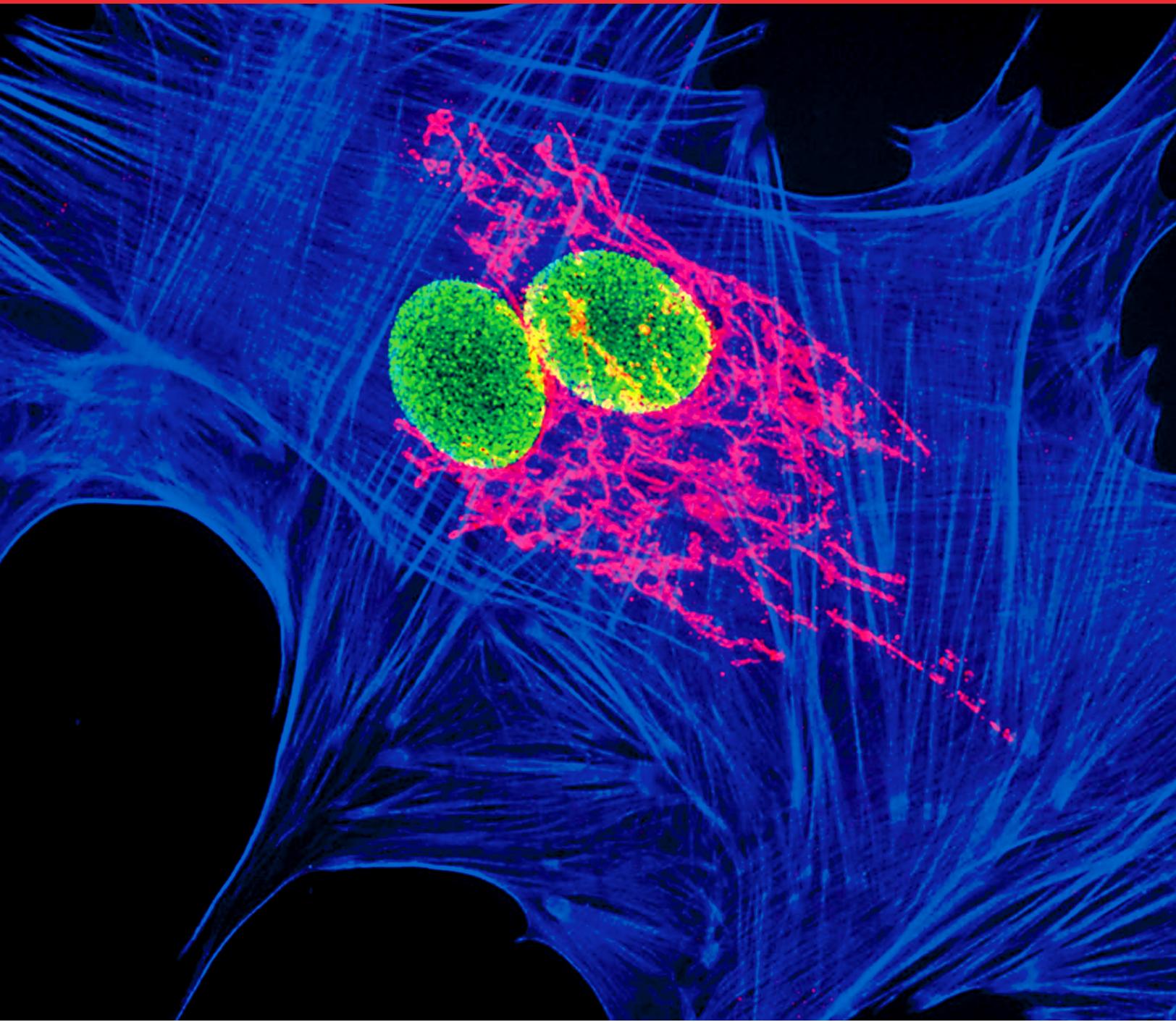


invitrogen



Five steps for publication-quality cell imaging the first time

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

ThermoFisher
SCIENTIFIC

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 and the wrong path can lead to missteps 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 are cited more frequently in published research than any others. Leverage our experience to enable your success and avoid costly missteps.

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 cell images are publication-ready the first time.



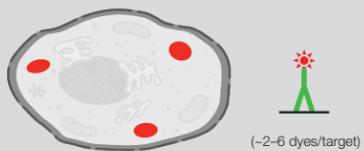
1



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

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

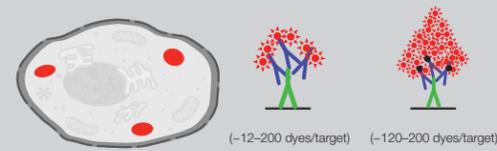
2



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

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

3

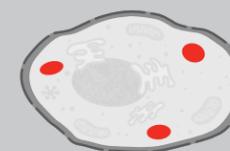


Detect—fine-tune fluorescence signal for detailed observation.

- Invitrogen Alexa Fluor™ Secondary Antibodies
- Streptavidin conjugates
- Invitrogen™ SuperBoost™ tyramide kits with Alexa Fluor™ tyramides using Tyramide Signal Amplification (TSA™) technology
- Invitrogen™ Image-iT™ FX Signal Enhancer ReadyProbes™ Reagent

— Primary Ab
— Secondary Ab
— Fluorophore
— Enzyme

4



Protect—maintain photostability of fluorescence signals of samples.

- Invitrogen™ ProLong™ Diamond antifade reagents

5



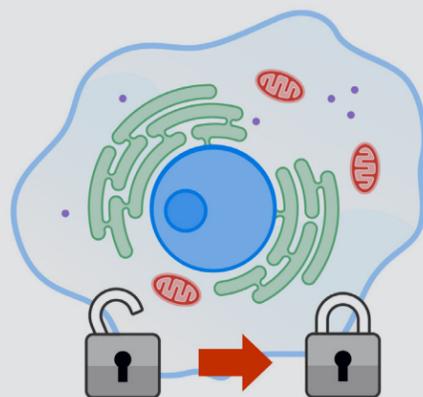
Image—capture imaging discoveries with maximum clarity and definition.

- Invitrogen™ EVOS™ Imaging Systems

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 minimizes the signal-to-noise ratio, thereby preventing the fluorescent labels from inadvertently binding to proteins that are not relevant to your research.

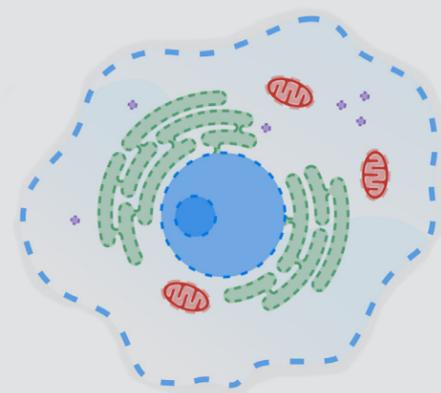


Fixation locks cellular structures in place.

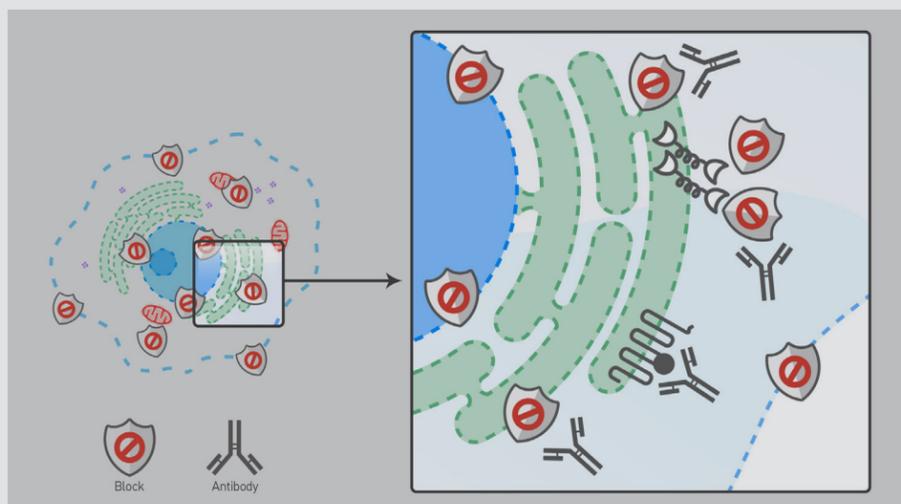
Product highlight

Image-iT Fixation/Permeabilization Kit

The Image-iT Fixation/Permeabilization Kit is a complete fixation and permeabilization solution containing all the reagents necessary to prepare your cells for antibody staining and imaging. The high-quality components come in a box with single-use vials and easy-to-follow protocols to help preserve cell morphology and reduce background staining.



Permeabilization removes cellular membrane lipids—opening access to the interior of cells for labeling and detection reagents.



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

2

Step 2—label

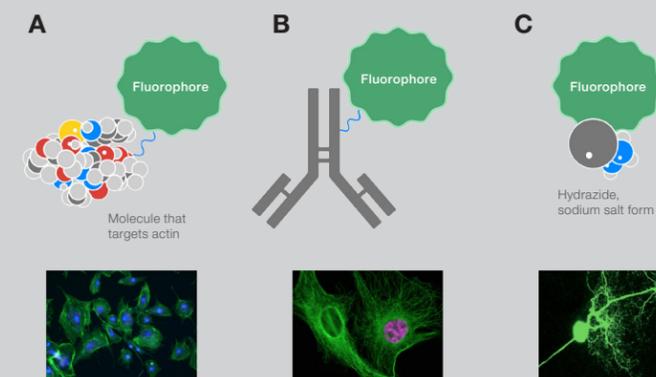
Labeling various targets with separate fluorescent colors allows you to visualize different structures or proteins within a cell in the same sample. Ways to label your target fluorescently include fluorescent dyes, immunolabeling, and fluorescent fusion proteins—all of which can provide a means to selectively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

Product highlight

Primary antibodies

The Invitrogen portfolio offers more than 48,000 high-quality primary antibodies. Some of these antibodies are attached directly to the broad range of intensely fluorescent markers and labels including Invitrogen™ Alexa Fluor™ dyes.

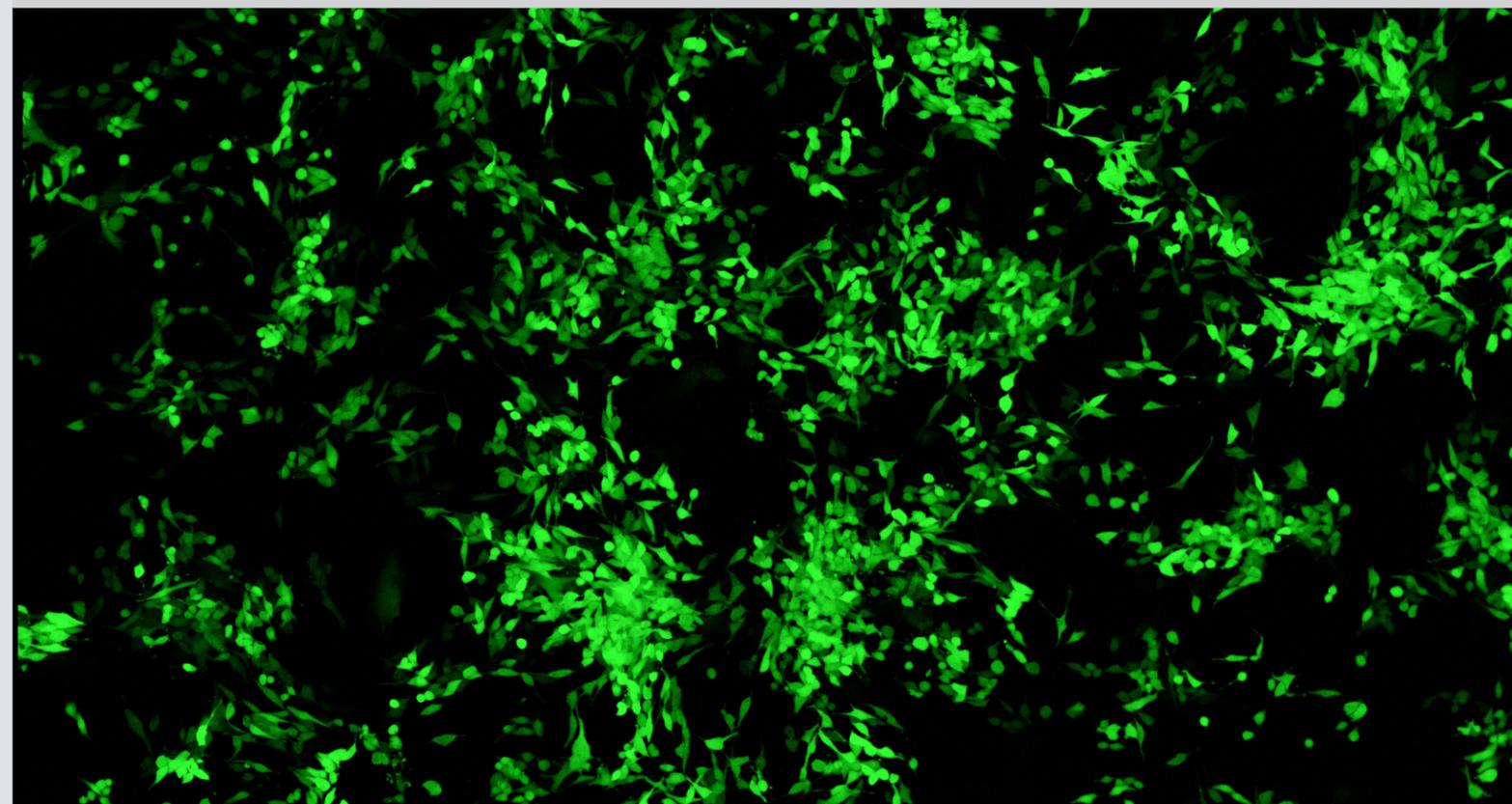
Explore our extensive portfolio of antibodies at thermofisher.com/primaryantibodies



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, (B) tubulin, and (C) salt forms for whole-cell staining.

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 a number of variant forms, each with a different specificity. For example, the green-fluorescent Invitrogen™ Alexa Fluor™ 488 dye molecule can be modified to target actin filaments, can be attached to an IgG for use in immunolabeling, or can act as a whole-cell stain.



3

Step 3—detect Fine-tune fluorescence signal for observation

Detecting the 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.

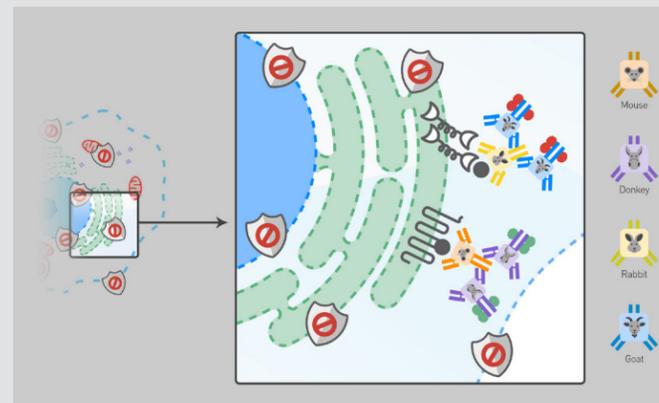
- Achieve modest amplification with secondary antibody conjugates for high- to medium-abundance protein targets
- Achieve signal elevation with streptavidin conjugates for medium- to low-abundance protein targets
- Achieve maximum signal enhancement with TSA technology for low-abundance protein targets

Quickly and easily choose the labeling solution you need with our immunofluorescence selection guide at [thermofisher.com/immunofluorescence](https://www.thermofisher.com/immunofluorescence)

High- to medium-abundance protein targets

Secondary antibodies are used for the indirect detection of target antigens. While primary antibodies bind directly to the target, secondary antibodies bind indirectly by using the primary antibody as a bridge to the targeted biomolecule. This methodology serves to amplify the signal and increase sensitivity to maximize detection.

Find out more about secondary antibodies at [thermofisher.com/secondaryantibodies](https://www.thermofisher.com/secondaryantibodies)

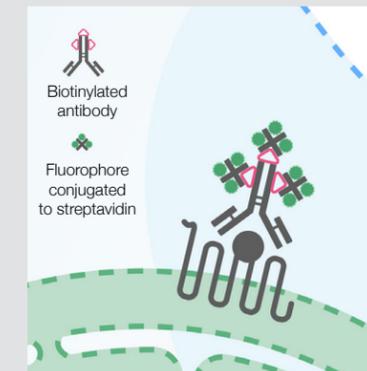


For secondary detection, the primary antibodies (orange and yellow) bind to their respective epitopes and fluorophore-labeled secondary antibodies (purple and blue) have specificity for and bind to their respective primary antibodies.

Medium- to low-abundance protein targets

Streptavidin conjugates can result in 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.

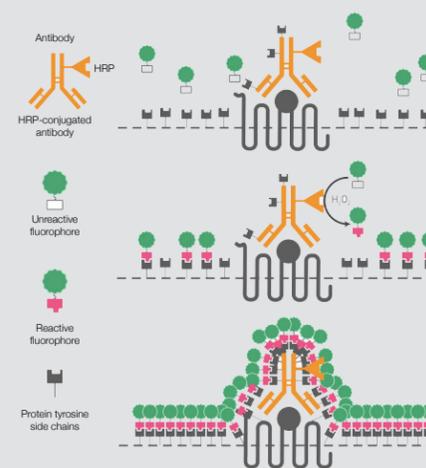
Find out more about imaging with streptavidin at [thermofisher.com/streptavidin](https://www.thermofisher.com/streptavidin)



Antibodies labeled using fluorophore–streptavidin conjugates can boost low signals in your labeling experiments because more fluorophores can bind to each antibody molecule, amplifying the signals.

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.



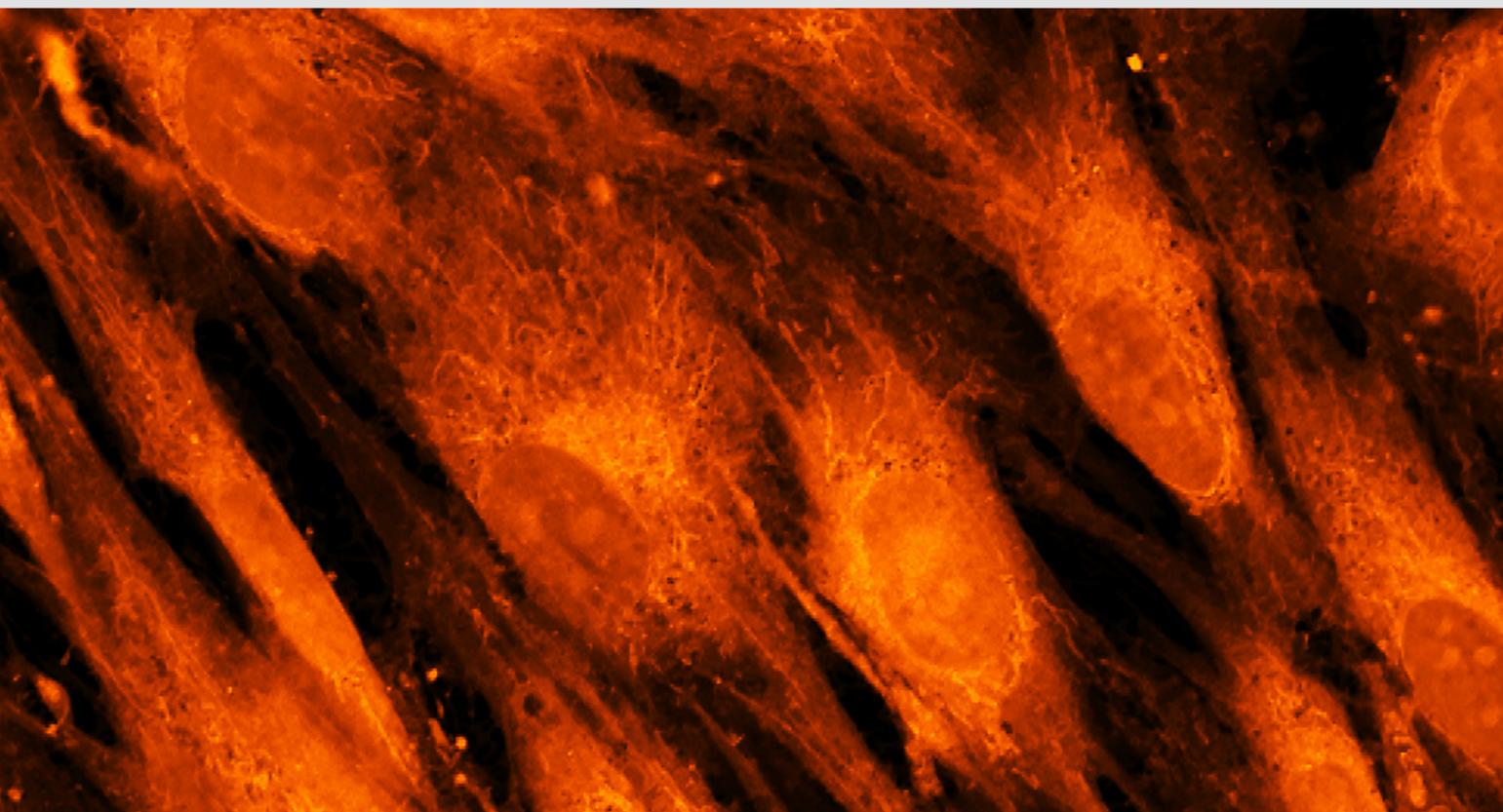
Tyramide signal amplification provides sensitive detection by adding fluorophores to epitopes resulting in superior sensitivity without compromising resolution.

Product highlight

Tyramide SuperBoost Kits

The SuperBoost technology is the most sensitive fluorescence imaging detection method for low-abundance protein targets. Offering sensitivity 10–200 times that 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 to 10 times above that of standard treatments, including TSA™ reagents (PerkinElmer).

Learn more at [thermofisher.com/superboost](https://www.thermofisher.com/superboost)



4

Step 4—protect

Maintain photostability of fluorescence signals of samples

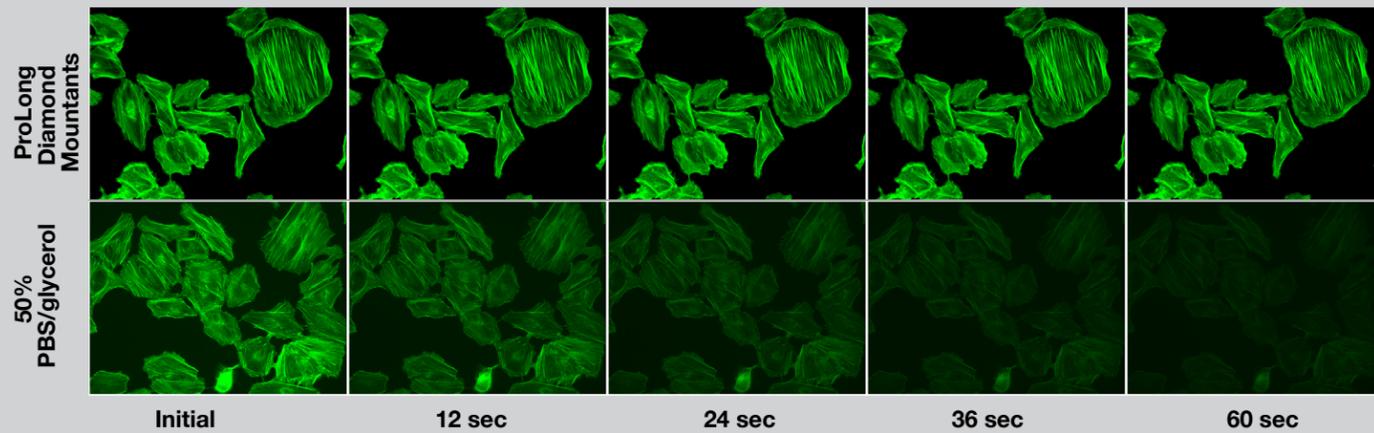
Fluorophores are ideal for high-quality cell imaging but are inevitably prone to photobleaching, a photochemical degradation or fading of fluorescence signals. Any reduction in photosensitivity can skew your data and yield false results. Antifade mountants are designed to protect the photostability of fluorescently labeled proteins and maintain image integrity from weeks to months.

Product highlight

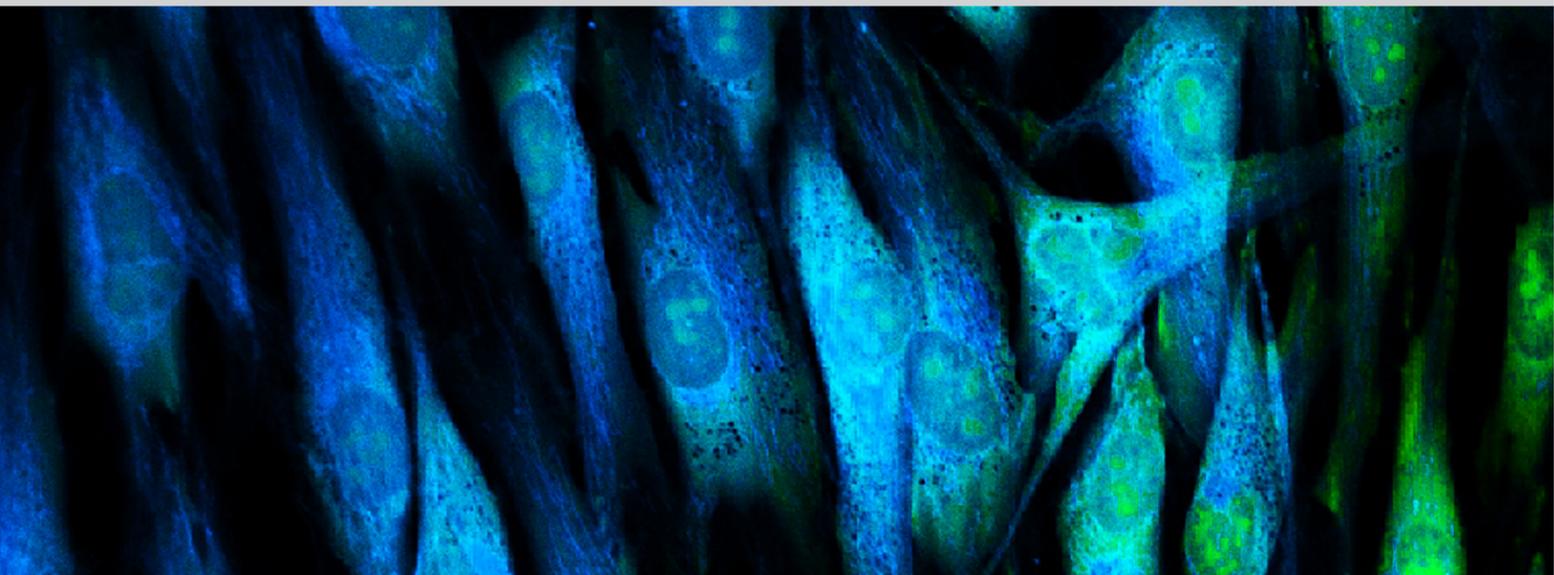
ProLong Diamond Antifade Mountants

To minimize photobleaching of experimental samples, Invitrogen™ ProLong™ Diamond Antifade Mountants increase the photostability of fluorophores. It cures within 24 hours, forming a semi-rigid gel with a refractive index of 1.46 and preserves the signal of fluorescently labeled proteins for long-term archival.

Learn more at thermofisher.com/antifades



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



5

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

Product highlight

EVOS cell imaging systems

EVOS cell imaging systems help you perform a variety of routine and specialty applications to capture images for publication.

Eliminate the complexities of microscopy without compromising performance. The EVOS line of cell imaging systems makes cell imaging accessible to almost every lab and budget. Find out which EVOS cell imaging system is right for you.

Explore the EVOS lineup at thermofisher.com/evos

Basic transmitted-light digital inverted system



EVOS XL Core system
Perfect for cell culture and routine cell maintenance

Advanced transmitted-light digital inverted system



EVOS XL system
Perfect for more advanced colorimetric assays

Basic fluorescence system



EVOS FLoid system
Perfect for quick fluorescence visualization

Advanced fluorescence system

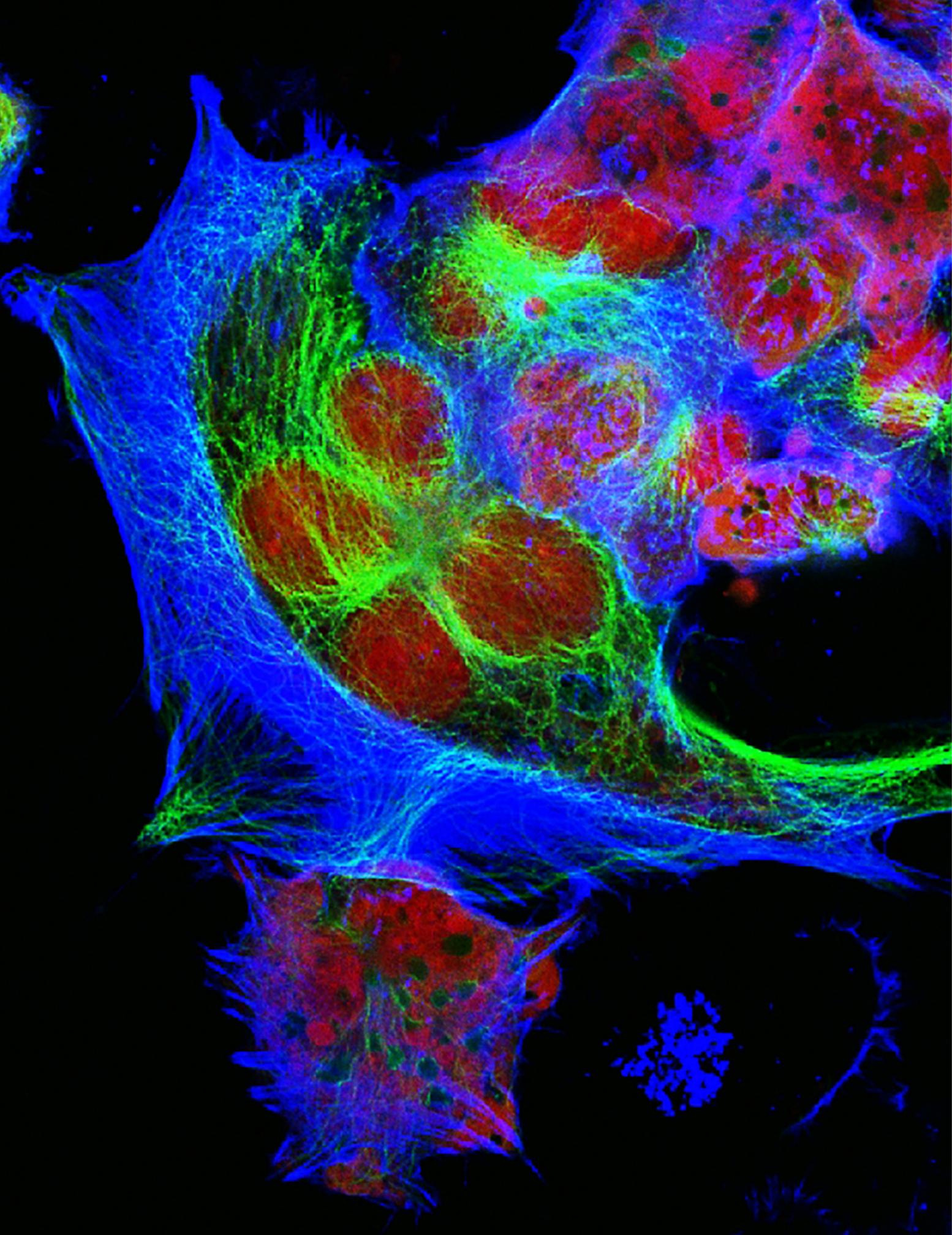


EVOS FL system
Perfect for multichannel fluorescence imaging and transfections

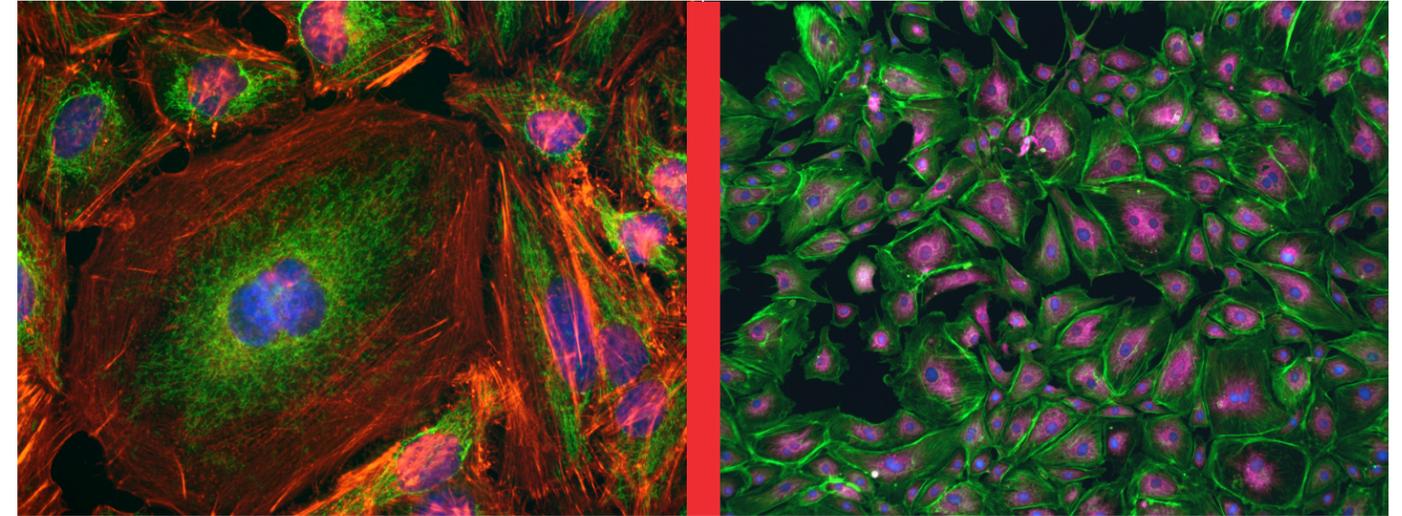
Fully automated fluorescence system



EVOS FL Auto system
Perfect for a variety of advanced, automated applications



Examples of image-based contextual information



- Cells prepared with Image-iT Fixation/Permeabilization Kit
- Treated with BlockAid Blocking Solution
- Labeled with primary antibody to mitochondria, FITC-conjugated secondary antibody (green), NucBlue cell stain (blue), ActinRed 555 ReadyProbes Reagent (red)
- Mounted with ProLong antifade reagent
- Detected with the Invitrogen™ EVOS™ FL Auto Imaging System

- Cells prepared with Image-iT Fixation/Permeabilization Kit
- Treated with BlockAid Blocking Solution
- Labeled with primary antibody to mitochondria, Invitrogen™ Alexa Fluor™ 750-conjugated secondary antibody (purple), NucBlue cell stain (blue), ActinGreen 488 ReadyProbes Reagent (green)
- Mounted with ProLong antifade reagent
- Detected with the EVOS FL Auto Imaging System

Ordering information

		Cat. No.				
		Tyramide SuperBoost kits				
Labeled	Ex/Em	Streptavidin	Goat anti-mouse IgG	Goat anti-rabbit IgG	Goat anti-mouse IgG	Goat anti-rabbit IgG
Alexa Fluor 488	495/519	S11223	B40912	B40922	A-21121	A-11070
Alexa Fluor 555	555/565	S21381	B40913	B40923	A-21137	A-21430
Alexa Fluor 594	591/617	S11227	B40915	B40925	A-21044	A-11072
Alexa Fluor 647	650/668	S21374	B40916	B40926	A-21240	A-21245
Biotin-XX	–	–	B40911	B40921	A10519	B-2770

Product	Quantity	Cat. No.
Image-iT Fixation/Permeabilization Kit	<ul style="list-style-type: none"> Fixative: high-purity 4% formaldehyde in PBS, pH = 7.3 	R37602
	<ul style="list-style-type: none"> Permeabilization solution: 0.5% Triton X-100 	
	<ul style="list-style-type: none"> Blocking buffer: 3% BSA, fraction V, delipidated, New Zealand source, in DPBS 	
	<ul style="list-style-type: none"> Wash solution: PBS, pH 7.4 	
BlockAid Blocking Solution	50 mL	B10710
ProLong Diamond Antifade Mountant with DAPI		P36962
ProLong Gold Antifade Mountant with DAPI	10 mL	P36931
ProLong Diamond Antifade Mountant		P36961
NucBlue Fixed Cell ReadyProbes Reagent	6 x 2.5 mL	R37606
NucRed Dead 647 ReadyProbes Reagent	6 x 2.5 mL	R37113
ActinGreen 488 ReadyProbes Reagents	1 kit	R37110
ActinRed 555 ReadyProbes Reagents	1 kit	R37112
Image-iT FX Signal Enhancer ReadyProbes Reagent	1 kit	R37107

Find out more at thermofisher.com/5-steps



In the United States:

For customer service, call 1-800-766-7000
 To fax an order, use 1-800-926-1166
 To order online: fishersci.com

In Canada:

For customer service, call 1-800-234-7437
 To fax an order, use 1-800-463-2996
 To order online: fishersci.ca

ThermoFisher
 SCIENTIFIC