fluorescence signals. Antifade mountants are designed to protect the photostability of fluorophores and maintain image integrity from several weeks to months.

Product highlight

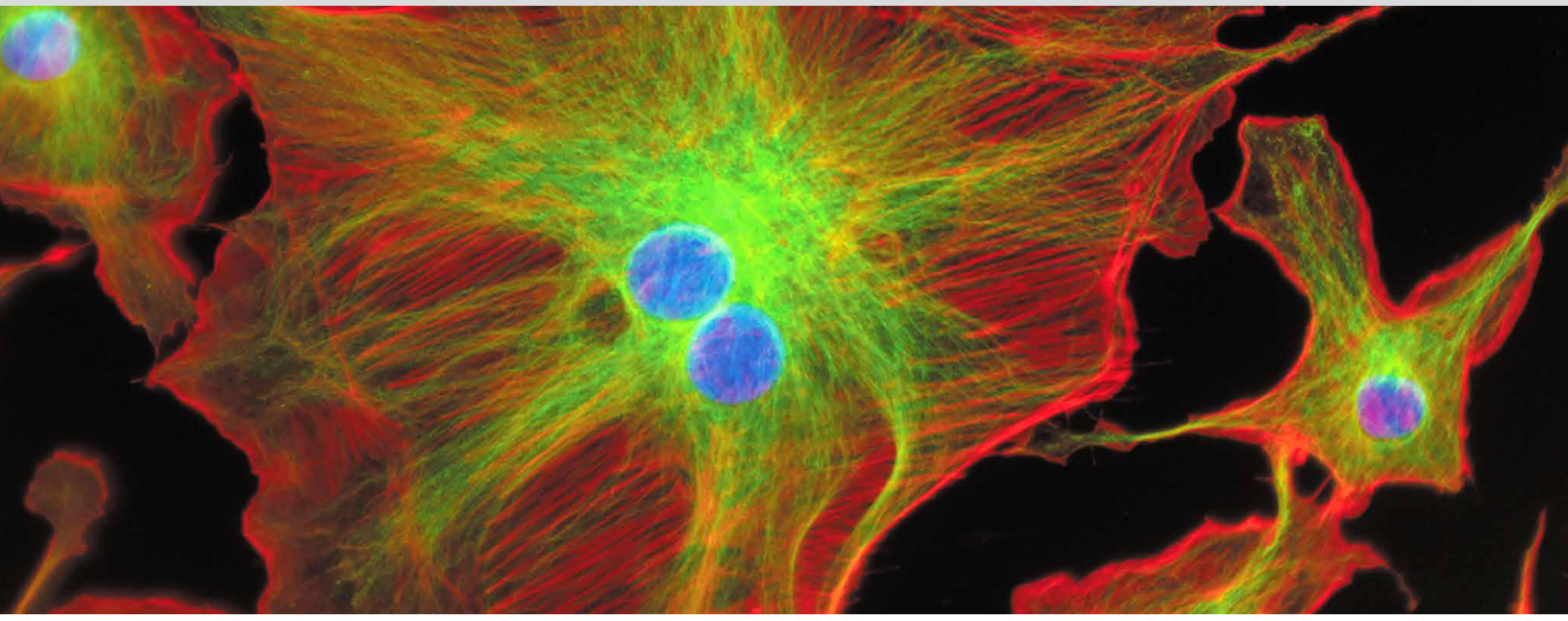
ProLong Glass antifade mountants

ProLong Glass mountants are designed to provide unparalleled antifade protection across the entire visible and near-infrared spectrum. With a refractive index of 1.52, ProLong Glass mountants can be used with many fluorescent dyes on virtually any cell or tissue sample ranging from as thin as 0.1 µm to as thick as 150 µm for bright, high-resolution Z-stack, 3D, and 2D images, and they work particularly well with oil-immersion lenses and confocal microscopes due to decreased distortion of the image.

Learn more at **thermofisher.com/antifades**



A 60 sec time course shows the resistance to photobleaching achieved with ProLong Glass antifade mountant. Fixed HeLa cells were labeled with Invitrogen™ Fluorescein Phalloidin and mounted in ProLong Glass antifade mountant or 50% phosphate-buffered saline (PBS)/glycerol solution. Images were acquired at 12 sec intervals using a 20x objective with continuous illumination from a standard 100 W Hg-arc lamp.



Step 5. Image

Capture research discoveries with maximum clarity and definition

In today's competitive scientific environment, generating publication-quality images is critical to your success. To capture top-quality images, you need an imaging platform with top-of-the-line imaging components, including:

- High-quality cameras and optics to capture high-resolution images
- LED illumination to produce superior signal-to-noise ratios
- Easy-to-use image capture and processing software for ready-to-publish images
- The ability to analyze images for extensive quantitative and statistical analysis

Product highlight

Customizable instruments for your fluorescence experiments

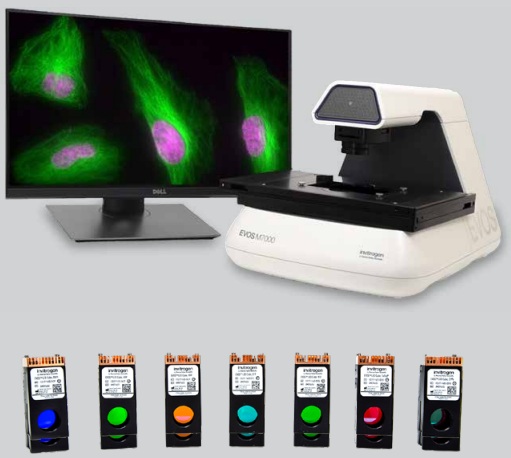
You can get more out of your research with easy-to-use, modular systems that can adjust to fit your experimental needs. We offer imaging systems that can be customized with a variety of LED light cubes, vessel holders, and objectives. There are more than 14 Invitrogen™ EVOS™ LED light cubes to choose from, covering a broad range of fluorescence excitation and emission.

Explore the EVOS lineup at
thermofisher.com/evos

Advanced fluorescence system

EVOS M5000 Imaging System
Perfect for multichannel fluorescence and transmitted light applications

Fully automated fluorescence system



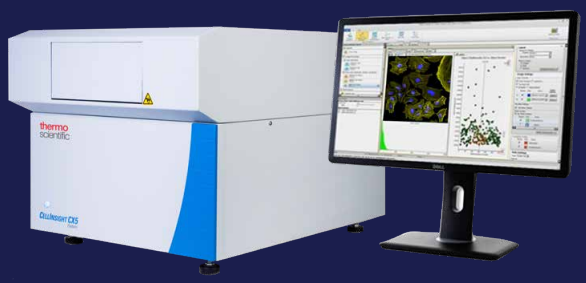
EVOS M7000 Imaging System
Powerful, flexible, and fast imaging system with the option of live-cell imaging

Need to acquire more quantitative data from your images while increasing sample throughput?

With the compact and powerful CellInsight CX5 High Content Analysis Platform, achieve faster time-to-data, regardless of sample size.

Automated image capture with simultaneous data analysis allows you to analyze up to half a million phenotypic cell measurements in under 5 minutes.

Learn about the CellInsight HCA portfolio at **thermofisher.com/hca**



CellInsight CX5 High Content Analysis Platform

Selection guide for fixed-cell imaging

Use the table below to find the tools you need for each step

		EVOS Light Cube, DAPI 2.0 (AMEP4950) Ex: 357/44 nm; Em: 447/60 nm	EVOS Light Cube, GFP 2.0 (AMEP4951) Ex: 470/22 nm; Em: 510/42 nm
Step 1. Fix, permeabilize, and block	Buffers	Image-iT Fixation/Permeabilization Kit (R37602), Image-iT Fixative Solution (FB002), BlockAid Blocking Solution (B10710), ReadyP ReadyProbes 2.5% Normal Chicken Serum (1X) (R37626), ReadyProbes Endogenous HRP and AP Blocking	
Step 2. Label	Mitochondria		Alexa Fluor 488 ATP Synthase Beta Monoclonal Antibody (4.3E8.D10) (MA1930A488)
	Cytoskeleton	Alexa Fluor 350 Phalloidin (A22281)	Alexa Fluor 488 Phalloidin (A12379) ActinGreen 488 ReadyProbes Reagent (R37110) Alexa Fluor 488 Beta Tubulin Monoclonal Antibody (2 28 33) (MA322600A488) CellMask Green Actin Tracking Stain (A57243)
	Plasma membrane	Wheat Germ Agglutinin, Alexa Fluor 350 Conjugate (W11263)	Wheat Germ Agglutinin, Alexa Fluor 488 Conjugate (W11261) Alexa Fluor 488 ATP1A1 Monoclonal Antibody (M7-PB-E9) (MA3928A488) Alexa Fluor 488 ZO-1 Monoclonal Antibody (ZO1-1A12) (MA339100A488)
	Nucleus	NucBlue Fixed Cell ReadyProbes Reagent (R37606) NucBlue Live ReadyProbes Reagent (R37605)	SYTO 9 Green Fluorescent Nucleic Acid Stain (S34854) SYTOX Green Nucleic Acid Stain (S7020) Alexa Fluor 488 Histone H3 Recombinant Rabbit Monoclonal Antibody (17H2L9) (MA702023A488) Alexa Fluor 488 HDAC1 Polyclonal Antibody (PA1860A488) Alexa Fluor 488 HDAC2 Polyclonal Antibody (PA1861A488)
Step 3. Detect	Antibody direct labeling kits	Alexa Fluor 350 Antibody Labeling Kit (A20180)	Alexa Fluor 488 Antibody Labeling Kit (A20181)
	Zenon kits	Zenon Alexa Fluor 350 Mouse IgG1 Labeling Kit (Z-25000) Zenon Alexa Fluor 350 Rabbit IgG Labeling Kit (Z-25300)	Zenon Alexa Fluor 488 Mouse IgG1 Labeling Kit (Z-25002) Zenon Alexa Fluor 488 Rabbit IgG Labeling Kit (Z-25302)
	Secondary antibodies	Alexa Fluor 350 Goat Anti–Mouse IgG (H+L) Secondary Antibody (A11045) Alexa Fluor 350 Goat Anti–Rabbit IgG (H+L) Secondary Antibody (A11046)	Alexa Fluor Plus 488 Goat Anti–Mouse IgG (H+L) Secondary Antibody (A32723) Alexa Fluor Plus 488 Goat Anti–Rabbit IgG (H+L) Secondary Antibody (A32731)
	Streptavidin	Alexa Fluor 350 Streptavidin (S11249)	Alexa Fluor 488 Streptavidin (S11223)
	SuperBoost TSA kits		Alexa Fluor 488 Tyramide SuperBoost Kit, Goat Anti–Mouse IgG (B40912) Alexa Fluor 488 Tyramide SuperBoost Kit, Goat Anti–Rabbit IgG (B40922)
Step 4. Protect and enhance	Signal enhancer		
	Mountants and antifades	ProLong Glass Hard-Set Antifade Mountant (P36980), ProLong Glass Antifade Mountant with	
Step 5. Image	Imaging and analysis		

To learn more, go to **thermofisher.com/fixedcellimaging**

Perform multiplex experiments by selecting a combination of fluorescent reagents for detection using interchangeable EVOS light cubes. These LED cubes are compatible with our EVOS M5000 and EVOS M7000 Imaging Systems and the Invitrogen™ Countess™ II FL Automated Cell Counter, each of which can be configured in your lab. There are more than 14 light cubes to choose from, to cover a broad range of fluorescence excitation and emission; and they can easily be transferred between systems.

EVOS Light Cube, RFP 2.0 (AMEP4952) Ex: 531/40 nm; Em: 593/40 nm	EVOS Light Cube, Texas Red 2.0 (AMEP4955) Ex: 585/29 nm; Em: 624/40 nm	EVOS Light Cube, Cy®5 2.0 (AMEP4956) Ex: 628/40 nm; Em: 693/40 nm
robes Mouse-on-Mouse IgG Blocking Solution (30X) (R37621), ReadyProbes 2.5% Normal Goat Serum (1X) (R37624), ReadyProbes 2.5% Normal Horse Serum (1X) (R37625), Solution (1X) (R37629), ReadyProbes Avidin/Biotin Blocking Solution (1X) (R37627), ReadyProbes Solvent-Resistant Permanent Marking Pen (R37623)		
Alexa Fluor 555 ATP Synthase Beta Monoclonal Antibody (4.3E8.D10) (MA1930A555)		Alexa Fluor 647 ATP Synthase Beta Monoclonal Antibody (4.3E8.D10) (MA1930A647)
Alexa Fluor 555 Phalloidin (A34055) ActinRed 555 ReadyProbes Reagent (R37112) CellMask Orange Actin Tracking Stain (A57247)	Alexa Fluor 594 Phalloidin (A12381)	Alexa Fluor 647 Phalloidin (A22287) Alexa Fluor 647 Beta Tubulin Monoclonal Antibody (2 28 33) (MA322600A647) Alexa Fluor 647 Alpha Tubulin Monoclonal Antibody (TU-01) (MA138000A647) CellMask Deep Red Actin Tracking Stain (A57245) Tubulin Tracker Deep Red (T34077)
Wheat Germ Agglutinin, Alexa Fluor 555 Conjugate (W32464) Alexa Fluor 555 ATP1A1 Monoclonal Antibody (M7-PB-E9) (MA3928A555) Alexa Fluor 555 ZO-1 Monoclonal Antibody (ZO1-1A12) (MA339100A555)	Wheat Germ Agglutinin, Alexa Fluor 594 Conjugate (W11262)	Wheat Germ Agglutinin, Alexa Fluor 647 Conjugate (W32466) Alexa Fluor 647 ZO-1 Monoclonal Antibody (ZO1-1A12) (MA339100A647) Alexa Fluor 647 ATP1A1 Monoclonal Antibody (M7-PB-E9) (MA3928A647)
SYTO 82 Orange Fluorescent Nucleic Acid Stain (S11363) Alexa Fluor 555 Histone H3 Recombinant Rabbit Monoclonal Antibody (17H2L9) (MA702023A555) Alexa Fluor 555 HDAC1 Polyclonal Antibody (PA1860A555) Alexa Fluor 555 HDAC2 Polyclonal Antibody (PA1861A555)		TO-PRO-3 Iodide (T3605) HCS NuclearMask Deep Red Stain (H10294) Alexa Fluor 647 Histone H3 Recombinant Rabbit Monoclonal Antibody (17H2L9) (MA702023A647) Alexa Fluor 647 HDAC1 Polyclonal Antibody (PA1860A647) Alexa Fluor 647 HDAC2 Polyclonal Antibody (PA1861A647) SYTOX Deep Red Nucleic Acid Stain, for fixed/dead cells (S11380) SYTO Deep Red Nucleic Acid Stain, for live cells (S34900)
Alexa Fluor 555 Antibody Labeling Kit (A20187)	Alexa Fluor 594 Antibody Labeling Kit (A20185)	Alexa Fluor 647 Antibody Labeling Kit (A20186)
Zenon Alexa Fluor 555 Mouse IgG1 Labeling Kit (Z-25005) Zenon Alexa Fluor 555 Rabbit IgG Labeling Kit (Z-25305)	Zenon Alexa Fluor 594 Mouse IgG1 Labeling Kit (Z-25007) Zenon Alexa Fluor 594 Rabbit IgG Labeling Kit (Z-25307)	Zenon Alexa Fluor 647 Mouse IgG1 Labeling Kit (Z-25008) Zenon Alexa Fluor 647 Rabbit IgG Labeling Kit (Z-25308)
Alexa Fluor 555 Goat Anti–Mouse IgG (H+L) Secondary Antibody (A21422) Alexa Fluor 555 Goat Anti–Rabbit IgG (H+L) Secondary Antibody (A21429) Alexa Fluor Plus 555 Goat Anti–Mouse IgG (H+L) Secondary Antibody (A32727) Alexa Fluor Plus 555 Goat Anti–Rabbit IgG (H+L) Secondary Antibody (A32732)	Alexa Fluor 594 Goat Anti–Mouse IgG (H+L) Secondary Antibody (A11032) Alexa Fluor 594 Goat Anti–Rabbit IgG (H+L) Secondary Antibody (A11012)	Alexa Fluor Plus 647 Goat Anti–Mouse IgG (H+L) Secondary Antibody (A32728) Alexa Fluor Plus 647 Goat Anti–Rabbit IgG (H+L) Secondary Antibody (A32733)
Alexa Fluor 555 Streptavidin (S21381)	Alexa Fluor 594 Streptavidin (S11227)	Alexa Fluor 647 Streptavidin (S21374)
Alexa Fluor 555 Tyramide SuperBoost Kit, Goat Anti–Mouse IgG (B40913) Alexa Fluor 555 Tyramide SuperBoost Kit, Goat Anti–Rabbit IgG (B40923)	Alexa Fluor 594 Tyramide SuperBoost Kit, Goat Anti–Mouse IgG (B40915) Alexa Fluor 594 Tyramide SuperBoost Kit, Goat Anti–Rabbit IgG (B40925)	Alexa Fluor 647 Tyramide SuperBoost Kit, Goat Anti–Mouse IgG (B40916) Alexa Fluor 647 Tyramide SuperBoost Kit, Goat Anti–Rabbit IgG (B40926)
Image-iT FX Signal Enhancer ReadyProbes Reagent (R37107)		
NucBlue Stain (P36981), Hoechst 33342 (H1399), SlowFade Glass Soft-Set Antifade Mountant (S36917), SlowFade Glass Soft-Set Antifade Mountant, with DAPI (S36920)		
EVOS Imaging Systems, CellInsight High Content Analysis Platforms		


Countess 3 FL Automated Cell Counter


EVOS M5000 Imaging System


EVOS M7000 Imaging System

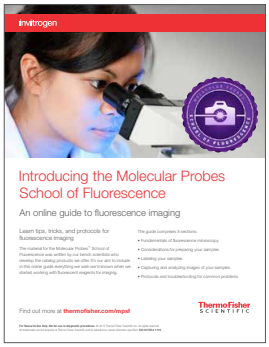

EVOS Light Cubes

Educational resources

Molecular Probes School of Fluorescence

The modules within the School of Fluorescence were developed by our in-house bench scientists. It was our aim to cover everything we wish we'd known when we first started working with fluorescent reagents and antibodies, including background information on the basics of fluorescence and practical tips for experimental design and protocols.

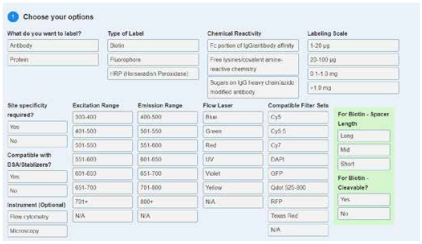
Find protocols, troubleshooting guides, and more at **thermofisher.com/mpsf**



Antibody labeling kits selection guide

Plan your antibody or protein labeling experiment with this interactive guide to find, compare, and select the right reagents for your imaging needs based on chemical reactivity, labeling scale, and excitation/emission of the fluorophores desired.

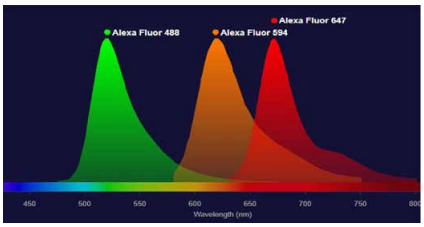
Find out more at **thermofisher.com/ablabelingguide**



Fluorescence SpectraViewer

Plot and compare spectra, check spectral compatibility for multiple fluorophores, and email the configuration to yourself in a clear printable format.

Try it now at **thermofisher.com/spectraviewer**



Find more 5-step workflows at **thermofisher.com/5steps**

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