



# Fluorescent Cellular Stains

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# Fluorescent Membrane Stains

## CellBrite™ Fix: Novel Membrane Dyes That Tolerate Permeabilization

CellBrite™ Fix dyes are a new class of dyes developed by Biotium to rapidly stain the outer plasma membranes of live cells. While other lipophilic membrane dyes can be fixed with formaldehyde, they are not compatible with detergent permeabilization or methanol fixation. In contrast, CellBrite™ Fix Membrane Stains are unique in that their surface staining can withstand permeabilization and methanol fixation, allowing membrane staining to be combined with intracellular staining with antibodies (Figure 2). Unlike lectins such as WGA, which bind specific targets, CellBrite™ Fix dyes are general membrane stains. However, unlike original CellBrite™ dyes and lectins, CellBrite™ Fix dyes cannot be used to stain cells that are already fixed.

CellBrite™ Fix dyes are amine-reactive and are designed to accumulate at the cell membrane, where they become covalently attached to membrane proteins. As a result, surface staining is well-retained after permeabilization or methanol fixation, with only a slight increase in intracellular fluorescence compared to formaldehyde fixation alone. CellBrite™ Fix dyes are only weakly fluorescent in aqueous media but become intensely fluorescent upon membrane staining. This fluorogenic property of the dyes makes the staining very specific with low background. Due to their better water solubility, CellBrite™ Fix dyes yield much more uniform staining compared to lipophilic carbocyanine dyes like DiO and Dil. The dyes are non-cytotoxic and do not readily transfer between cells. They also can be used to stain yeast and bacteria (gram-positive or gram-negative).

CellBrite™ Fix dyes are available with visible red and far-red fluorescence. More colors are in development.

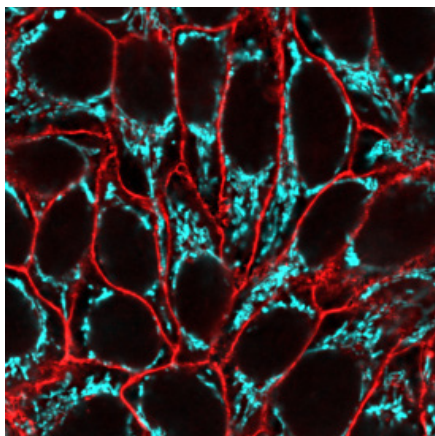


Figure 2. HeLa cells were stained with CellBrite™ Fix 555 (magenta), then fixed in methanol and stained with CF@640R Monoclonal anti-Mitochondrial Marker Clone 113-1 (cyan).

## CellBrite™ Fix Cytoplasmic Membrane Dyes

| Catalog No. | Product                           | Ex/Em (nm) | Unit Size |
|-------------|-----------------------------------|------------|-----------|
| 30088-T     | CellBrite™ Fix 555 Membrane Stain | 542/571    | 1 vial    |
| 30088       |                                   |            | 5 vials   |
| 30089-T     | CellBrite™ Fix 640 Membrane Stain | 638/667    | 1 vial    |
| 30089       |                                   |            | 5 vials   |

## CellBrite™ Cytoplasmic Membrane Dyes

| Catalog No. | Product   | Ex/Em (nm) | Unit Size |
|-------------|---|------------|-----------|
| 30024       | CellBrite™ Blue Cytoplasmic Membrane Labeling Kit   | 366/441    | 50 assays |
| 30021       | CellBrite™ Green Cytoplasmic Membrane Labeling Dye  | 484/501    | 1 mL      |
| 30022       | CellBrite™ Orange Cytoplasmic Membrane Labeling Dye | 549/565    | 1 mL      |
| 30023       | CellBrite™ Red Cytoplasmic Membrane Labeling Dye    | 644/665    | 1 mL      |
| 30070       | CellBrite™ NIR680 Cytoplasmic Membrane Labeling Dye | 683/724    | 100 uL    |
| 30077       | CellBrite™ NIR750 Cytoplasmic Membrane Labeling Dye | 748/780    | 100 uL    |
| 30078       | CellBrite™ NIR770 Cytoplasmic Membrane Labeling Dye | 767/806    | 100 uL    |
| 30079       | CellBrite™ NIR790 Cytoplasmic Membrane Labeling Dye | 786/820    | 100 uL    |

## CellBrite™ Cytoplasmic Membrane Dyes

Lipophilic carbocyanine dyes label membranes in a wide variety of cell types. The dyes are weakly fluorescent in aqueous phase, but become highly fluorescent in lipid bilayers. Staining is very stable with low toxicity and very little dye transfer in between cells, making the dyes suitable for long-term cell labeling and tracking studies. Cell populations can be labeled with different fluorescent colors for identification after mixing. Double labeling can identify cells that have fused or formed stable clusters. Cells can be fixed with formaldehyde either before or after staining, but plasma membrane staining has poor tolerance for detergent permeabilization.

Unlike PKH dyes, CellBrite™ dyes do not require a complicated hypoosmotic labeling protocol. They are ready-to-use dye delivery solutions that can be added directly to normal culture media to label suspended or adherent cells in culture. We offer a selection of dyes with fluorescence ranging from blue to near-infrared. The CellBrite™ NIR dyes are suitable for near-IR small animal imaging.

## Other Lipophilic Carbocyanine Dyes for Membrane Labeling

Biotium offers a selection of stand-alone carbocyanine dyes. Neuro-DiO, Neuro-Dil, and diinoleyl dyes have structural features designed to make the probes diffuse faster in cell membranes. Near-infrared DiR is useful for small animal imaging.

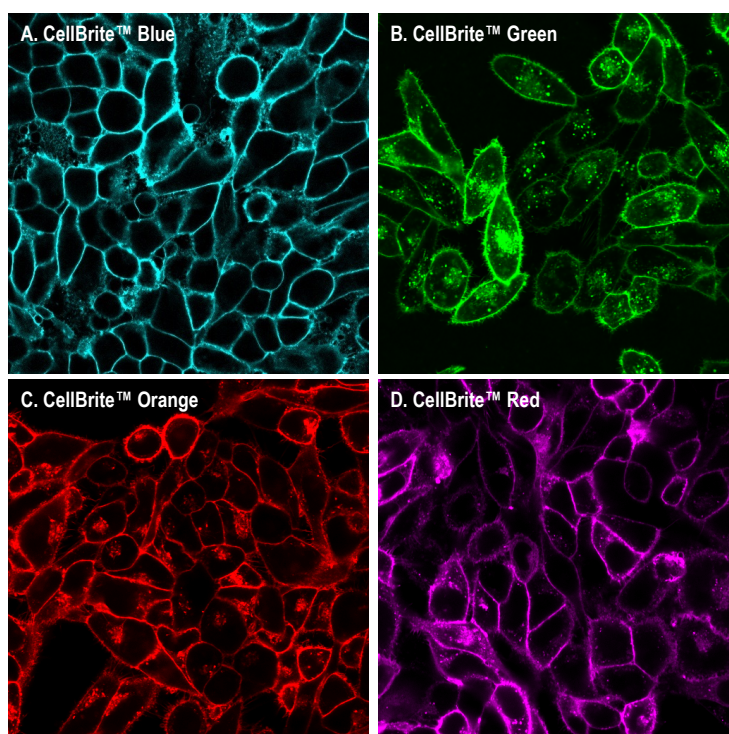


Figure 1. HeLa cells stained with CellBrite™ Blue after formaldehyde fixation (A). Live cell staining with CellBrite™ Green (B), CellBrite™ Orange (C), or far-red CellBrite™ Red (D).

## Other Lipophilic Carbocyanine Dyes

| Catalog No. | Product  | Ex/Em (nm) | Unit Size |
|-------------|--|------------|-----------|
| 60011       | DiO  | 484/501    | 50 mg     |
| 60015       | Neuro-DiO                                      | 484/501    | 25 mg     |
| 60019       | Neuro-DiO in vegetable oil                     | 484/501    | 200 uL    |
| 60035       | Dilinoleyl DiO (FAST DiO™)                     | 484/499    | 5 mg      |
| 60012       | DiOC <sub>14</sub> (3), hydroxyethanesulfonate | 484/501    | 50 mg     |
| 60010       | Dil  | 549/565    | 50 mg     |
| 60018       | Dil in vegetable oil                           | 549/565    | 0.5 mL    |
| 60034       | Dilinoleyl Dil (FAST Dil™)                     | 549/565    | 5 mg      |
| 60016       | Neuro-Dil                                      | 549/565    | 25 mg     |
| 60014       | DiD  | 644/655    | 50 mg     |
| 60017       | DiR  | 748/780    | 25 mg     |

Fast Dil and Fast DiO are trademarks of Thermo Fisher Scientific

# Lipid Stains and Membrane Potential Dyes

## LipidSpot™ Lipid Droplet Stains

Intracellular lipid droplets are cytoplasmic organelles involved in the storage and regulation of triglycerides and cholesterol esters. LipidSpot™ dyes are fluorogenic neutral lipid stains that rapidly stain lipid droplets in live cells with no wash step required. Cells also can be fixed and permeabilized before or after staining. LipidSpot™ stains show minimal background staining of cellular membranes or other organelles, unlike traditional dyes like Nile Red. Available with green or far-red fluorescence.

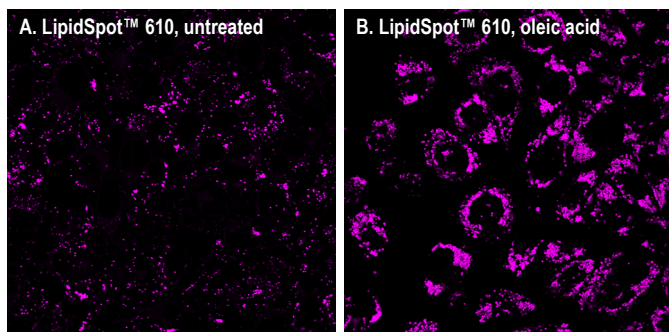


Figure 1. Live HeLa cells stained with LipidSpot™ 610. Prior to staining, cells were untreated (A) or treated with oleic acid to induce lipid droplet formation (B).

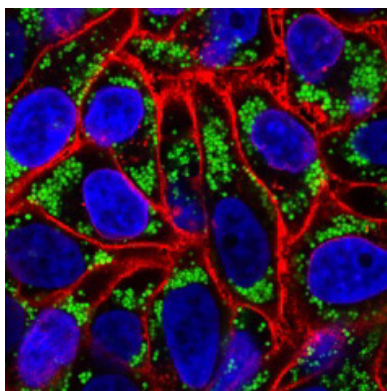


Figure 2. Oleic acid-treated HeLa cells were fixed with formaldehyde and stained with LipidSpot™ 488 (green), CF@594 WGA (red), and Hoechst (blue).

## LipidSpot™ Lipid Droplet Stains

| Catalog No. | Product   | Ex/Em (nm) | Unit Size |
|-------------|---|------------|-----------|
| 70065-T     | LipidSpot™ 488 Lipid Droplet Stain, 1000X in DMSO | 420/511    | 20 uL     |
| 70065       |   |            | 125 uL    |
| 70069-T     | LipidSpot™ 610 Lipid Droplet Stain, 1000X in DMSO | 610/663    | 20 uL     |
| 70069       |   |            | 125 uL    |

## Phospholipid Membrane Probes

These membrane probes are derived from natural phospholipids by modifying the head group with a fluorescent dye or biotin. The probes are useful for studies of vesicle trafficking and membrane fusion. Red fluorescent phospholipids like TRITC-, Rhodamine-, and Texas Red®-DHPE have been used as fluorescence acceptors in combination with NBD-DHPE in membrane-fusion assays.

## Phospholipid Probes

| Catalog No. | Product          | Ex/Em (nm) | Unit Size |
|-------------|------------------|------------|-----------|
| 60022       | Biotin-DHPE      | N/A        | 10 mg     |
| 60023       | Biotin-X-DHPE    | N/A        | 5 mg      |
| 60024       | Fluorescein-DHPE | 496/519    | 5 mg      |
| 60025       | NBD-PE           | 463/536    | 10 mg     |
| 60028       | TRITC-DHPE       | 540/566    | 1 mg      |
| 60026       | Rhodamine-DHPE   | 560/581    | 5 mg      |
| 60027       | Texas Red®-DHPE  | 582/601    | 1 mg      |

## Slow-Responding Membrane Potential Dyes

Translational (or slow-responding) membrane potential dyes undergo a change in their membrane distribution as a result of changes in membrane potential.

The fluorescence of DiBAC<sub>4</sub>(3) is enhanced with membrane depolarization. The rate of fluorescence response of the dye is slower than styryl dyes like the ANEPPS dyes, but the fluorescence change is significantly larger. DiOC<sub>2</sub>(3) has been used for measuring membrane potential in bacteria. The green fluorescent dye forms red fluorescent aggregates with increasing membrane potential, allowing ratiometric potential measurements. DiOC<sub>5</sub>(3) and DiOC<sub>6</sub>(3) are two of the most widely used carbocyanine dyes for membrane potential measurements. Tetramethylrhodamine ethyl ester (TMRE) and Tetramethylrhodamine methyl ester (TMRM) can be used for quantitative measurements of membrane potential and mitochondrial membrane potential.

## Fast-Responding Membrane Potential Dyes

Fast-responding membrane potential dyes are styryl dyes that undergo changes in fluorescence intensity in response to changes in membrane potential, on the order of 2-10% change in fluorescence per 100 mV. The dyes also undergo spectral shift with changes in membrane potential, allowing ratiometric measurements. Fast response dyes have been used to measure electrical activity in neural and cardiac cells.

Di-8-ANNEPPS is more hydrophobic and better retained in the outer leaflet of the plasma membrane than Di-4-ANNEPS, and therefore is more suitable for long-term membrane potential studies. It is also more photostable and less phototoxic than Di-4-ANNEPS. Di-4-ANNEPS has been used for studies of human stem cell-derived cardiomyocytes.

Di-2-ANEPEQ (also known as JPW 1114) is a highly water soluble fast-responding dye that is usually introduced into cells by microinjection. Di-8-ANEPEQ and Di-12-ANEPEQ are successively more hydrophobic, and have been used for potential-sensitive retrograde labeling of neurons.

RH237, RH414, RH421, and RH795 are fast-responding potentiometric probes generally used for functional imaging of neurons. RH421 exhibits >20% fluorescence change per 100 mV on neuroblastoma cells. These dyes can differ in their physiological effects, for example RH414 causes arterial constriction during cortex staining, while the spectrally similar dye RH795 does not.

## DiO/DPA Membrane Potential Kit

The membrane localization of the fluorescence quencher dipicrylamine (DPA) is a function of the polarity and magnitude of membrane potential. The DiO/DPA system detects cytoplasmic membrane potential changes using the principle of fluorescence resonance energy transfer (FRET). The green fluorescent membrane dye DiO is a "stationary" FRET donor while DPA acts as a mobile FRET acceptor, resulting in a membrane potential-dependent quenching of fluorescence by FRET. The DiO/DPA system has been reported to produce a fluorescence signal change of >56% in HEK-293 cells and >25% in neuronal cultures and brain slices per 100 mV membrane potential change.

## Membrane Potential Dyes

| Catalog No. | Product                        | Ex/Em (nm) (MeOH) <sup>1</sup> | Unit Size |
|-------------|--------------------------------|--------------------------------|-----------|
| 61011       | DiBAC <sub>4</sub> (3)         | 493/516                        | 25 mg     |
| 70008       | DiOC <sub>2</sub> (3)          | 482/497                        | 100 mg    |
| 70007       | DiOC <sub>5</sub> (3)          | 482/497                        | 100 mg    |
| 70009       | DiOC <sub>6</sub> (3)          | 484/501                        | 100 mg    |
| 70016       | TMRE                           | 549/574                        | 25 mg     |
| 70017       | TMRM                           | 548/573                        | 25 mg     |
| 61010       | Di-4-ANEPPS                    | 496/705                        | 5 mg      |
| 61012       | Di-8-ANEPPS                    | 498/713                        | 5 mg      |
| 61013       | Di-2-ANEPEQ (JPW 1114)         | See note 2                     | 5 mg      |
| 61014       | Di-8-ANEPPQ                    | See note 2                     | 5 mg      |
| 61015       | Di-12-ANEPPQ                   | See note 2                     | 5 mg      |
| 61018       | RH237                          | 528/782                        | 5 mg      |
| 61016       | RH414                          | 532/706                        | 5 mg      |
| 61017       | RH421                          | 515/704                        | 25 mg     |
| 61019       | RH795                          | 530/712                        | 5 mg      |
| 30037       | DiO/DPA Membrane Potential Kit | 484/501 (DiO)<br>406/- (DPA)   | 1 kit     |

<sup>1</sup>Spectral properties of styryl dyes are highly dependent on environment. In cell membranes, spectra are typically blue shifted by as much as 20 nm for Abs/Ex and 80 nm for Em.

<sup>2</sup>Spectra expected to be similar to Di-4-ANEPPS and Di-8-ANEPPS, but red-shifted 10-20 nm.

# Nerve Terminal Probes

## SynaptoGreen™ and SynaptoRed™ Nerve Terminal Dyes

Nerve terminal probes were originally called FM® dyes. Now they are available from Biotium under the trademark names SynaptoGreen™ and SynaptoRed™, depending on their fluorescence emission. They are a series of fluorescent cationic styryl dyes developed to follow synaptic activity at neuromuscular junctions or synapses. The dyes label synaptic vesicles in neuronal tissues (Fig. 1) and cultured neurons (page 5, Fig. 1) in an activity-dependent fashion. They also can be used to label endocytic vesicles in other cell types. These dyes have a lipophilic tail at one end and a highly hydrophilic, cationically charged head group at the other end as illustrated by the general structure below, where m is the number of carbons in the lipophilic tail and n is the number of double bonds linking the two aromatic rings in the dye.

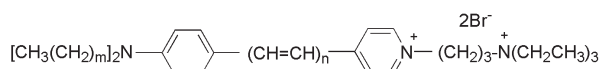


Figure 1. General structure of SynaptoGreen™ and SynaptoRed™ dyes, where m = 0-17 and n = 1 or 3.

Nerve terminal dyes are virtually non-fluorescent in aqueous solution, but become intensely fluorescent in membranes. Following nerve stimulation, the dye molecules are internalized in newly formed endocytic vesicles. During exocytosis, the dyes are released from the vesicles along with neurotransmitters, causing a decrease in fluorescence signal. As a result, the change in fluorescent intensity reflects the amount of endocytosis/exocytosis or synaptic activity. The rate of fluorescence increase during endocytosis, the “on-rate”, and the rate of fluorescence decrease during exocytosis, the “off-rate”, vary from dye to dye. In general, dyes with longer lipophilic tails and more double bonds have a higher affinity toward membrane and thus a higher on-rate and lower off-rate. Some styryl dyes can enter cells through ion channels; SynaptoGreen™ C18 and AM3-25 are high molecular weight dyes that cannot pass through ion channels, and have been used as controls to distinguish mechanisms of dye uptake.

### Fixable Synaptic Vesicle Dyes

AM dyes and HM dyes are fixable nerve terminal dyes. After staining with these dyes, cells can be fixed and permeabilized for subsequent immunostaining. The AM dyes have an aldehyde-fixable amino group attached to the positively-charged head group of the dyes. HM1-43 is similar except that the amino group is replaced by a hydrazide, which is more reactive with aldehyde fixatives. AM dyes are more water-soluble (and therefore have a lower on-rate and higher off-rate) than the corresponding FM dye counterparts, while HM1-43 is more lipophilic than AM1-43 due to its neutral hydrazide group.

### Background Quenchers and Nerve Terminal Staining Kits

A common problem encountered with nerve terminal dyes is background fluorescence due to residual membrane staining, even after extensive washing. To reduce this background fluorescence, we offer three quencher or dye-clearing agents. ADVASEP-7, a sulfonated β-cyclodextrin, forms a water soluble inclusion complex with SynaptoGreen™ C4 that can be removed more effectively by washing. Biotium's unique quencher, SCAS, reduces background fluorescence as soon as it is added to the preparation without the need for washing. Sulforhodamine 101 quenches SynaptoGreen™ background staining via fluorescent resonance energy transfer (FRET). We offer these reagents as individual products and in kits with dyes and the quencher/dye-clearing agents.

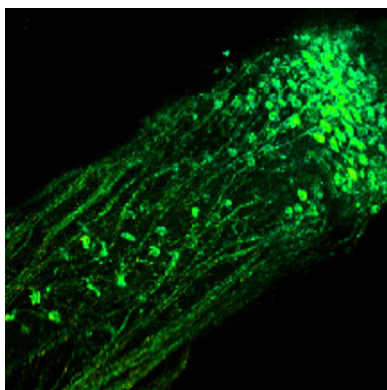


Figure 1. The apical half of a single mouse vibrissal follicle, labeled with AM1-43. Image courtesy of Dr. David Corey, Harvard Medical School.

## Nerve Terminal Dyes

| Catalog No. | Size     | Product                                   | m* | n* | Ex/Em (nm) in membranes | Fixable? |
|-------------|----------|---|----|----|-------------------------|----------|
| 70042       | 5 mg     | SynaptoGreen™ C1                          | 0  | 1  | ~480/600                | No       |
| 70043       | 5 x 1 mg |   |    |    |                         |          |
| 70044       | 5 mg     | SynaptoGreen™ C2 (equivalent to FM®2-10)  | 1  | 1  | ~480/600                | No       |
| 70045       | 5 x 1 mg |   |    |    |                         |          |
| 70023       | 5 mg     | SynaptoGreen™ C3                          | 2  | 1  | ~480/600                | No       |
| 70026       | 5 x 1 mg |   |    |    |                         |          |
| 70020       | 5 mg     | SynaptoGreen™ C4 (equivalent to FM®1-43)  | 3  | 1  | ~480/600                | No       |
| 70022       | 5 x 1 mg |   |    |    |                         |          |
| 70046       | 5 mg     | SynaptoGreen™ C5 (equivalent to FM®1-84)  | 4  | 1  | ~480/600                | No       |
| 70047       | 5 x 1 mg |   |    |    |                         |          |
| 70048       | 5 mg     | SynaptoGreen™ C18 (equivalent to FM®3-25) | 17 | 1  | ~480/600                | No       |
| 70049       | 5 x 1 mg |   |    |    |                         |          |
| 70024       | 1 mg     | AM1-43                                    | 3  | 1  | ~480/600                | Yes      |
| 70038       | 1 mg     | AM1-44                                    | 4  | 1  | ~480/600                | Yes      |
| 70036       | 1 mg     | AM2-10                                    | 1  | 1  | ~480/600                | Yes      |
| 70051       | 1 mg     | AM3-25                                    | 17 | 1  | ~480/600                | Yes      |
| 70053       | 1 mg     | HM1-43                                    | 3  | 1  | ~480/600                | Yes      |
| 70040       | 5 mg     | SynaptoRed™ C1                            | 0  | 3  | ~510/750                | No       |
| 70041       | 5 x 1 mg |   |    |    |                         |          |
| 70021       | 5 mg     | SynaptoRed™ C2 (equivalent to FM®4-64)    | 1  | 3  | ~510/750                | No       |
| 70027       | 5 x 1 mg |   |    |    |                         |          |
| 70019       | 5 mg     | SynaptoRed™ C2M** (equivalent to FM®5-95) | 1  | 3  | ~510/750                | No       |
| 70028       | 5 x 1 mg |   |    |    |                         |          |
| 70025       | 1 mg     | AM4-64                                    | 1  | 3  | ~510/750                | Yes      |
| 70039       | 1 mg     | AM4-65                                    | 3  | 3  | ~510/750                | Yes      |
| 70050       | 1 mg     | AM4-66                                    | 4  | 3  | ~510/750                | Yes      |

\*See Figure 1 for general dye structures. \*\*The positively-charged end of SynaptoRed C2M is a trimethylammonium group. FM is a registered trademark of Thermo Fisher Scientific.

### Background Quenchers

| Catalog No. | Product            | Unit Size |
|-------------|--------------------|-----------|
| 70029       | ADVASEP-7          | 250 mg    |
| 70037       | SCAS               | 100 mg    |
| 80101       | Sulforhodamine 101 | 100 mg    |

### Nerve Terminal Staining Kits

| Catalog No. | Product                            | Kit Components   |
|-------------|------------------------------------|--|
| 70030       | Nerve Terminal Staining Kit I      | 5 x 1 mg SynaptoGreen™ C4 (70022)<br>250 mg ADVASEP-7 (70029)          |
| 70031       | Nerve Terminal Staining Kit II (A) | 1 mg AM1-43 (70024)<br>100 mg ADVASEP-7 (70029-1)                      |
| 70031-1     | Nerve Terminal Staining Kit II (B) | 1 mg of AM1-43 (70024)<br>100 mg SCAS (70037)                          |
| 70032       | Nerve Terminal Staining Kit III    | 5 x 1 mg SynaptoGreen™ C4 (70022)<br>100 mg Sulforhodamine 101 (80101) |
| 70034       | Nerve Terminal Staining Kit V      | 5 x 1 mg SynaptoRed™ C2 (70027)<br>250 mg ADVASEP-7 (70029)            |

# Cytosolic Tracers and Cell Division Tracking

## CF® Dye Hydrazides

We offer a wide selection of our bright and photostable CF® dyes as water-soluble, aldehyde-fixable hydrazides for microinjection as neuronal tracers (Figure 1). See page 11.

## Calcein and Calcein AM

Calcein is a water soluble fluorescein derivative widely used for the study of cell membrane integrity that can be introduced by microinjection. Calcein AM is a membrane-permeant AM ester of calcein that can be loaded into cells by incubation, where it is cleaved by esterases to release the dye. Calcein AM also can be used to quantitate viable cells (see page 10).

## Other Fluorescent Tracers

Lucifer Yellow CH and Lucifer Yellow cadaverine are aldehyde-fixable green fluorescent cytosolic tracers. Hydroxystilbamidine (equivalent to Fluoro-Gold™) has been used extensively as a retrograde tracer for neurons and as a histochemical stain. Sulforhodamines are highly water soluble red fluorescent dyes that can be used as polar tracers for neuronal morphology and intercellular junctions. Sulforhodamine B also has been used as a fixed cell protein stain for colorimetric quantitation of cell number.

## Biotin Derivatives

Biocytin is a cellular tracer that can be introduced by microinjection; biocytin hydrazide is an aldehyde-fixable analog. Biotin ethylenediamine (equivalent to Neurobiotin™) is used as an anterograde and transneuronal tracer. Fluorescent biotin derivatives can be used for cell tracing or detection of biotin binding sites. We also offer conjugates of biotin and the biotin-binding protein streptavidin with our bright and photostable CF® dyes. See page 11.

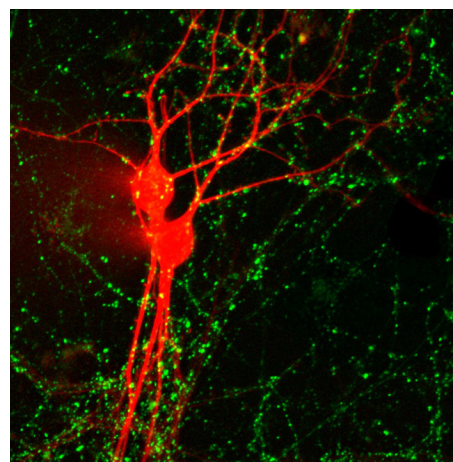


Figure 1. Cultured rat hippocampal neurons microinjected with CF@647 hydrazide (red) and stained with SynptoGreen™ C4 (green, synaptic vesicles). Image courtesy of Professor Guosong Liu, Tsinghua University, Beijing.

## Cytosolic Tracers

| Catalog No. | Product  | Ex/Em (nm)                      | Unit Size   |
|-------------|--|---------------------------------|-------------|
| 80013       | Calcein (high purity)                                      | 494/517                         | 100 mg      |
| 30026       | Calcein AM Cell Viability Assay Kit                        | 494/517<br>(hydrolyzed product) | 1000 assays |
| 80011       | Calcein AM   |                                 | 1 mg        |
| 80011-1     | Calcein AM, 4 mM in anhydrous DMSO                         |                                 | 100 uL      |
| 80011-2     | Calcein AM, 1 mg/mL in anhydrous DMSO                      |                                 | 1 mL        |
| 80011-3     | Calcein AM   |                                 | 20 x 50 ug  |
| 80015       | Lucifer Yellow CH, lithium salt                            | 428/536                         | 25 mg       |
| 80016       | Lucifer Yellow CH, potassium salt                          |                                 | 25 mg       |
| 80018       | Lucifer Yellow Cadaverine                                  |                                 | 25 mg       |
| 80017       | Lucifer Yellow Cadaverine Biotin-X, dipotassium salt       | 428/532                         | 10 mg       |
| 80014       | Hydroxystilbamidine (Fluoro-Gold™)                         | 361/536                         | 10 mg       |
| 80023       | Hydroxystilbamidine (Fluoro-Gold™), 4% in H <sub>2</sub> O | 361/536                         | 200 uL      |
| 80100       | Sulforhodamine B   | 565/586                         | 5 g         |
| 80101       | Sulforhodamine 101   | 586/605                         | 500 mg      |
| 80102       | Sulforhodamine G   | 529/548                         | 5 g         |
| 90055       | Biocytin   | N/A                             | 100 mg      |
| 90060       | Biocytin hydrazide   | N/A                             | 25 mg       |
| 90057       | Biotin ethylenediamine, hydrobromide (Neurobiotin™)        | N/A                             | 25 mg       |
| 90075       | Biotin ethylenediamine, hydrochloride                      | N/A                             | 25 mg       |
| 80019       | Fluorescein-biotin   | 494/518                         | 5 mg        |
| 90062       | Biotin-4-fluorescein                                       | 494/523                         | 10 mg       |
| 80022       | Biotin-rhodamine 110                                       | 502/524                         | 5 mg        |
| 80017       | Lucifer Yellow Cadaverine Biotin-X, dipotassium salt       | 428/532                         | 10 mg       |

## Membrane Fusion Dyes

| Catalog No. | Product                                 | Ex/Em (nm)    | Unit Size |
|-------------|---|---------------|-----------|
| 80104       | DBA/Terbium for membrane fusion assay   | 276/490, 545* | 1 set     |
| 80105       | SDIP/Europium for membrane fusion assay | 250-320/~610* | 1 set     |
| 80012       | DPX                                     | N/A           | 500 mg    |
| 90010       | ANTS                                    | 353/520       | 500 mg    |

\*For complex.

Neurobiotin is a trademark of Vector Laboratories. Fluoro-Gold is a trademark of Fluorochrome.

## Reagents for membrane fusion assays

The principle of DPA/Terbium vesicle fusion assays is based on the fact that contact of the chelator dipicolinic acid (DPA) with terbium (III) instantly forms a complex that is ~10,000 times more fluorescent than free Tb<sup>3+</sup>. In the assay, separate vesicle populations are loaded with DPA or terbium. Fusion of the two types of vesicles results in fluorescence at 490 and 545. Biotium developed SDIP/Europium as an alternative to DPA/Tb<sup>3+</sup> for vesicle fusion assays. The combination of SDIP/Eu<sup>3+</sup> generates intense red fluorescence at 610, with brighter intensity compared to DPA/Tb<sup>3+</sup>. Each set includes 10 mg SDIP and 25 mg EuCl<sub>3</sub>.

DPX is a positively charged quencher that has been used with the fluorescent dye ANTS for studies of vesicle fusion or membrane permeability. The complex of ANTS-DPX has minimal fluorescence, but when it is diluted due to membrane fusion or leakage, the dye becomes increasingly fluorescent.

## ViaFluor® SE Cell Proliferation Dyes

ViaFluor® SE Cell Proliferation Kits diffuse passively into cells covalently label intracellular proteins throughout the cell. They can be used as cell-filling stains for imaging morphology, or to track cell division by dye dilution. With each cell division, daughter cells inherit roughly half of the fluorescent label, allowing the number of cell divisions to be detected by the appearance of successively dimmer fluorescent peaks on a flow cytometry histogram. Staining is formaldehyde fixable.

ViaFluor® CFSE is the classic cell proliferation dye, detected in the FITC channel. Biotium created ViaFluor® 488, a new improved green dye that is less toxic, less leaky and more fixable than CFSE. We also offer blue fluorescent ViaFluor® 405 for the violet laser. ViaFluor® 405 has improved brightness and less toxicity than CFSE.

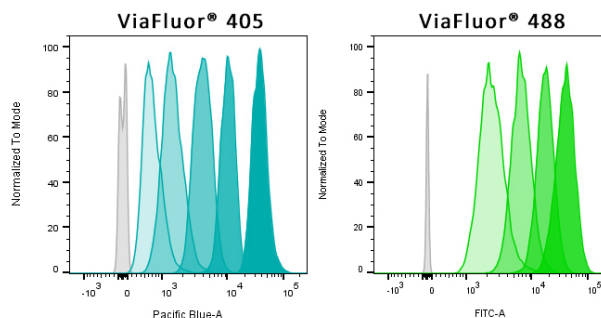


Figure 2. Cell division tracking in Jurkat cells over successive days. Cells were labeled with ViaFluor® 405 (left) or ViaFluor® 488 (right) on day 0, and analyzed by flow cytometry on each following day. Each successively dimmer peak represents one cell division. Unstained cells are in gray.

## ViaFluor® SE Cell Proliferation Dyes

| Catalog No. | Product                                 | Ex/Em (nm) | Unit Size |
|-------------|---|------------|-----------|
| 30068       | ViaFluor® 405 SE Cell Proliferation Kit | 408/452    | 1 kit     |
| 30086       | ViaFluor® 488 Cell Proliferation Kit    | 493/532    | 1 kit     |
| 30050       | ViaFluor® CFSE Cell Proliferation Kit   | 495/519    | 1 kit     |
| 90041       | 5(6)-CFDA, SE                           | 495/519    | 25 mg     |

# Mitochondrial Dyes

## MitoView™ Dyes

MitoView™ dyes are fluorogenic mitochondrial stains for live cells (Fig. 1). The dyes rapidly stain mitochondria without a wash step, and are non-toxic for live cell imaging. They are available with blue, green, far-red, and near-infrared fluorescence. MitoView™ 633 can be used to monitor mitochondrial membrane potential during apoptosis (Fig. 2). MitoView™ Green is membrane potential-independent, and can be used for fixed cell staining.

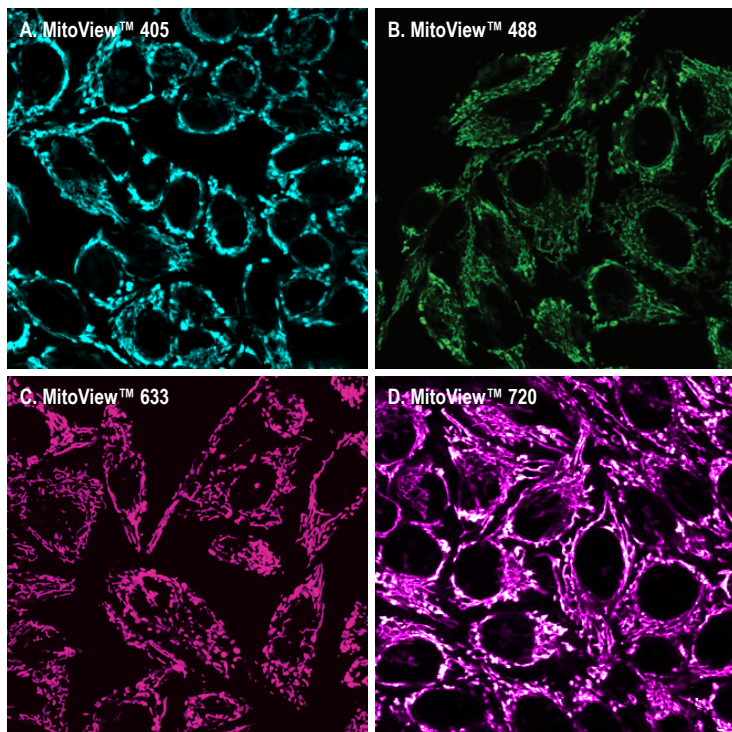


Figure 1. HeLa cells stained with A. MitoView™ Blue, B. MitoView™ Green, C. MitoView™ 633, or D. MitoView™ 720.

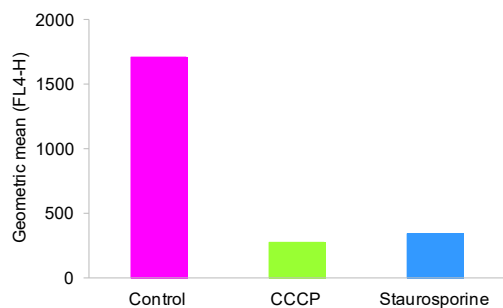


Figure 2. Flow cytometry analysis of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, resulting in decreased MitoView™ 633 staining.

## JC-1 and Other Mitochondrial Dyes

In healthy cells, JC-1 dye aggregates in mitochondria as a function of membrane potential, resulting in red fluorescence (Ex/Em 585/590 nm) with brightness proportional to the membrane potential. Conversely, in apoptotic and necrotic cells with diminished mitochondrial membrane potential, JC-1 exists in a green fluorescent (Ex/Em 510/527 nm) monomeric form in the cytosol, allowing cell viability to be assessed by measuring the ratio of red to green fluorescence by flow cytometry or fluorescence plate reader.

We also offer a selection of classic potentiometric mitochondrial stains in a variety of fluorescent colors. Red fluorescent TMRM (tetramethylrhodamine methyl ester) and TMRE (tetramethylrhodamine ethyl ester) are preferred dyes for quantitative membrane potential measurements. Rhodamine 123 is a green fluorescent dye widely useful for flow-based studies of mitochondrial membrane potential. DASPEI is a dye with red fluorescence emission that is used to stain mitochondria in live cells. DiI<sub>C<sub>1</sub>(5)</sub> is a near-infrared carbocyanine dye that has been used to detect loss of mitochondrial membrane potential in apoptotic cells. Nonyl acridine orange (NAO) is a green fluorescent mitochondrial dye with staining that is not membrane potential dependent.

## MitoView™ Dyes

| Catalog No. | Product         | Ex/Em (nm)   | Potential-dependent? | Unit Size  |
|-------------|-----------------|--------------|----------------------|------------|
| 70070-T     | MitoView™ 405   | 398/440      | Partial†             | 50 ug      |
| 70070       |                 |              |                      | 20 x 50 ug |
| 70054-T     | MitoView™ Green | 490/523      | No                   | 50 ug      |
| 70054       |                 |              |                      | 20 x 50 ug |
| 70055-T     | MitoView™ 633   | 622/648*     | Yes                  | 50 ug      |
| 70055       |                 |              |                      | 20 x 50 ug |
| 70068-T     | MitoView™ 720   | 720/758 nm** | Partial†             | 50 ug      |
| 70068       |                 |              |                      | 20 x 50 ug |

\*MitoView™ 633 also has visible red fluorescence in the Cy@3/rhodamine channel. It is not recommended for imaging with other visible red probes.

\*\*While optimal for Cy@7 settings, MitoView™ 720 is bright enough to be imaged in the Cy@5 channel, and can be combined with visible red fluorescent probes.

†Dyes with partial mitochondrial membrane potential dependence localize to the cytoplasm after mitochondrial depolarization, but still retain fluorescence.

## Other Mitochondrial Dyes

| Catalog No. | Product   | Ex/Em (nm)                                  | Potential-dependent? | Unit Size  |
|-------------|---|---|----------------------|------------|
| 30001       | JC-1 Mitochondrial Membrane Potential Detection Kit |   | Yes                  | 100 assays |
| 70011       | JC-1, chloride salt                                 | 510/527 (cytosol)<br>585/590 (mitochondria) | Yes                  | 5 mg       |
| 70014       | JC-1, iodide salt                                   |   | Yes                  | 5 mg       |
| 70016       | TMRE  | 548/573                                     | Yes                  | 25 mg      |
| 70005       | TMRE, 2 mM in DMSO                                  | 548/573                                     | Yes                  | 0.5 mL     |
| 70017       | TMRM  | 548/573                                     | Yes                  | 25 mg      |
| 70010       | Rhodamine 123                                       | 505/534                                     | Yes                  | 50 mg      |
| 70018       | DASPEI  | 461/589                                     | Yes                  | 100 mg     |
| 70015       | DiI <sub>C<sub>1</sub>(5)</sub>                     | 638/658                                     | Yes                  | 100 mg     |
| 70012       | Nonyl acridine orange                               | 495/522                                     | No                   | 50 mg      |

## LysoView™ Dyes

LysoView™ dyes are fluorescent stains for imaging lysosome localization and morphology in live cells. LysoView™ dyes belong to a family of lysosomotropic dyes that contain weakly basic amines that accumulate in acidic organelles. LysoView™ dyes are available with blue, green, visible red, and far-red fluorescence. Red-fluorescent LysoView™ 540 and far-red fluorescent LysoView™ 633 dye fluorescence is pH-sensitive (Figure 2), resulting in specific lysosomal staining without a wash step. We also offer LysoView™ 650, a far-red lysosome dye that is compatible with super-resolution imaging by SIM and STED.

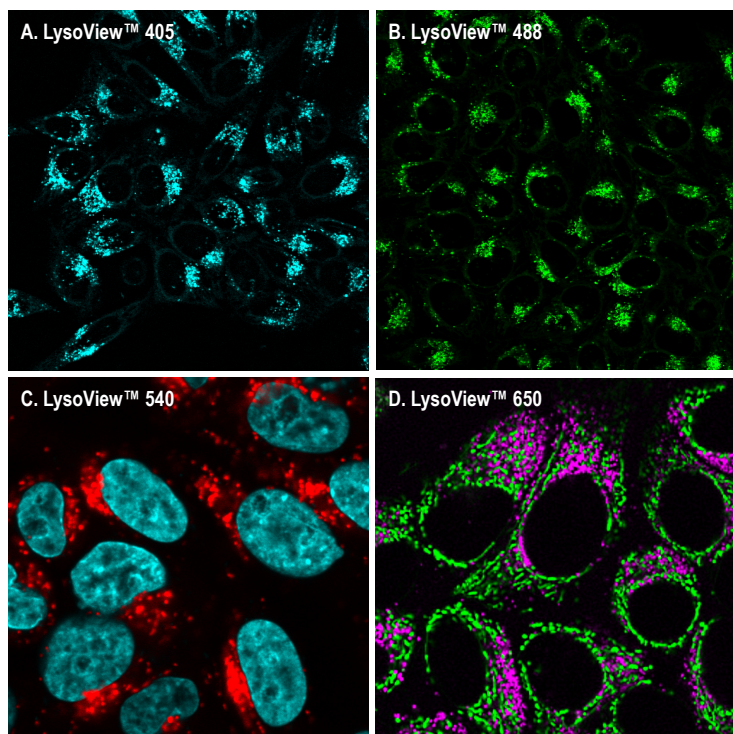


Figure 1. Live HeLa cells stained with (A) LysoView™ 405, (B) LysoView™ 488, (C) LysoView™ 540 (red) and Hoechst 33342 (blue), or (D) LysoView™ 650 (magenta) and MitoView™ Green.

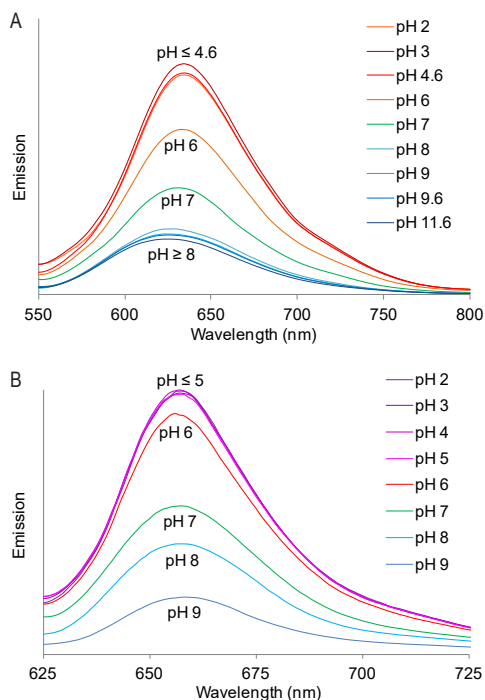


Figure 2. pH dependence of LysoView™ 540 (A) and LysoView™ 633 (B) fluorescence emission.

## “Light-On” LysoView™ 555: a unique photo-activated dye

We also offer “Light-On” LysoView™ 555, a UV-activatable lysosome stain. In cells, the dye initially shows low fluorescence, but brief exposure to UV excitation from a mercury arc lamp activates bright red fluorescence localizing to lysosomes (Figure 4). Lysosomal fluorescence fades over the course of several minutes after UV exposure, but can be re-activated in the same cells multiple times by exposure to UV light. Therefore the dye provides a novel tool for UV-activated, reversible fluorescence imaging of lysosomes.

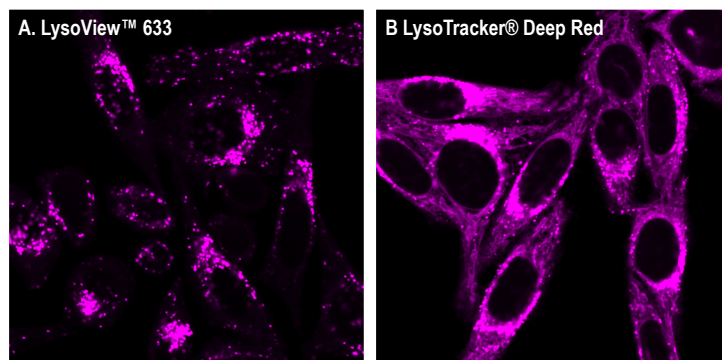


Figure 3. LysoView™ 633 compared to LysoTracker® Deep Red. Live HeLa cells were stained for 10 minutes at 37°C with 1X LysoView™ 633 or 50 nM LysoTracker® Deep Red (Thermo Fisher Scientific). LysoView™ 633 (A) showed more specific lysosomal staining with less cytoplasmic staining compared to LysoTracker® Deep Red (B).

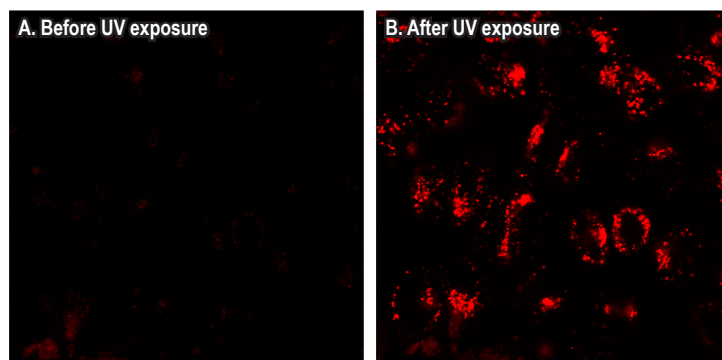


Figure 4. UV-activated lysosomal fluorescence with “Light-On” LysoView™ 555. HeLa cells were stained with 1  $\mu$ M Light-on LysoView™ 555 for 15 minutes at 37°C, then imaged using a Zeiss LSM 700 confocal microscope using a 40X objective and imaging settings for Cy@3. A. Before UV exposure, fluorescence was not detectable. B. After five seconds of exposure to UV light from a short arc lamp, bright red fluorescence localized to lysosomes was observed.

## LysoView™ Dyes

| Catalog No. | Product                                    | Ex/Em (nm)   | Unit Size          |
|-------------|--|--------------|--------------------|
| 70061-T     | LysoView™ 405, 1000X in DMSO               | 318, 400/464 | 10 $\mu$ L         |
| 70061       |  |              | 50 $\mu$ L         |
| 70061-T     | LysoView™ 488, 1000X in DMSO               | 506/532      | 10 $\mu$ L         |
| 70061       |  |              | 50 $\mu$ L         |
| 70061-T     | LysoView™ 540, 1000X in DMSO               | 541/634 *    | 10 $\mu$ L         |
| 70061       |  |              | 50 $\mu$ L         |
| 70058-T     | LysoView™ 633 (1000X after reconstitution) | 634/659*     | 100 $\mu$ L**      |
| 70058       |  |              | 10 x 100 $\mu$ L** |
| 70059-T     | LysoView™ 650, 1000X in DMSO               | 650/675      | 10 $\mu$ L         |
| 70059       |  |              | 50 $\mu$ L         |
| 70060-T     | “Light-On” LysoView™ 555, 1 mM in DMSO     | 554/583 *    | 10 $\mu$ L         |
| 70060       |  |              | 50 $\mu$ L         |

\*pH  $\leq$  5

\*\*After reconstitution

# Cytoskeleton Probes

## ViaFluor® Live Cell Microtubule Stains

ViaFluor® Live Cell Microtubule Stains are fluorescent cell-permeable taxol probes for imaging the microtubule cytoskeleton in live cells (Fig.1). They are simple, rapid and sensitive stains. Taxol binds to polymerized tubulin and stabilizes microtubules, resulting in inhibition of mitosis. However, fluorescent taxol compounds like ViaFluor® stains are less disruptive of microtubule dynamics and cell division, presumably due to lower binding affinity of the fluorescent probe compared to taxol itself. However, immortalized cells show signs of cytotoxicity after 24 hours of exposure to ViaFluor® microtubule stains. The stains are supplied with a vial of verapamil, an efflux pump inhibitor that may improve probe retention and staining in certain cell types. Biotium offers blue fluorescent ViaFluor® 405, green fluorescent ViaFluor® 488, and far-red fluorescent ViaFluor® 647 Live Cell Microtubule Stains. ViaFluor® 647 Microtubule Stain is compatible with super-resolution imaging by STED.

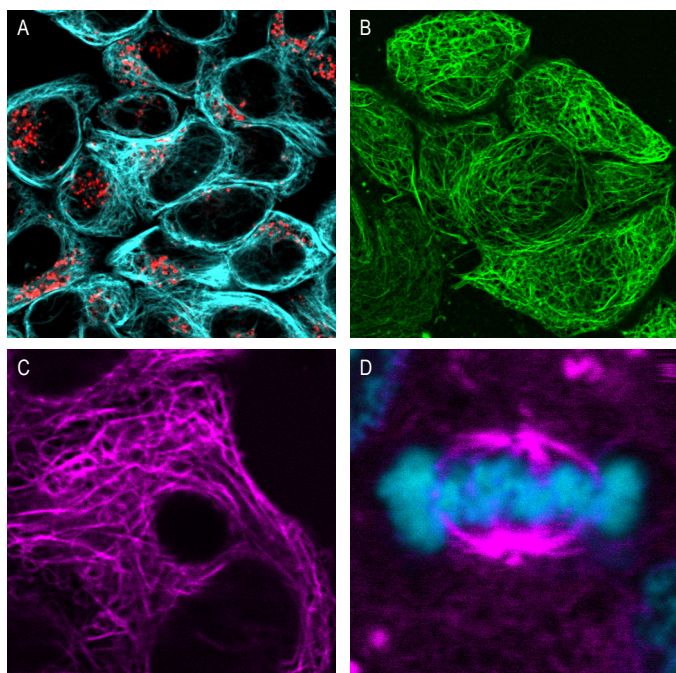


Figure 1. ViaFluor® Live Cell Microtubule Stains. A. Live HeLa cells stained with ViaFluor® 405 microtubule stain (blue) and LysoView™ 540 (red). B. HeLa cells stained with ViaFluor® 488 microtubule stain. C. MCF-7 cell stained with ViaFluor® 647 microtubule stain. D. Dividing HeLa cell stained with ViaFluor® 647 microtubule stain showing mitotic spindle (magenta). DNA is stained with Hoechst 33342 (blue).

## ViaFluor® Live Cell Microtubule Stains

| Catalog No. | Product  | Ex/Em (nm) | Unit Size |
|-------------|--|------------|-----------|
| 70064-T     | ViaFluor® 405 Live Cell Microtubule Stain, 1000X in DMSO | 408/452    | 10 uL     |
| 70064       |  |            | 50 uL     |
| 70062-T     | ViaFluor® 488 Live Cell Microtubule Stain, 1000X in DMSO | 500/515    | 10 uL     |
| 70062       |  |            | 50 uL     |
| 70063-T     | ViaFluor® 647 Live Cell Microtubule Stain, 1000X in DMSO | 650/675    | 10 uL     |
| 70063       |  |            | 50 uL     |

## Phalloidin conjugates

Phalloidin is a toxin isolated from the *Amanita phalloides* mushroom. It is a bicyclic peptide that binds specifically to F-actin. Fluorescently labeled phalloidins stain F-actin at nanomolar concentrations. Unlike antibodies, the binding of phalloidin is not species-specific, and non-specific staining is negligible. Biotium offers phalloidin conjugated to a large selection of CF® dyes. CF® dyes have advantages in brightness, photostability, and water solubility compared to dyes like Alexa Fluor®, DyLight®, and Cy® dyes. A number of our CF® dyes have been validated in super-resolution imaging by STORM, STED, SIM, and other methods. See our CF® dye selection guide at [www.biotium.com](http://www.biotium.com) for more information.

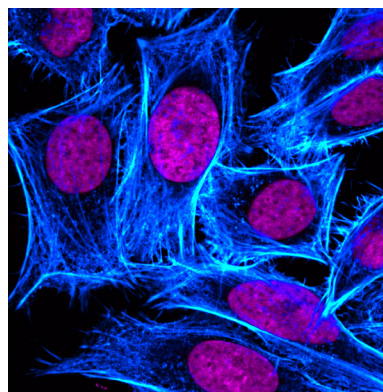


Figure 2. HeLa cells stained with CF®405M phalloidin (blue) and RedDot™2 Far-Red Nuclear Stain (magenta).

## Phalloidin Conjugates

| Catalog No. | Product            | Ex/Em (nm) | Unit Size |
|-------------|--------------------|------------|-----------|
| 00049-T     | CF®350 Phalloidin  | 347/448    | 50 U      |
| 00049       |                    |            | 300 U     |
| 00034-T     | CF®405M Phalloidin | 408/452    | 50 U      |
| 00034       |                    |            | 300 U     |
| 00054-T     | CF®430 Phalloidin  | 426/498    | 50 U      |
| 00054       |                    |            | 300 U     |
| 00055-T     | CF®440 Phalloidin  | 440/515    | 50 U      |
| 00055       |                    |            | 300 U     |
| 00042-T     | CF®488A Phalloidin | 490/515    | 50 U      |
| 00042       |                    |            | 300 U     |
| 00051-T     | CF®532 Phalloidin  | 527/558    | 50 U      |
| 00051       |                    |            | 300 U     |
| 00043-T     | CF®543 Phalloidin  | 541/560    | 50 U      |
| 00043       |                    |            | 300 U     |
| 00040-T     | CF®555 Phalloidin  | 555/565    | 50 U      |
| 00040       |                    |            | 300 U     |
| 00044-T     | CF®568 Phalloidin  | 562/583    | 50 U      |
| 00044       |                    |            | 300 U     |
| 00045-T     | CF®594 Phalloidin  | 593/614    | 50 U      |
| 00045       |                    |            | 300 U     |
| 00046-T     | CF®633 Phalloidin  | 630/650    | 50 U      |
| 00046       |                    |            | 300 U     |
| 00050-T     | CF®640R Phalloidin | 642/662    | 50 U      |
| 00050       |                    |            | 300 U     |
| 00041-T     | CF®647 Phalloidin  | 650/665    | 50 U      |
| 00041       |                    |            | 300 U     |
| 00052-T     | CF®660C Phalloidin | 667/685    | 50 U      |
| 00052       |                    |            | 300 U     |
| 00047-T     | CF®660R Phalloidin | 663/682    | 50 U      |
| 00047       |                    |            | 300 U     |
| 00053-T     | CF®680 Phalloidin  | 681/698    | 50 U      |
| 00053       |                    |            | 300 U     |
| 00048-T     | CF®680R Phalloidin | 680/701    | 50 U      |
| 00048       |                    |            | 300 U     |

Alexa Fluor and DyLight are registered trademarks of Thermo Fisher Scientific. Cy Dye is a registered trademark of GE Healthcare.



## NucSpot® 470 Nuclear Stain for dead or fixed cells

NucSpot® 470 is a cell membrane-impermeant green fluorescent DNA stain. While other green nucleic acid stains like TOTO®, TO-PRO®, or SYTOX® dyes stain both the nucleus and cytoplasm, NucSpot® 470 specifically stains the nucleus of fixed and permeabilized cells. It also can be used to selectively stain dead cells in living cultures. It can be imaged using standard settings for FITC, and is an excellent match for instruments with blue LED excitation.

## NucSpot® Live 488 and NucSpot® Live 650 Nuclear Stains

NucSpot® Live Nuclear Stains specifically stain nuclei in live or fixed cells without the need for washing. NucSpot® Live 488 has green fluorescence and NucSpot® Live 650 has far-red fluorescence for the Cy@5 channel (Fig. 1). Unlike Draq5™ and RedDot™1, NucSpot® Live dyes have low cytotoxicity and can be used for long term imaging. NucSpot® Live 650 dye is compatible with super-resolution imaging by SIM and STED.

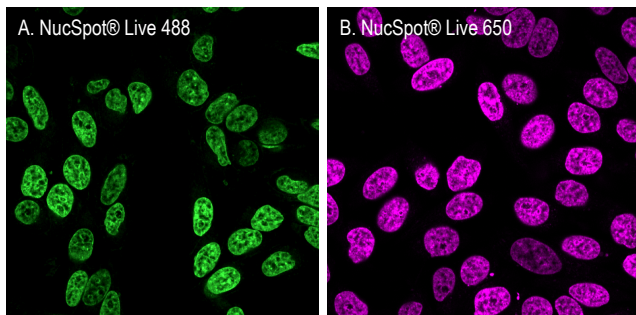


Figure 1. A. Live HeLa cells stained with 1X NucSpot® Live 488. B. Live HeLa incubated overnight at 37°C with 1X NucSpot® Live 650.

## RedDot™1 and RedDot™2 Far-Red Nuclear Stains

RedDot™1 and RedDot™2 are far-red nuclear counterstains for the Cy@5 channel. RedDot™1 rapidly and specifically stains nuclei in live cells (see page 11, Fig. 3), and can be used for cell cycle analysis by flow cytometry or for cell normalization for In Cell Western™. It also has been used to stain nuclei in live flatworms. Note: Similar to Draq5™, RedDot™1 shows cytotoxicity within four hours of staining. For long-term live cell imaging, we recommend using NucSpot® Live dyes.

RedDot™2 is membrane impermeant and can be used to selectively stain dead cells, or as a nuclear counterstain for fixed cells. RedDot™2 shows better nuclear specificity in fixed cells than Draq7™, which requires a blocking step for nuclear-specific staining (Fig. 2).

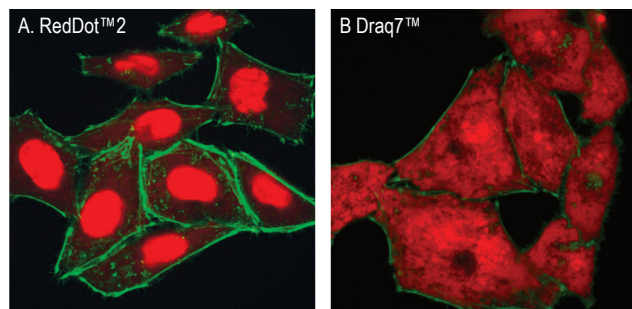


Figure 2. Formaldehyde fixed and detergent permeabilized HeLa cells stained with RedDot™2 (A) or Draq7™ (B). Actin is stained with CF@488A phalloidin (green).

## Other Nucleic Acid Dyes

DAPI and Hoechst are widely used blue fluorescent nuclear counterstains. They are minor groove-binding DNA dyes that are minimally fluorescent in solution, but have strong fluorescence enhancement upon binding DNA. Hoechst dyes are membrane permeant and can be used for live or fixed cell staining and cell cycle analysis.

DMAO is a green fluorescent nucleic acid dye for staining bacteria.

Acridine Orange (AO) is cell membrane permeant and stains double-stranded DNA green (525 emission) and RNA red (650 emission).

Propidium iodide (PI) is a membrane impermeant red fluorescent DNA/RNA stain commonly used to selectively stain dead cells for flow cytometry and for cell cycle analysis. Our Live-or-Dye NucFix™ Red is a fixable alternative to PI for dead cell nuclear staining for microscopy or flow cytometry. Ethidium homodimer III and 7-AAD are dead-cell selective stains. These dyes are also available in live/dead staining kits (see page 10).

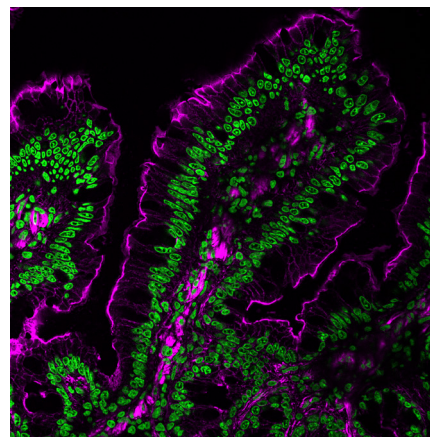


Figure 3. Intestine section stained with CF@640R phalloidin (magenta) and NucSpot@ 470 (green).

## Nuclear Stains

| Catalog No. | Product   | Ex/Em (nm)             | Unit Size  |        |
|-------------|---|------------------------|------------|--------|
| 40081-T     | NucSpot® 470 Nuclear Stain,<br>1000X in DMSO      | 500/515                | 10 uL      |        |
| 40081       |   |                        | 50 uL      |        |
| 40081-T     | NucSpot® Live 488 Nuclear Stain,<br>1000X in DMSO | 500/515                | 10 uL      |        |
| 40081       |   |                        | 50 uL      |        |
| 40082-T     | NucSpot® Live 650 Nuclear Stain,<br>1000X in DMSO | 650/675                | 10 uL      |        |
| 40082       |   |                        | 50 uL      |        |
| 40060-T     | RedDot™1 Far-Red Nuclear Stain,<br>200X in water  | 662/694*               | 25 uL      |        |
| 40060       |   |                        | 250 uL     |        |
| 40060-1     |   |                        | 1 mL       |        |
| 40061-T     | RedDot™2 Far-Red Nuclear Stain,<br>200X in DMSO   | 662/694*               | 25 uL      |        |
| 40061       |   |                        | 250 uL     |        |
| 40061-1     |   |                        | 1 mL       |        |
| 40011       | DAPI  | 358/461*               | 10 mg      |        |
| 40009       | DAPI, dilactate                                   |                        | 10 mg      |        |
| 40043       | DAPI, 10 mg/mL in H <sub>2</sub> O                |                        | 1 mL       |        |
| 23002       | EverBrite™ Mounting Medium with DAPI              |                        | 10 mL      |        |
| 23004       | EverBrite™ Hardset Mounting Medium with DAPI      |                        | 10 mL      |        |
| 40044       | Hoechst 33258, 10 mg/mL in H <sub>2</sub> O       |                        | 10 mL      |        |
| 40045       | Hoechst 33258, pentahydrate                       |                        | 100 mg     |        |
| 40046       | Hoechst 33342, 10 mg/mL in H <sub>2</sub> O       |                        | 10 mL      |        |
| 40047       | Hoechst 33342, trihydrochloride trihydrate        |                        | 100 mg     |        |
| 40012       | DMAO, 2 mM in DMSO                                |                        | 503/530*   | 1 mL   |
| 40016       | Propidium iodide (PI)                             |                        | 535/617 ** | 100 mg |
| 40017       | Propidium iodide, 1 mg/mL in H <sub>2</sub> O     |                        |            | 10 mL  |
| 40048       | Propidium iodide buffer, 50 ug/mL in PBS          | 2 mL                   |            |        |
| 40039       | Acridine Orange, 10 mg/mL in H <sub>2</sub> O     | 500/525*<br>460/650*** | 10 mL      |        |
| 40037       | 7-AAD   | 546/647 *              | 1 mg       |        |
| 40010       | Ethidium Homodimer I                              | 528/617**              | 1 mg       |        |
| 40014       | Ethidium Homodimer I, 2 mM in DMSO                |                        | 0.5 mL     |        |
| 40050       | Ethidium Homodimer III                            | 530/620**              | 1 mg       |        |
| 40051       | Ethidium Homodimer III, 1 mM in DMSO              |                        | 200 uL     |        |

\*With DNA \*\*With DNA or RNA \*\*\*With RNA

Draq7 is a trademark of Biostatus, Ltd. Cy Dye is a registered trademark of GE Healthcare. In Cell Western is a trademark of LI-COR® Biosciences. SYTOX, TOTO, and TO-PRO are registered trademarks of Thermo Fisher Scientific.

# Apoptosis and Viability Stains

## NucView® Caspase-3 Substrates

NucView® Caspase-3 Substrates are cleaved by caspases to stain the nuclei of apoptotic cells with fluorescence. Unlike FLICA substrates, NucView® substrates do not inhibit caspase activity, allowing caspase-3/7 activity to be monitored in individual intact cells in real time.

Green fluorogenic NucView® 488 Caspase-3 Substrate has been validated in more than a hundred published studies and cell types, and has been validated in real-time kinetic studies using the IncuCyte® Live Cell Analysis System (Essen Bioscience). We also offer blue fluorogenic NucView® 405 and orange fluorogenic NucView® 530 for multi-color flexibility.

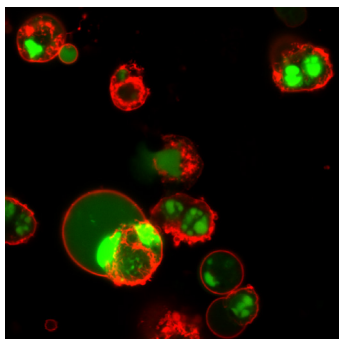


Figure 1. Apoptotic Jurkat cells stained with NucView® 488 Caspase-3 Substrate (green) and CF®594 Annexin V (red).

## Apoptosis and Viability Assays

| Catalog No. | Product  | Ex/Em (nm)   |
|-------------|--|--|
| 10402       | NucView® 488 Caspase-3 Substrate, 1 mM in DMSO                                   | 504/534  |
| 10403       | NucView® 488 Caspase-3 Substrate, 1 mM in PBS                                    | 504/534  |
| 10405       | NucView® 405 Caspase-3 Substrate, 1 mM in DMSO                                   | 429/469  |
| 10407       | NucView® 405 Caspase-3 Substrate, 1 mM in PBS                                    | 429/469  |
| 10406       | NucView® 530 Caspase-3 Substrate, 1 mM in DMSO                                   | 528/563  |
| 10408       | NucView® 530 Caspase-3 Substrate, 1 mM in PBS                                    | 528/563  |
| 30067       | Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®594 Annexin V  | 504/534 (NucView®) 593/614 (Annexin)                   |
| 30073       | Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®640R Annexin V | 504/534 (NucView®) 642/662 (Annexin)                   |
| 30062       | NucView® 488 and MitoView™ 633 Apoptosis Kit                                     | 504/534 (NucView®) 622/648 (MitoView™)                 |
| 30072       | NucView® 488 and RedDot™2 Apoptosis & Necrosis Kit                               | 504/534 (NucView®) 662/694 (RedDot™)                   |
| 30060       | CF®488A Annexin V and PI Apoptosis Assay Kit                                     | 490/515 (Annexin) 535/617 (PI)                         |
| 30061       | CF®488A Annexin V and 7-AAD Apoptosis Assay Kit                                  | 490/515 (Annexin) 546/647 (7-AAD)                      |
| 30065       | Apoptosis and Necrosis Quantitation Kit Plus                                     | 490/515 (Annexin) 530/620 (EthD-III)                   |
| 30066       | Apoptotic, Necrotic and Healthy Cells Quantitation Kit Plus                      | 358/461 (Hoechst) 490/515 (Annexin) 530/620 (EthD-III) |
| 30026       | Calcein AM Cell Viability Assay Kit  | 494/517  |
| 30002       | Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells                    | 494/517 (Calcein) 530/620 (EthD-III)                   |
| 32002       | Live-or-Dye™ 350/448   | 347/448  |
| 32003       | Live-or-Dye™ 405/452   | 408/452  |
| 32009       | Live-or-Dye™ 405/545   | 395/545  |
| 32004       | Live-or-Dye™ 488/515   | 490/515  |
| 32005       | Live-or-Dye™ 568/583   | 562/583  |
| 32006       | Live-or-Dye™ 594/614   | 561/624  |
| 32007       | Live-or-Dye™ 640/662   | 642/662  |
| 32008       | Live-or-Dye™ 750/777   | 755/777  |
| 32010       | Live-or-Dye NucFix™ Red  | 520/610  |

## CF® Dye Annexin V Conjugates and Cell Viability Kits

Fluorescent conjugates of Annexin V can be used to stain phosphatidylserine on the surface of apoptotic cells. We offer Annexin V conjugates of our exceptionally bright and photostable CF® dyes for flow cytometry or fluorescence microscopy. See p. 11 for a complete list. Our CF®488A and CF®594 Annexin V conjugates have been validated in real-time kinetic imaging studies using the IncuCyte® Live Cell Analysis System (Essen Bioscience). Near infrared CF® dye Annexin V conjugates (CF®680 through CF®790) are supplied as preservative-free lyophilized solids, suitable for in vivo imaging.

We also offer kits combining CF®488A Annexin V with red dead cell nucleic acid stains PI or 7-AAD. The Apoptosis and Necrosis Kit Plus includes CF®488A and EthD-III, while the Apoptotic, Necrotic and Healthy Cells Kit Plus also includes Hoechst to stain the total cell population. See page 9 for more information about nucleic acid stains.

## Live-or-Dye™ Fixable Viability Stains

Biotium offers a selection of eight different amine-reactive Live-or-Dye™ viability stains spanning the fluorescence spectrum, for maximal flexibility in multi-color analysis. The membrane-impermeant dyes selectively enter dead cells and covalently label intracellular proteins. They can be used to discriminate dead from live cells in flow cytometry or microscopy (Fig. 2). Live-or-Dye™ labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells.

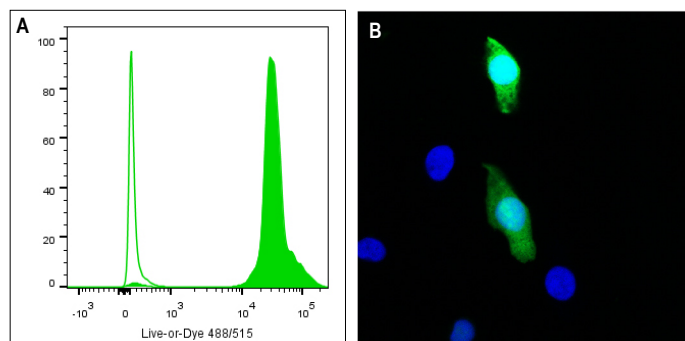


Figure 2. Discrimination of live and dead cells with Live-or-Dye™ 488/515 Fixable Viability Stain. A. Flow cytometry of live (white peak) or heat-killed (solid peak) Jurkat cells. B. Microscopy of ethanol-treated HeLa cells. Killed cells show bright green staining, compared to no staining in live cells (blue Hoechst-stained nuclei). Identical results are seen before and after fixation and permeabilization of stained cells.

## Live-or-Dye NucFix™ Red Viability Stain

Live-or-Dye NucFix™ Red is a unique, cell membrane-impermeant dye that specifically stains the nuclei of dead cells (Fig. 3). Unlike other nuclear viability stains such as propidium iodide (PI), NucFix™ labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells.

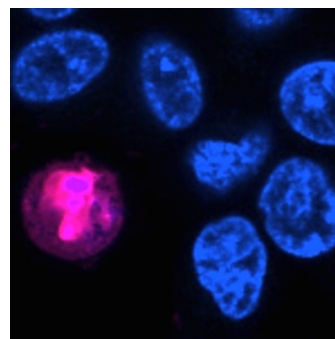


Figure 3. Ethanol-treated HeLa cells stained with Live-or-Dye NucFix™ Red (red) and Hoechst (blue). Dead cells show bright red nuclear fluorescence. Identical staining is seen when cells are fixed and permeabilized after staining.

# CF® Dye Lectins, Toxins, and Other Conjugates

## CF® Dye Conjugates

Biotium offers a wide selection of probes for cell staining conjugated to CF® dyes, our line of next-generation fluorescent dyes. CF® dyes have advantages in brightness, photostability, and water solubility compared to dyes like Alexa Fluor®, DyLight®, and Cy® dyes. A number of our CF® dyes have been validated in super-resolution imaging by STORM, STED, SIM, and other methods. See our CF® dye selection guide at [www.biotium.com](http://www.biotium.com) for more information.

**Table 1. Applications of Fluorescent Bioconjugates.**

| Conjugate                               | Application  |
|---|--|
| Annexin V                               | Binds phosphatidylserine, apoptotic cell surface marker                                    |
| Biotin                                  | Cytoplasmic tracer (see p. 5); biotin binding site detection                               |
| $\alpha$ -Bungarotoxin ( $\alpha$ -BTX) | Acetylcholine receptor/neuromuscular junction probe  |
| Bovine serum albumin (BSA)              | Fluid-phase endocytosis tracer; in vivo blood flow tracer                                  |
| Cholera Toxin Subunit B                 | Lipid raft, endocytic vesicle, and neuronal tracing  |
| Concanavalin A (Con A)                  | Binds $\alpha$ -D-mannosyl and $\alpha$ -D-glucosyl groups, stains yeast cell wall         |
| Dextran, anionic and fixable            | Fluid-phase endocytosis tracer available in a variety of molecular weights                 |
| Hydrazide                               | Fixable, water-soluble cytoplasmic tracer (see p. 5)                                       |
| Phalloidin                              | Filamentous actin probe (see p. 8 for product list)  |
| Peanut agglutinin (PNA)                 | Lectin specific for terminal $\beta$ -galactose  |
| Streptavidin                            | Detection of biotinylated probes   |
| Transferrin (human)                     | Recycling endosome tracer  |
| Wheat germ agglutinin (WGA)             | Binds N-acetyl-D-glucosamine and sialic acid; bacterial Gram stain, stains yeast bud scars |

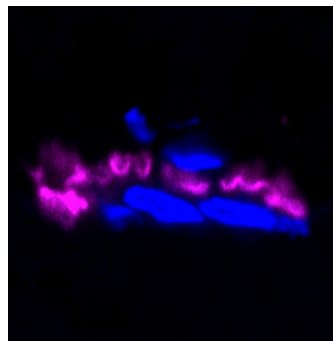


Figure 1. Rat neuromuscular junction stained with CF®633  $\alpha$ -bungarotoxin (magenta). Nuclei are stained with DAPI (blue).

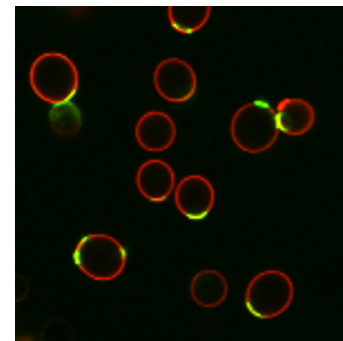


Figure 2. *S. cerevisiae* stained with CF®488A WGA (bud scars, green) and CF®594 ConA. ConA (cell wall, red).

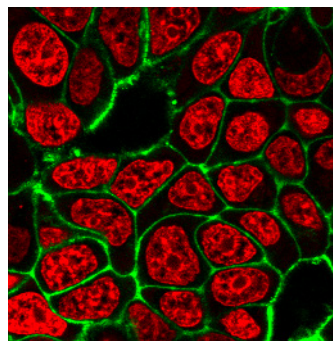


Figure 3. Live MCF7 cells stained with WGA conjugated to CF®488A (green). Nuclei are stained with RedDot™1 (red).

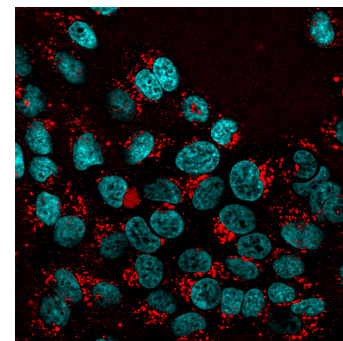


Figure 4. Endocytosis of CF®594 dextran (red) by HeLa cells. Nuclei are stained with Hoechst 33342 (cyan).

## CF® Dye Conjugates

| Dye Conjugate | Ex/Em (nm) | Annexin V | Biotin | $\alpha$ -BTX | BSA   | Cholera Toxin B | Con A | Dextran 10K MW | Dextran 40K MW | Dextran 70K MW | Dextran 150K MW | Dextran 250K MW | Hydrazide | PNA   | Streptavidin | Transferrin | WGA   |
|---------------|------------|-----------|--------|---------------|-------|-----------------|-------|----------------|----------------|----------------|-----------------|-----------------|-----------|-------|--------------|-------------|-------|
| CF@350        | 347/448    | 29012     |        |               |       |                 | 29015 |                |                |                |                 |                 | 92151     |       | 29031        |             | 29021 |
| CF@405S       | 404/431    |           |        | 00002         |       |                 | 29075 |                |                |                |                 |                 | 92183     |       | 29032        |             | 29027 |
| CF@405M       | 408/452    | 29009     |        |               |       |                 | 29074 |                |                |                |                 |                 |           |       | 29033        |             |       |
| CF@488A       | 490/515    | 29005     |        | 00005         | 20289 | 00070           | 29016 | 80110          | 80126          | 80117          | 80131           | 80134           | 92152     | 29060 | 29034        | 00081       | 29022 |
| CF@532        | 527/558    |           |        |               |       |                 |       |                |                |                |                 |                 |           |       | 29030        |             | 29064 |
| CF@543        | 541/560    |           |        | 00026         |       |                 |       | 80111          |                |                |                 |                 |           |       | 29043        | 00082       |       |
| CF@555        | 555/565    | 29004     |        | 00018         |       |                 |       | 80112          |                |                |                 |                 | 92153     |       | 29038        |             | 29076 |
| CF@568        | 562/583    | 29010     | 80029  | 00006         |       | 00071           |       | 80113          |                |                |                 |                 | 92154     | 29061 | 29035        | 00083       | 29077 |
| CF@594        | 593/614    | 29011     |        | 00007         | 20290 | 00072           | 29017 | 80114          |                |                |                 |                 | 92158     | 29062 | 29036        | 00084       | 29023 |
| CF@633        | 630/650    | 29008     | 80031  | 00009         |       |                 | 29018 |                |                |                |                 |                 | 92156     |       | 29037        |             | 29024 |
| CF@640R       | 642/662    | 29014     | 80032  | 00004         | 20291 | 00073           | 29019 | 80115          |                |                |                 |                 | 92157     | 29063 | 29041        | 00085       | 29026 |
| CF@647        | 650/665    | 29003     |        |               |       |                 |       |                |                |                |                 |                 | 92136     |       | 29039        |             |       |
| CF@660C       | 667/685    |           |        |               |       |                 |       |                |                |                |                 |                 |           |       |              |             |       |
| CF@660R       | 663/682    | 29069     |        |               |       | 00078           |       |                |                |                |                 |                 | 96024     |       | 29040        |             |       |
| CF@680        | 681/698    | 29007*    |        |               | 20292 |                 | 29020 | 80118          | 80127          | 80129          | 80132           | 80135           |           |       |              |             | 29029 |
| CF@680R       | 680/701    | 29070*    |        | 00003         |       | 00079           |       | 80116          |                |                |                 |                 | 96025     |       | 29042        | 00086       | 29025 |
| CF@750        | 755/777    | 29006*    |        |               |       |                 | 29080 | 80119          | 80128          | 80130          | 80133           | 80136           |           |       |              | 00087       |       |
| CF@770        | 770/797    | 29046*    |        |               |       |                 | 29058 | 80120          | 80122          | 80123          | 80124           | 80125           | 92192     |       |              |             | 29059 |
| CF@790        | 784/806    | 29047*    |        |               |       |                 |       | 80121          |                |                |                 |                 |           |       |              |             |       |
| CF@800        | 797/816    | 29078*    |        |               |       |                 |       |                |                |                |                 |                 |           |       |              |             |       |

\* Preservative-free lyophilized solid.

Don't see what you're looking for? We regularly add new CF® dye products to our catalog according to customer demand. Be sure to check our website for updates. If you are looking for a CF® dye product not listed in our catalog, contact us at [techsupport@biotium.com](mailto:techsupport@biotium.com). We may be able to add it as a new product, or perform a custom conjugation for you.



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