ReadyProbes® ready-to-use imaging reagents for brilliant results

Convenient dropper bottles mean no calculations or dilutions required: Simply add and image.

Producing high-quality images of live and fixed fluorescent cells and tissues can be quite challenging, with optimization required at every step—from sample preparation to dye and instrument selection. As scientists at Life Technologies, we faced similar barriers in our own experiments and wanted to make things less complicated. With simplification in mind, we have developed a variety of ReadyProbes® fluorescent labeling reagents that make sample staining and image analysis much easier (Table 1).

Whether you are new to fluorescence imaging or an experienced user trying to streamline your protocols, ReadyProbes® reagents allow you to spend less time preparing solutions and more time analyzing results.

Figure 1. ReadyProbes® ready-to-use imaging reagents. (A) ReadyProbes® reagents are packaged in convenient dropper bottles; shown here is the BackDrop® Background Suppressor. **(B)** The easy-to-follow protocol can be found right on the lid of the product box—no more hunting for product insert pages. This staining protocol is for the NucBlue®, NucGreen™, and NucRed™ ReadyProbes® Reagents.

ReadyProbes® imaging reagents: The take-home message

ReadyProbes® ready-to-use imaging reagents allow you to stain cells without pipetting solutions, making calculations, preparing dilutions, or enlisting the help of an experienced labmate (Figure 1). Simply apply 2 drops from the convenient dropper bottles per milliliter of sample and you're ready for the next step. Moreover, many of these user-friendly formulations can be kept at room temperature, right on your lab bench. (The new ReadyProbes® secondary antibodies should be stored at 4°C for long-term storage; see box at the end of this article.) All ReadyProbes® products are designed to provide brilliant results without compromising image quality.

ReadyProbes® nuclear stains for blue, green, and red fluorescence channels

ReadyProbes® nuclear stains include cell-impermeant DNA dyes for dead- and fixed-cell staining and cell-permeant DNA dyes for live-cell staining (Table 1). Each of these high-affinity DNA dyes is supplied as a ready-to-use solution in a dropper bottle—just tip and

Table 1. ReadyProbes® ready-to-use reagents for fluorescence imaging.

ReadyProbes® reagent	For live cells?	Ex/Em (nm)*	Optical filters	Quantity	Cat. No.
Fluorescent nuclear stains					
NucBlue® Live ReadyProbes® Reagent	Yes	358/461	DAPI	6 x 2.5 mL	R37605
NucBlue® Fixed Cell ReadyProbes® Reagent	No	352/461	DAPI	6 x 2.5 mL	R37606
NucGreen™ Dead 488 ReadyProbes® Reagent	No	504/523	FITC	6 x 2.5 mL	R37109
NucRed™ Live 647 ReadyProbes® Reagent	Yes	638/686	Cy®5	6 x 2.5 mL	R37106
NucRed™ Dead 647 ReadyProbes® Reagent	No	642/661	Cy®5	6 x 2.5 mL	R37113
Propidium Iodide ReadyProbes® Reagent	No	535/617	RFP	6 x 2.5 mL	R37108
Fluorescent actin stains					
ActinGreen™ 488 ReadyProbes® Reagent	No	495/518	FITC	2 x 2.5 mL	R37110
ActinRed™ 555 ReadyProbes® Reagent	No	540/565	RFP	2 x 2.5 mL	R37112
Fluorescent apoptosis indicator					
CellEvent® Caspase-3/7 Green ReadyProbes® Reagent	Yes	502/530	FITC	1 x 2.5 mL	R37111
Fluorescence imaging accessories					
BackDrop® Background Suppressor ReadyProbes® Reagent	Yes	NA	NA	6 x 2.5 mL	R37603
Image-iT® FX Signal Enhancer ReadyProbes® Reagent	No	NA	NA	6 x 2.5 mL	R37107

*Excitation (Ex) and emission (Em) maxima in nm for the reagents when bound to their respective targets in cells.

drip two drops per milliliter of cell sample, regardless of whether your cells are in complete media or buffer solutions. Incubations times between 5 and 30 minutes are typical for bright nuclear staining, though some optimization may be required for certain cell types and growth conditions. Washing steps are not usually necessary; however, staining intensity will increase with time if cells are not washed prior to imaging.

NucBlue® Fixed-Cell and NucBlue® Live Reagents are ReadyProbes® formulations of the classic DAPI and Hoechst® 33342 dyes, respectively. NucBlue® Fixed-Cell Reagent provides you with a high-purity DAPI solution for blue-fluorescent staining of nuclei in fixed cells and tissues. NucBlue® Live Reagent is an optimized and validated Hoechst® 33342 solution that stains nuclei of live and fixed cells and tissues with bright blue fluorescence. Both NucBlue® reagents have large Stokes shifts (>100 nm) and are ideal for multiplexing with green- and red-fluorescent labels in antibody-based experiments and for nuclear segmentation in high-content imaging analyses.

NucGreen™ Dead 488 Reagent is a cell-impermeant stain that emits exceptionally bright green fluorescence when bound to DNA in cells with compromised membranes (Figure 2). Cells that have lost plasma membrane integrity are stained within minutes with the NucGreen™ Dead 488 Reagent, making it useful for detecting damaged, dying, and dead cells in cell preparations and tissue sections, as well as for staining fixed cells. Because it does not penetrate healthy live-cell membranes, NucGreen™ Dead 488 Reagent is well suited as a counterstain for viability and cytotoxicity assays.

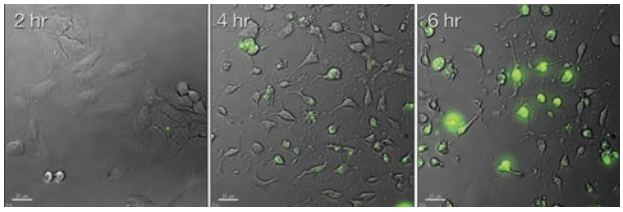


Figure 2. Visualizing the time course of apoptosis and cell death using NucGreen™ Dead 488 ReadyProbes® Reagent. HeLa cells were treated with 0.5 µM staurosporine to induce apoptosis. NucGreen™ Dead 488 Reagent (Cat. No. R37109) was applied to cells using 2 drops per milliliter complete medium; the dye remained in the sample throughout the course of the experiment. Differential interference contrast (DIC) and green-fluorescence channel images were collected over 6 hr using a DeltaVision® Core microscope with a 40x objective; images are gain- and exposure-matched.

The two NucRed™ reagents are far-red–fluorescent DNA dyes that can be easily combined with blue (DAPI, Hoechst®, CFP), green (Alexa Fluor® 488, FITC, GFP), and red (Alexa Fluor® 594, Texas Red®, TRITC) fluorophores in multicolor experiments. NucRed™ Dead 647 Reagent is a cell-impermeant DNA stain that provides a quick way to visualize cells with compromised membranes, including fixed cells, in the far-red fluorescence channel (Figure 3). The long-wavelength fluorescence of the NucRed™ Dead 647 Reagent is especially important when staining tissues, as its fluorescent signal is generally not obscured by tissue autofluorescence. The cell-permeant NucRed™ Live 647 Reagent stains the nuclei of both live and fixed cells with far-red fluorescence (Figure 4) and can be used for cell counting, DNA content measurements, and cell cycle assays. →

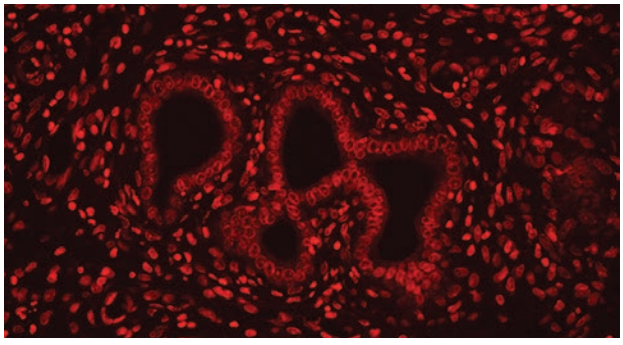


Figure 3. Staining nuclei in fixed uterine tissue with NucRed™ Dead 647 ReadyProbes® Reagent. An 8 µm section of formalin-fixed, paraffin-embedded rat uterine tissue was deparaffinized and stained with NucRed™ Dead 647 ReadyProbes® Reagent (Cat. No. R37113) in PBS (2 drops per milliliter of PBS) for 20 min. The section was then rinsed with PBS, mounted in ProLong® Gold Antifade Reagent (Cat. No. P36934), and imaged using a DeltaVision® Core microscope with a 20x objective.

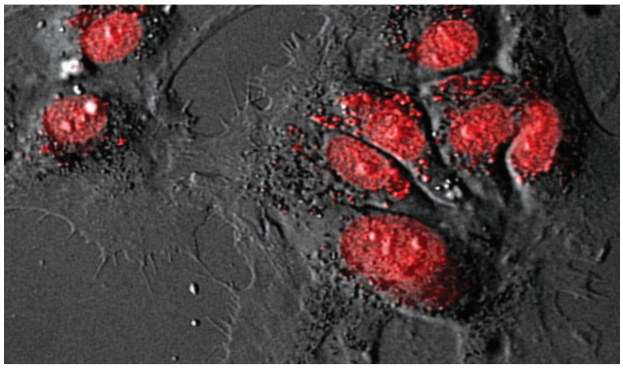


Figure 4. Visualizing nuclei in live cells with NucRed™ Live 647 ReadyProbes® Reagent. hUVECs were grown on MatTek glass-bottom culture dishes overnight. Nuclei were stained with NucRed™ Live 647 ReadyProbes® Reagent (Cat. No. R37106) by adding 2 drops per milliliter of complete medium and incubating for 20 min at 37°C. DIC and fluorescence images were collected using a DeltaVision® Core microscope with a 40x objective.

The ReadyProbes® family of nuclear stains also includes a room temperature–stable version of the classic cell-impermeant DNA stain propidium iodide. Extensively used as a dead-cell stain in fluorescence-activated cell sorting (FACS), propidium iodide exhibits a sufficiently large Stokes shift (Ex/Em = 535/617 nm) to allow simultaneous detection of both nuclear DNA and green-fluorescent FITC- or Alexa Fluor® 488–labeled antibodies with a single excitation source such as the 488 nm line of the argon-ion laser.

Label filamentous actin with green or red fluorescence

ActinGreen™ 488 and ActinRed™ 555 ReadyProbes® Reagents (Table 1) are high-affinity fluorescent phalloidin conjugates for visualizing F-actin in fixed and permeabilized cells and tissues and are fully compatible with other fixed-cell probes, including antibodies, fluorescent dyes, and Qdot® probes. ActinGreen™ 488 Reagent is a green-fluorescent Alexa Fluor® 488 conjugate of phalloidin that is both extremely bright and photostable (Figure 5); similarly, ActinRed™ 555 Reagent is a red-orange–fluorescent tetramethylrhodamine phalloidin conjugate.

Fluorescently labeled phalloidin has several advantages over antibodies for actin labeling, including virtually identical binding properties with actin from different species of plants and animals and low nonspecific binding. Whether you are studying the cytoskeleton in the context of stem cell differentiation or cancer progression, or as a target in drug discovery, ActinGreen™ and ActinRed™ ReadyProbes® Reagents are validated, robust actin stains. As with other ReadyProbes® reagents, the ActinGreen™ and ActinRed™ probes come in convenient dropper bottles and effectively stain fixed and permeabilized cells with just two drops per milliliter of buffer solution or complete media.

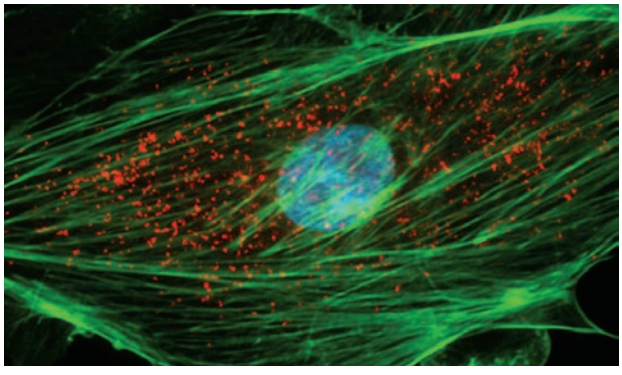


Figure 5. Three-color imaging of actin (green), nuclei (blue), and peroxisomes (red) in fixed BPAE cells. BPAE cells were fixed, permeabilized, and blocked using the Image-iT® Fixation/Permeabilization Kit (Cat. No. R37602). Peroxisomes were labeled using rabbit anti-PMP70 antibody (Cat. No. 718300) followed by detection with Alexa Fluor® 594 goat anti–rabbit IgG secondary antibody (ReadyProbes® Reagent, Cat. No. R37117). Actin was stained using ActinGreen™ 488 ReadyProbes® Reagent (Cat. No. R37110), and nuclei were counterstained with NucBlue® Fixed Cell ReadyProbes® Reagent (Cat. No. R37606).

Track apoptosis in its earliest stages with CellEvent® Caspase-3/7 Green ReadyProbes® Reagent

CellEvent® Caspase-3/7 Green ReadyProbes® Reagent (Table 1) is a no-wash fluorogenic probe for the detection of activated caspase-3 and caspase-7, which are early indicators of apoptosis. Compatible with both live- and fixed-cell fluorescence imaging applications, this ReadyProbes® reagent allows rapid and sensitive detection of cells destined for apoptosis. CellEvent® Caspase-3/7 Green Reagent is a 4–amino acid peptide (DEVD) conjugated to a nucleic acid–binding dye. Upon activation of caspase-3 or caspase-7, this peptide is cleaved such that the dye can bind to DNA and generate bright green fluorescence (Ex/Em = 502/530 nm), which can be observed using a standard FITC filter set.

The simple and fast no-wash protocol for the CellEvent® Caspase-3/7 Green Reagent helps preserve delicate apoptotic cells. Importantly, the fluorescent signal from CellEvent® Caspase-3/7 Green Reagent survives fixation and permeabilization, making it compatible with end-point and antibody-based assays.

Reduce background fluorescence in live or fixed cells to enhance signal-to-noise ratios

Image-iT® FX Signal Enhancer dramatically reduces background fluorescence resulting from nonspecific staining during secondary detection with fluorescent avidin or antibody conjugates (Figure 6, Table 1). The Image-iT® FX Signal Enhancer ReadyProbes® Reagent is a room temperature–stable solution that is applied directly to slides or coverslips containing fixed and permeabilized cells or tissue samples before staining with fluorescent probes. Simply wash your slides and then add enough drops to cover the sample. After a 30-minute incubation, you are ready to proceed with your staining protocol.

This reagent is a highly effective block for reducing background staining that results from nonspecific interactions between a variety of fluorescent conjugates and cell and tissue constituents. Image-iT® FX Signal Enhancer can significantly reduce this background binding, resulting in marked improvements in staining that

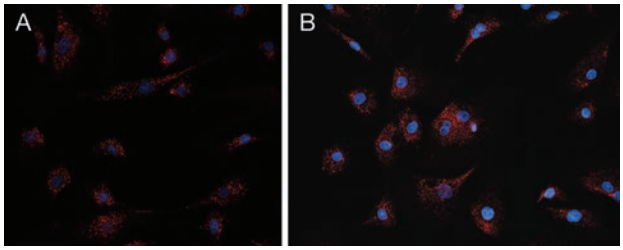


Figure 6. Reducing background fluorescence with Image-iT® FX Signal Enhancer. BPAE cells were fixed, permeabilized, and blocked using the Image-iT® Fixation/Permeabilization Kit (Cat. No. R37602). Samples were then incubated **(A)** without or **(B)** with Image-iT® FX Signal Enhancer ReadyProbes® Reagent (Cat. No. R37107) prior to labeling peroxisomes with rabbit anti-PMP70 antibody (Cat. No. 718300) in conjunction with Alexa Fluor® 594 goat anti–rabbit IgG secondary antibody (Cat. No. A11037). Nuclei were counterstained with NucBlue® Fixed Cell ReadyProbes® Reagent (Cat. No. R37606).

can mean the difference when pushing sensitivity limits to detect low-abundance targets.

If you are working with live cells, we recommend using the BackDrop® Background Suppressor ReadyProbes® Reagent (Table 1). Background signals from media components, nonspecific labeling, and unbound fluorophores can decrease signal-to-noise ratios and thus reduce assay sensitivity. Supplied as a set of two dropper bottles each of blue, green, and red fluorescence suppressors (Figure 1A), BackDrop® Background Suppressor is designed to effectively suppress background fluorescence in live-cell imaging samples without having to remove media or unincorporated dye. If you are experiencing high background signals or weak fluorescence in the blue, green, or red channels, see how a few drops of BackDrop® Background Suppressor can improve your image quality by cutting through the haze and increasing contrast. BackDrop® Background Suppressor is most effective if cells are photographed within one hour of application, though longer treatment will not harm cells.

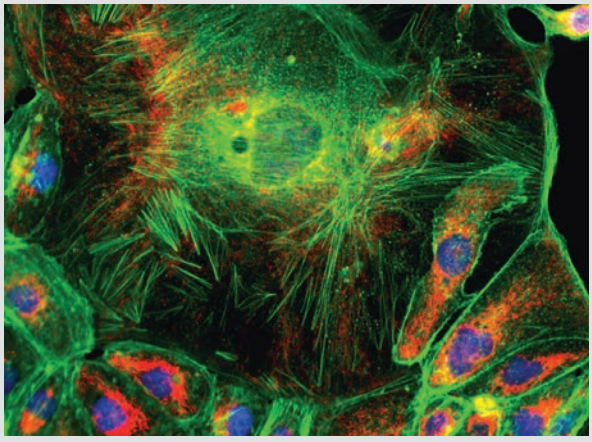
More ReadyProbes® reagents under development

We are continuing to develop user-friendly reagents and protocols to facilitate fluorescence imaging for beginners and experts alike. As always, we welcome your comments and suggestions. To learn more about our current selection of ReadyProbes® reagents, visit **lifetechnologies.com/readyprobesbp69**. ■

Our newest ready-to-use imaging reagents: ReadyProbes® secondary antibodies

We have recently introduced eight ready-to-use dropper bottle formulations of our donkey and goat secondary antibodies labeled with the bright and photostable Alexa Fluor® 488 and Alexa Fluor® 594 dyes. These Alexa Fluor® secondary antibodies react with IgG heavy chains and all classes of immunoglobulin light chains from either mouse or rabbit. Best of all, they are as convenient as our other ReadyProbes® formulations. Just apply 2 drops per milliliter of sample and you are ready to image—no pipetting or dilutions required.

ReadyProbes® secondary antibodies	Quantity	Cat. No.
Alexa Fluor® 488 Donkey Anti–Mouse IgG Antibody	2 x 2.5 mL	R37114
Alexa Fluor® 594 Donkey Anti–Mouse IgG Antibody	2 x 2.5 mL	R37115
Alexa Fluor® 488 Donkey Anti–Rabbit IgG Antibody	2 x 2.5 mL	R37118
Alexa Fluor® 594 Donkey Anti–Rabbit IgG Antibody	2 x 2.5 mL	R37119
Alexa Fluor® 488 Goat Anti–Mouse IgG Antibody	2 x 2.5 mL	R37120
Alexa Fluor® 594 Goat Anti–Mouse IgG Antibody	2 x 2.5 mL	R37121
Alexa Fluor® 488 Goat Anti–Rabbit IgG Antibody	2 x 2.5 mL	R37116
Alexa Fluor® 594 Goat Anti–Rabbit IgG Antibody	2 x 2.5 mL	R37117



Immunostaining with ReadyProbes® secondary antibodies. CAKI cells were fixed, permeabilized, and blocked using the Image-iT® Fixation/Permeabilization Kit (Cat. No. R37602). Mitochondria were labeled using anti–ATP synthase subunit IF1 mouse monoclonal antibody (clone 5E2D7, Cat. No. A21355) followed by detection with Alexa Fluor® 594 goat anti–mouse IgG antibody (ReadyProbes® Reagent, Cat. No. R37121). Actin was stained using ActinGreen™ 488 ReadyProbes® Reagent (Cat. No. R37110), and nuclei were counterstained with NucBlue® Fixed Cell ReadyProbes® Reagent (Cat. No. R37606).