

Comprehensive guide to imaging reagents

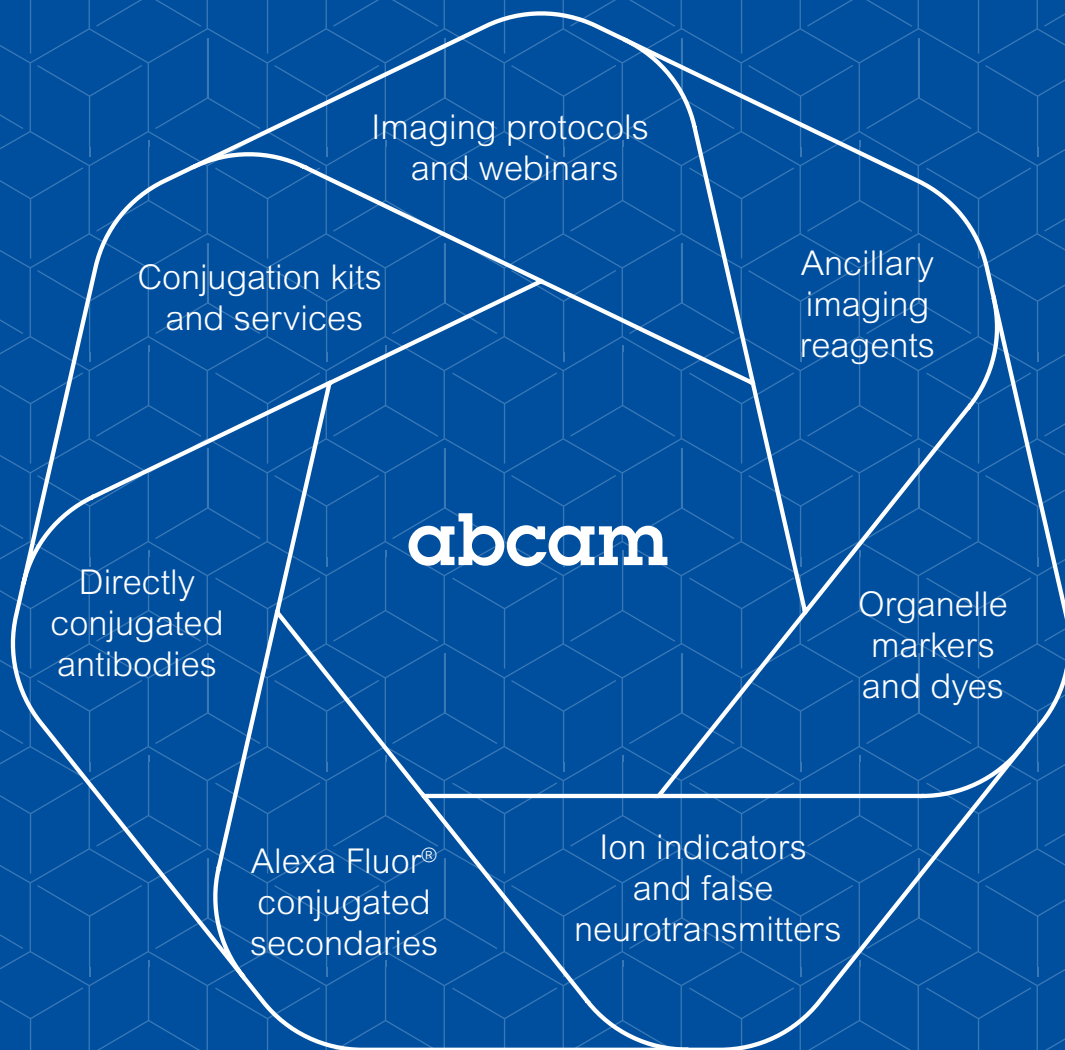
abcam.com/cell-imaging-resources

abcam

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Your total resource for cell imaging



‘I use Abcam antibodies because they are such good products.’

‘My experience with their products demonstrates to me that they perform very well in my experiments.’

Dr Scott Altmann, HDL Apomics

Our commitment to quality and validation

Whether it's antibodies, kits, proteins or small molecule activators and inhibitors, our products are continuously validated to provide consistent performance and relevant data.

Manufacturing excellence

Over 15 years of developing and manufacturing products in-house guarantees consistency and quality.

Validated in multiple applications and species

A team of dedicated research scientists feed current scientific knowledge into our product validation process.

Partnering with experts

We maintain close connections with academic and industry experts to perform specialized testing and to provide insight on our validation processes.

Stability testing

Our lab performs stability testing to provide you with accurate storage temperature recommendations for maximal performance.

Abpromise®

If for any reason the product does not work as stated on the datasheet, we will provide a replacement or refund up to 12 months from the date the product was delivered.

Numbers you can trust

39,000+

Validated
imaging
reagents

28,000+

Reagents with
a reference,
image or
Abreview®

25,000+

Protein targets
with validated
antibodies

Key tips for successful immunofluorescence experiments

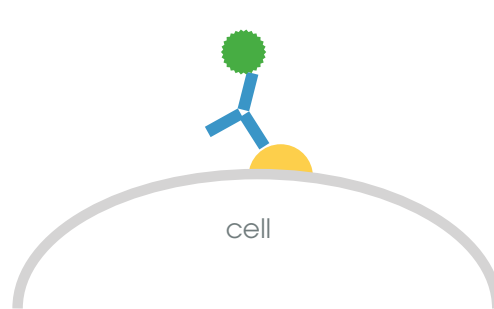
1. Proper data interpretation is extremely important in any imaging experiment. Autofluorescence, off-target binding of the primary antibody and non-specific binding of secondary antibodies can be mis-interpreted as genuine staining. It is therefore important to ensure the correct controls are used.
 - No primary or secondary antibodies – to rule out the possibility of autofluorescence
 - Secondary alone – to dismiss the possibility of the secondary antibody binding non-specifically to the endogenous cell proteinsOften, immunofluorescence images consist of only a few cells so it is important to make sure the image is typical and representative of the population.
2. It is important not to let your cells dry out at any point during staining as this can introduce artefacts. Drying of the samples during incubation steps can be prevented by using a humidifying chamber.
3. Fixation may or may not mask the epitope of an antibody (this is particularly relevant for monoclonal antibodies). It is therefore worth adding the antibody to cells that have and have not been fixed to identify if fixation is or is not required.
4. The cell membrane must be permeabilized to allow antibodies access to the intracellular components. For each antibody the optimal permeabilization step will be different and therefore a variety of methods should be investigated, for instance trying different percentages of Triton X-100 (0.1–0.25%).
5. For multi-target staining, it is important to make sure your secondary antibodies are pre-adsorbed against the host species of your primary and secondary antibodies. Pre-adsorbed secondary antibodies minimize cross-species reactivity and non-specific binding with endogenous proteins within the sample.
6. Non-specific staining may be reduced by
 - a. Blocking with serum from the host species of your secondary antibody.
 - b. Using less antibody and/or decreasing the incubation times (overnight at 4°C or 1 hour at room temperature).
 - c. Quenching residual aldehydes following formaldehyde fixation using 0.1M glycine.
7. After adding the fluorescently labeled antibodies to your cells, make sure all subsequent incubation steps are performed in the dark to prevent quenching of the fluorescent dye/proteins.

Direct vs indirect immunofluorescence

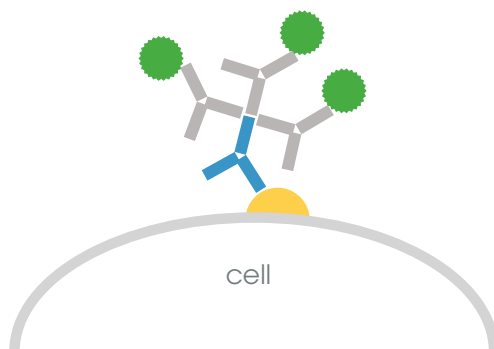
Immunofluorescence (IF) or cell imaging techniques rely on the use of chemically conjugated antibodies to label a specific target antigen with a fluorescent dye (known as fluorophores or fluorochromes) such as fluorescein isothiocyanate (FITC). The fluorophore allows visualization of the target distribution in the sample under a fluorescent microscope (eg epifluorescence and confocal microscopes). We distinguish between two immunocytochemistry (ICC)/IF methods depending on whether the fluorophore is conjugated to the primary or the secondary antibody:

- Direct ICC/IF: uses a single primary antibody directed against the target of interest. The antibody is directly conjugated to a fluorophore.
- Indirect ICC/IF: uses two antibodies. The primary antibody is unconjugated and a fluorophore-conjugated secondary antibody directed against the primary antibody is used for detection.

Direct



Indirect



Indirect and direct methods have their advantages and disadvantage as shown in the table below.

	Direct	Indirect
Time	Protocols for direct IF are usually shorter as they only require one labeling step.	The fact that you have to use a conjugated secondary antibody to detect the primary antibody results in additional steps.
Cost	Conjugated primary antibodies are usually more expensive than their unconjugated counterparts.	Secondary antibodies are relatively inexpensive compared to primary antibodies. Further cost savings may be realized by the possibility of using the same conjugated secondary antibody to detect different primary antibodies.
Complexity	Less steps in the protocol simplify direct methods.	Added complexity in indirect methods may result from having to select the appropriate secondary antibody. This is particularly relevant in multiplex experiments where several secondary antibodies, each targeting a different species and conjugated to different dyes, are needed.
Flexibility	Commercially available pre-conjugated primary antibodies limit your flexibility.	The possibility of using different conjugated secondary antibodies adds greater flexibility.
Sensitivity	The signal obtained in direct methods may seem weak when compared to indirect methods as signal amplification provided by the use of secondary antibodies does not occur.	Several secondary antibodies will bind to the primary antibody resulting in an amplified signal.
Species cross-reactivity	Species cross-reactivity is minimized in direct methods as the fluorophore is already conjugated to the primary antibody.	Secondary antibodies may cross-react with species other than the target. The use of pre-adsorbed secondary antibodies can prevent cross-reactivity.
Background	Non-specific binding is reduced through the use of conjugated primary antibodies.	Samples with endogenous immunoglobulins may exhibit a high background with indirect methods.

Cell imaging protocols and webinars

Our website offers you a wide range of cell imaging protocols and webinars. Highlighted below are some of our most popular ones.

Protocols

Immunocytochemistry and immunofluorescence protocol

Our detailed guide on ICC/IF is a must-have resource to ensure you get optimal results in your cell imaging experiments. Whether you are doing single staining or multiple staining, the guide provides a step-by-step protocol that includes hints and tips for:

- Fixation
- Antigen retrieval (optional)
- Permeabilization
- Blocking and incubation
- Multicolor staining (optional):
 - Simultaneous incubation
 - Sequential incubation
- Counter staining
- Mounting

You can find the protocol here: www.abcam.com/ICC-protocol

Find more protocols at: www.abcam.com/protocols

Webinars

Our webinar series can be viewed on demand, and provides detailed examples to help you with your cell imaging experiments. Topics include:

Introduction to ICC

An overview of the key principles of the technique

Single and multiple labeling in ICC

Strategies to overcome common pitfalls in multiple labeling experiments

Principles and practices of confocal microscopy

Outlines basic and advanced principles of the technique

Optimizing ICC

Hands-on tips to improve your experiments

Find more webinars at: www.abcam.com/webinars

Directly conjugated antibodies

Rapid advances in fluorescence microscopy and flow cytometry have led to better and brighter imaging reagents. Whether you are performing single cell imaging or multicolor flow cytometry, our dye-labeled antibodies are the ideal immunoreagents to light up your experiment.

Feature	Benefit
Powered by RabMAb® technology	Higher affinity and specificity than traditional monoclonals
Free dye removal step	Higher signal to noise ratios
Optimized fluorophore:protein (F:P) labeling	Superior sensitivity for each dye conjugate
Suitable for multicolor analysis	Eliminates cross-reactivity seen with two-step labeling
Thorough validation	Batch tested in cellular imaging applications

Validated for cell imaging applications

We validate each batch of antibody conjugate in key imaging applications to ensure they perform in your hands, every time.

- ICC/IF – high-resolution cell and tissue section images taken using a confocal microscope are provided on the datasheet. Nuclear and cytoskeletal counterstains are overlaid to increase the contrast of subcellular localization.
- Flow cytometry – histogram is provided on the datasheet showing positive events as indicated by a peak shift. Two negative controls – an isotype control, and an unlabeled control – are overlaid to confirm positive signal.

The choice is yours

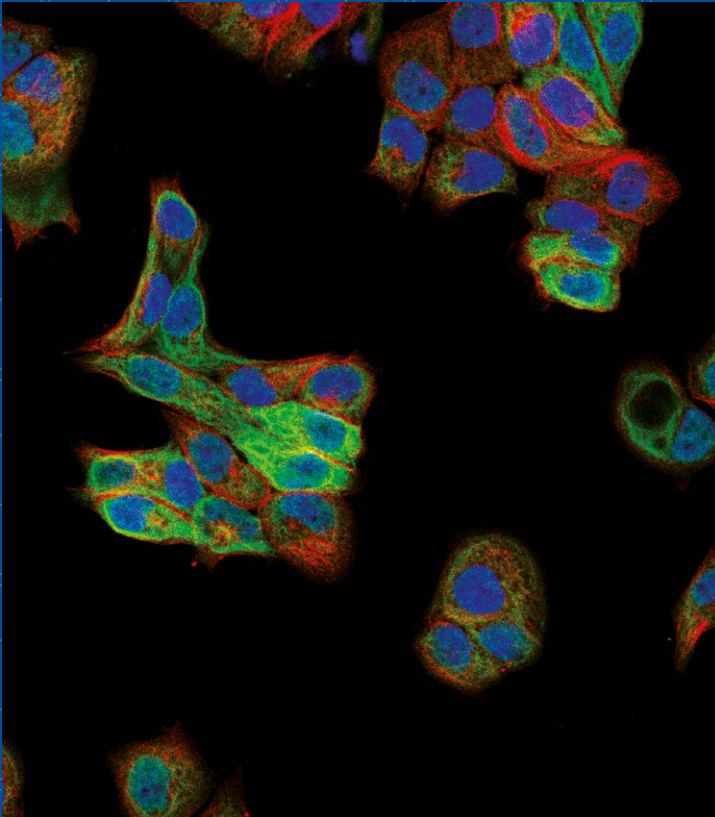
We manufacture antibodies labeled with nine distinct Alexa Fluor® dyes, covering a wide range of UV, visible, and near-IR wavelengths, and reducing the chances of spectral overlap.

Our Alexa Fluor® series



Discover more www.abcam.com/primary-conjugates

Product highlights



Sample

PFA-fixed T47D cells

Green staining

Primary antibody ab185048 – rabbit monoclonal to cytokeratin (Alexa Fluor® 488)

Red staining

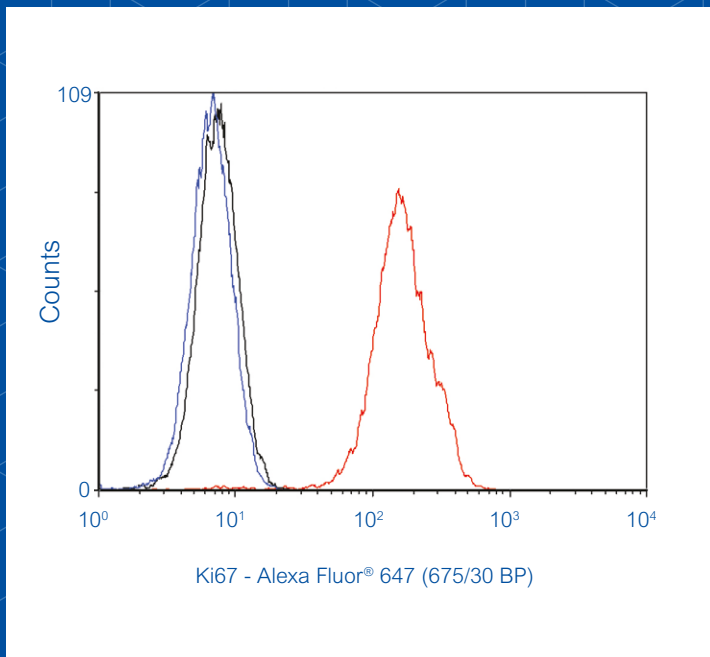
Ab195889 – mouse monoclonal to alpha tubulin (Alexa Fluor® 594)

Blue staining

Nuclear counterstain with DAPI

Instrument

Leica-Microsystems TCS SP8 confocal microscope



Sample

Methanol fixed HeLa cells

Red Peak

Primary antibody ab194724 – rabbit monoclonal to Ki67 (Alexa Fluor® 647)

Blue Peak

Unlabeled methanol fixed HeLa cell control

Black Peak

Rabbit monoclonal (Alexa Fluor® 647) isotype control

Instrument

Beckman Coulter FC500 MPL

Using multiple conjugates in flow panels?

Our multicolor flow selector lets you search and compare conjugated antibodies for up to three targets of interest.

Search for up to three targets or clone numbers 

CD44 CD45 CD90

Refine by		Clear All		46 Matching results found in 3 conjugations								
+ Conjugate		Clear		Filtered By	Conjugate	FITC	or	Phycoerythrin	or	Allophycocyanin	Reactivity	Mouse
+ Target / Protein				Target / Protein	Clone	Isotype	FITC	PE	APC			
+ Clonality							ex : 493 nm em : 528 nm	ex : 488 nm em : 575 nm	ex : 645 nm em : 660 nm			
+ Host Species												
+ Isotype												
+ Other Applications												
+ Reactivity		Clear										
	CD44	5035-4...	IgG1				ab23557					
	CD44	IM7	IgG2b					ab95519			ab95515	
	CD44	KM201	IgG1				ab25064	ab25224			ab25451	
	CD44	KM81	IgG2a				ab112165	ab112174				
	CD44	T2-F4	IgG2				ab41615					
	CD45	104	IgG2a				ab95785					
	CD45	104-2	IgG2a					ab25480			ab25326	
	CD45	I3/2.3	IgG2b				ab25670	ab25603			ab25519	
	CD45	IBL-5/25	IgG1				ab112507	ab112508				
	CD45	YW 62.3	IgG2b				ab22475					
	CD45R	RA3-6B2	IgG2a				ab24897	ab25551			ab25523	
	CD45RB	16A	IgG2a				ab112464	ab113407				
	CD45RB	C363.16A	IgG2a				ab25006	ab25647				
	CD45RC	C455.1F	IgG2a				ab24937	ab25521				
	CD90 /...	30-H12	IgG2b				ab62009					
	CD90 /...	5a-8	IgG2b				ab112463	ab112471			ab112207	
	CD90 /...	FITC.M...	IgG1				ab226					
	CD90 /...	G7	IgG2c				ab25672	ab24904			ab25322	

Build your next multicolor flow experiment at abcam.com/flow-cytometry

Alexa Fluor® conjugated secondary antibodies

Feature	Benefit
Brightest dyes	Alexa Fluor® dyes outperform other spectrally-similar dyes providing greater sensitivity
Greater photostability	Allows for longer periods of image capture
Broad pH tolerance	Intensity of Alexa Fluor® dyes remains high over a wide pH range
Good water solubility	Prevents precipitation and aggregation of Alexa Fluor® conjugated antibodies

We have implemented a robust manufacturing process that guarantees the quality of our Alexa Fluor® conjugated secondary antibodies:

- Conjugation – the number of dye molecules per antibody (F:P ratio) are optimized to provide superior sensitivity
- Purification – the highest possible purity grade is achieved by removing free dye after conjugation
- Validation – each individual antibody is validated in ICC/IF to ensure bright staining and low background

“The spectral location in the red made this perfect for a double label with the other fluorophore in the blue.

It has stood up well to storage in the refrigerator and gives a good signal after six months.”

Researcher from Station Biologique de Roscoff, France

Alexa Fluor®	405	488	555	594	647
Anti-mouse IgG H&L	ab175658	ab150105	ab150114	ab150116	ab150115
Anti-rabbit IgG H&L	ab175652	ab150077	ab150074	ab150080	ab150075
Anti-rat IgG H&L	ab175671	ab150157	ab150154	ab150160	ab150155
Anti-goat IgG H&L	ab175664	ab150129	ab150130	ab150132	ab150131
Anti-chicken IgG H&L	ab175674	ab150169	ab150174	ab150172	ab150171

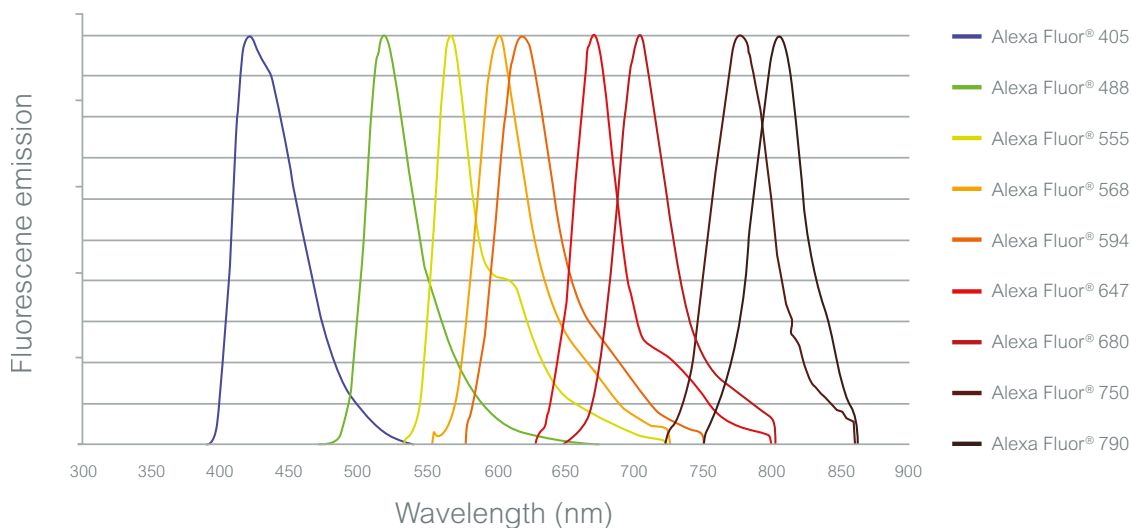
Explore our broad offering at www.abcam.com/alexafluorsecondaries

Ideal for multicolor staining

We currently offer secondary antibodies conjugated to nine different Alexa Fluor® dyes covering the whole spectrum from the UV to the near infra-red regions with minimal spectral overlap:









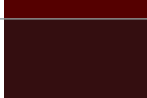
- Raised in different species including donkey, goat, and rabbit
- Targeting several species and their isotypes such as rabbit, mouse, rat, goat, and chicken
- A large range of pre-adsorbed antibodies ensures low species cross-reactivity
- Many fragments as well as whole antibodies

Emission spectra – Alexa Fluor® conjugated secondary antibodies



If you are looking for common combinations in double and triple immunostaining experiments we recommend the following combinations:

Immunostaining	Antibody combination
Double	Alexa Fluor® 488 and Alexa Fluor® 647
Triple	Alexa Fluor® 405, Alexa Fluor® 555 and Alexa Fluor® 647

Alexa Fluor®	Absorption max (nm)	Emission max (nm)	Emission color*	Matching dyes	Extinction coefficient	Quantum yield
405	402	421		Cascade Blue	35,000	-
488	495	519		Cy2, FITC (fluorescein)	73,000	0.92
555	555	555		Cy3, TRITC (Rhodamine)	155,000	0.1
568	578	603		Rhodamine Red	88,000	0.69
594	590	617		Texas Red	92,000	0.66
647	650	668		APC, Cy5	270,000	0.33
680	679	702		Cy5, IR680	184,000	0.36
750	749	775		Cy7	290,000	0.12
790	784	814		IR800	270,000	-

* Typical emission color seen through a conventional fluorescence microscope with appropriate filters

** Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes

For more information on how to handle and use Alexa Fluor® conjugated secondary antibodies, please visit our FAQs page at www.abcam.com/alexafluorsecondariesfaqs

Organelle markers and dyes

The subcellular localization of a protein is often tied to its function, so it is important to determine where the protein of interest is located. High resolution imaging allows researchers to track the location and movement of proteins within the cellular environment. To ensure a proper interpretation of these experiments, it is necessary to confirm whether the protein is actually located in the subcellular environment you expect.

Tracking your protein of interest

There are two different approaches that can be used to confirm the subcellular localization of a protein: organelle-specific antibodies and organelle stains. Organelle stains can be used as counterstains to help identify the location of specific proteins and targets of interest within the cell, while antibodies against proteins associated with a specific organelle can lead to a better understanding of cellular function.

Organelle marker antibodies

Feature	Benefit
Over 60 targets for multiple organelles and structures	Easily find the right commercial antibody for your experiment
The brightest dyes available	Greater sensitivity with Alexa Fluor® dyes
Multiple host species and clonalities	Easily study multiple targets with RabMAb® reagents

CytoPainter organelle dyes

Feature	Benefit
Use in live and fixed cells	Easy to implement in your current staining protocols
Suitable for proliferating and non-proliferating cells	Can be used in most cellular or tissue samples
High photostability	Minimal photobleaching to allow long exposures

When might a CytoPainter dye work best for you?

- Working with live cells – the current range of antibodies available in the market can only be used on fixed cells, therefore they cannot be used when studying a time-lapsed event in live cells. Most organelle dyes will stain subcellular compartments in live cells as well as being retained within said compartments after a fixation step. This versatile characteristic means that organelle dyes can be used for co-staining experiments with antibodies.
- Studying multiple proteins – the number of proteins that you can study using antibodies is limited by the number of species in which fluorescence-linked antibodies are available. Organelle dyes bypass this limitation as they are chemical compounds, making them an excellent alternative to antibodies when your experiment requires multiple targets.
- Morphology/distribution studies – when studying disease models and mutated cells, it is possible that the protein of interest will not be localized to the expected subcellular compartment, or the morphology of the organelle may be compromised. Organelle stains will stain the subcellular structures as long as they are intact, even if its morphology has been changed.

DRAQ™ dyes for nuclear staining

DRAQ5™ and DRAQ7™ are far-red fluorescent dyes that are used for nuclear staining.

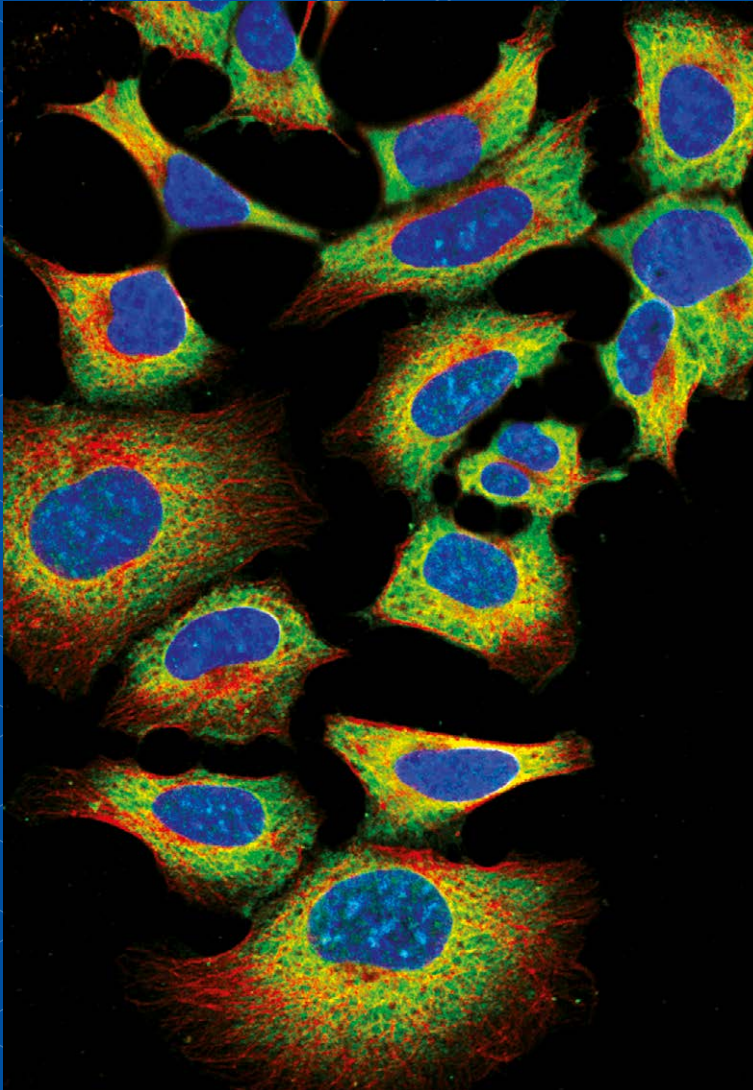
Key features of DRAQ5™:

- Can be used in both live/non-fixed and fixed cells in flow cytometry, live cell imaging, and cell-based assays
- Rapid uptake into live cells
- No compensation needed with common FITC/GFP + PE combinations in flow cytometry
- No photobleaching

Key features of DRAQ7™:

- Only stains nuclei in fixed/permeabilized cells and does not enter intact live cells
- No compensation required with common FITC/GFP + PE combinations in flow cytometry
- Low photobleaching

Product highlights



Sample

Methanol fixed Hek293 cells

Green staining

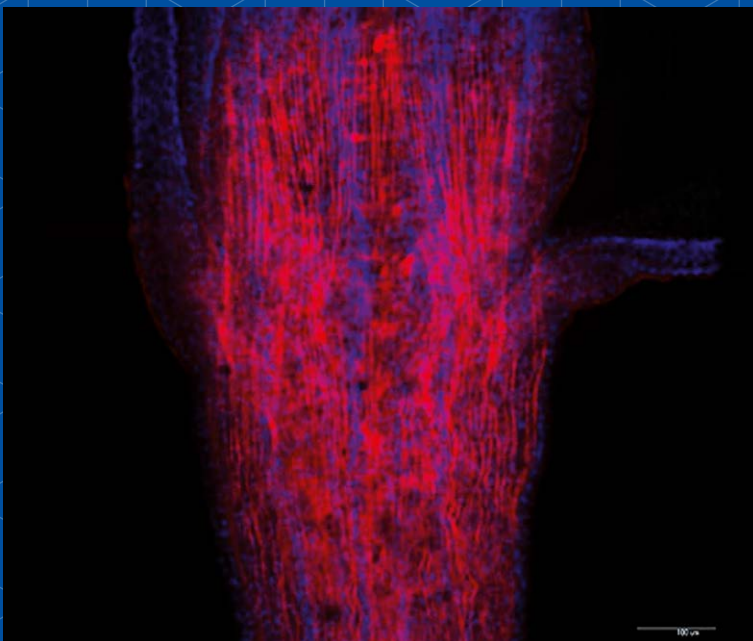
Plasma membrane marker ab197496 – mouse monoclonal to alpha 1 Sodium potassium ATPase (Alexa Fluor® 488)

Red staining

Microtubule marker ab195889 – mouse monoclonal to alpha tubulin (Alexa Fluor® 594)

Blue staining

Nucleus labeled with DAPI



Sample

Whole Hydractinia fixed in 4% PFA

Red staining

Actin filaments stained with CytoPainter F-actin Staining Kit –

Red Fluorescence (ab112127) 1:500

Blue staining

Nucleus labeled with Hoechst nuclear staining

Ion indicators and false neurotransmitters for cell imaging

Imaging and monitoring intracellular ion changes is vital for our understanding of signaling and functional pathways in cellular systems. These are central to many fundamental processes such as muscle contraction and synaptic nerve signal transmission. Measuring the ionic concentrations with both spatial and temporal resolution has become critical in research ranging from drug discovery to neuronal function studies.

We provide a wide range of ion indicators to track calcium and other ion concentrations with intense fluorescent signals over a range of different wavelengths.

Ca²⁺ indicators

Indicator	Excitation (nm)	Emission (nm)	Kd (nM)	Notable features
Fura-2	340/380	505	145	Available with enhanced characteristics - Low affinity - Leakage resistant - Near membrane
Indo-1	346	475/405	230	Available with enhanced characteristics - Low affinity - Leakage resistant - Near membrane
Fluo-8	490	520	390	Brighter than Fluo-4 due to improved cell loading
Rhod-2	552	581	570	Ideal for use in cells and tissues that have high levels of auto fluorescence. Rhod-2 AM is cationic, which facilitates uptake into mitochondria.

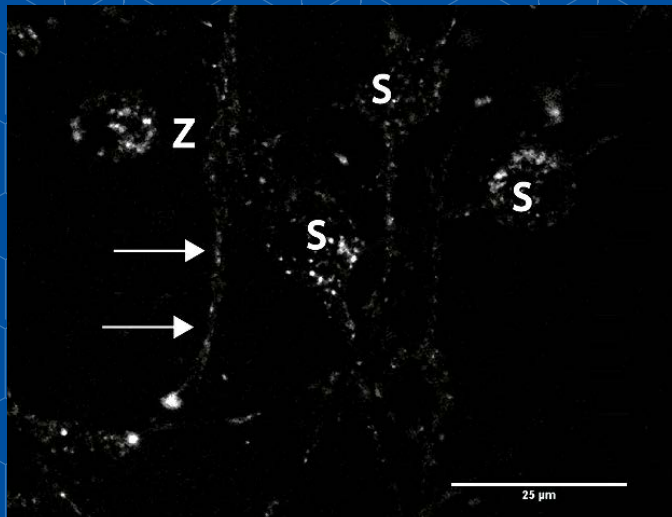
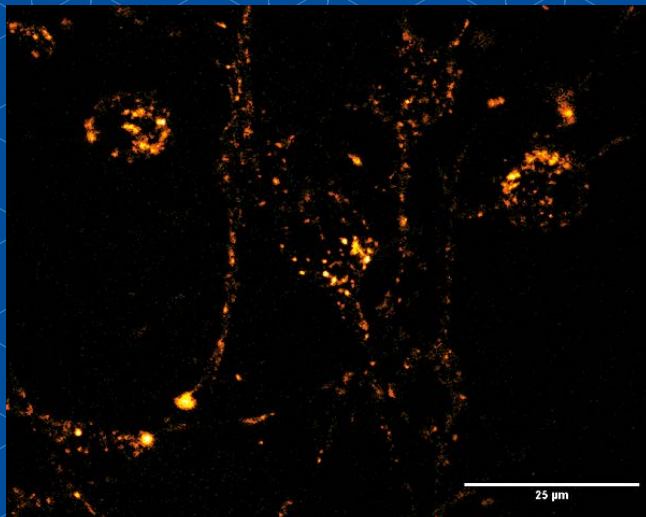
Find your ion, pH and membrane potential indicator today at [abcam.com/indicators](https://www.abcam.com/indicators)

Product highlights

Neuronal mapping with novel pH-responsive fluorescent false neurotransmitters

Discovery of fluorescent false neurotransmitters

The fluorescent false neurotransmitters FFN102, FFN202, and FFN511 have been designed to loosely mimic the overall topology and physical properties of monoamine neurotransmitters and have been engineered to have fluorescence properties.



Images show a group of neuronal cells stained with 50 μM FFN102 (sum projection of a confocal stack). FFN102 localizes to structures on the cell soma (S) as well as neurites (arrows). Z indicates the area zoomed in for an additional z-stack.

FFN102 allows measurement of activity at dopamine synapses and enables the study of synaptic plasticity by allowing optical imaging of dopaminergic presynaptic terminals.

Applications suitable for FFN102, FFN202 and FFN511:

- Measure localization and activity of dopaminergic presynaptic terminals
- Measure pH of secretory vesicles
- Visualize dopamine release from individual presynaptic terminals

Advantages of using fluorescent false neurotransmitters:

- Optically study various aspects of synaptic transmission
- Compatible with GFP tags including Alexa Fluor® 488
- Sufficiently bright, photostable and suitable for two-photo fluorescence microscopy
- Suitable for standard fluorescent microscopy

To find out more please visit abcam.com/ffn

Ancillary imaging reagents

We offer a wide range of high-quality ancillary reagents to support your imaging experiments, including mounting media and sera for blocking.

Mounting media

Fluoroshield is an aqueous mounting medium with a unique formulation that prevents rapid photobleaching of commonly used fluorescent labels during your experiment. In addition, the fluorescence of the sample is retained during prolonged storage at 4°C in the dark.

www.abcam.com/fluoroshield

Selected references

Lambert KG et al. Contingency-based emotional resilience: effort-based reward training and flexible coping lead to adaptive responses to uncertainty in male rats. *Front Behav Neurosci* 8:124 (2014).

Chung CY et al. Progressive Proximal-to-Distal Reduction in Expression of the Tight Junction Complex in Colonic Epithelium of Virally-Suppressed HIV+ Individuals. *PLoS Pathog* 10:e1004198 (2014).

Hernandez-Delgadillo R et al. Bismuth oxide aqueous colloidal nanoparticles inhibit *Candida albicans* growth and biofilm formation. *Int J Nanomedicine* 8:1645-52 (2013).

CyGEL™ is a novel thermoreversible hydrogel that enables easy immobilization of live non-adherent cells and organisms, and their subsequent recovery after microscopy. CyGEL™ is liquid when cold and a gel at ~21°C. It is optically clear with low autofluorescence. For imaging experiments longer than 2 hours, we recommend CyGEL Sustain™, which is specially formulated to allow the addition of RPMI and similar culture media, enabling cells to survive for hours.

www.abcam.com/cygels

Sera for blocking

Most immunostaining protocols include a blocking step to reduce non-specific binding of the antibody. We offer high-quality sera from a number of different species, including goat, donkey, guinea pig, mouse and rabbit. Simply match the serum species to the species that the secondary antibody was raised in.

www.abcam.com/blockingsera

Selected references

Guo Y et al. PEG-Like Nanoprobes: Multimodal, Pharmacokinetically and Optically Tunable Nanomaterials. *PLoS One* 9:e95406 (2014).

Najm FJ et al. Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells. *Nat Biotechnol* 31:426-33 (2013).

Witasz A et al. Elevated circulating levels and tissue expression of pentraxin 3 in uremia: a reflection of endothelial dysfunction. *PLoS One* 8:e63493 (2013).

Dzinic SH et al. Identification of an intrinsic determinant critical for maspin subcellular localization and function. *PLoS One* 8:e74502 (2013).

Conjugation kits and custom conjugation services

Can't find a commercially available antibody in the conjugate or buffer format you need? Try one of our fast and reliable conjugation kits to accelerate your research. If you are working on a project requiring a larger supply, our custom reformulation and conjugation services have successfully delivered many projects.

If you are struggling to find your antibody of interest labeled with the right dye, you can easily conjugate the label to the antibody yourself using one of our antibody conjugation kits. Our conjugation kits are quick and easy to use:

- Less than one minute hands-on time
- Conjugated antibody ready in under 20 minutes when using our fast conjugation kits
- (3 hours when using the standard kits)
- One-step labelling method, no separation steps required
- Label small amounts of antibody (as little as 10 µg)

We offer a wide range of fluorescent and enzymatic labels.

abcam.com/kits/antibody-conjugation-kits

Selected references

Guo Y et al. PEG-Like Nanoprobes: Multimodal, Pharmacokinetically and Optically Tunable Nanomaterials. *PLoS One* 9:e95406 (2014).

Najm FJ et al. Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells. *Nat Biotechnol* 31:426-33 (2013).

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Custom conjugation services

Abcam delivers many custom conjugates to our customers each month. We encourage you to inquire about your specific conjugation or reformulation needs.

- We can conjugate milligrams of material to Alexa Fluor® dyes and other common labels, to provide you with a consistent supply for your research
- We can purify and reformulate products to suit your particular applications

[Learn more at abcam.com/custom-conjugation](http://abcam.com/custom-conjugation)

Alexa Fluor® is a registered trademark of Life Technologies.

Alexa Fluor® dye conjugates contain(s) technology licensed to Abcam by Life Technologies.

abcam.com