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Overview of Organelle & Cytoskeleton Stains

Membrane & Cell Surface

- Cell surface stains for long-term imaging of live cells (p. 4)
- Lipophilic carbocyanine dyes for live or fixed cells (p. 5)
- Covalent membrane & surface stains for fixable live-cell staining (p. 5)
- Lectin conjugates for staining cell surface glycoproteins in live or fixed cells (p. 6)

Cytoskeleton

- Live cell microtubule stains (p. 10)
- Phalloidin conjugates for fixed cells (p. 10)

Lipid Droplets

Neutral lipid stains for live or fixed cells (p. 6)

Nucleus

Blue, green, and red stains with various properties for labeling fixed, dead, or live cells (p. 7)

Mitochondria

Membrane-permeant dyes that accumulate in mitochondria due to membrane potential & lipophilicity (p. 8)

Lysosomes

Membrane-permeant, pH-sensitive dyes that accumulate and fluoresce in the acidic environment of lysosomes (p. 9)

Vesicle Trafficking

- Fluorescent toxins and ligands for receptor-mediated endocytosis (p. 11)
- Fluorescent dextrans for fluid phase tracing (p. 11)

Cytoplasm

- Stable, covalent live cell cytoplasmic stains for cell division analysis by flow or tracking cells in culture (p. 3)
- Non-covalent cytoplasm stains for cell viability & dye efflux assays (p. 3)

Cytoplasmic Stains and Cell Division Tracking

ViaFluor® SE Cell Proliferation Kits

ViaFluor® SE Cell Proliferation Kits use amine-reactive dyes to covalently label cells throughout the cell cytoplasm and intracellular compartments. While developed for cell division tracking by flow cytometry, stable whole-cell labeling is also useful for imaging cell morphology, or to track cell populations in mixed co-culture experiments (Fig. 1). In addition, the labeling is covalent and can therefore withstand fixation and permeabilization for downstream immunostaining.

ViaFluor® CFSE is the classic cell proliferation dye, detected in the FITC channel. Biotium created ViaFluor® 488 as an improved alternative to CFSE that is less toxic, less leaky, and has a greater tolerance for fixation. We also offer blue fluorescent ViaFluor® 405 for the Pacific Blue® channel which provides bright and stable staining with low toxicity.

ViaFluor® SE Cell Proliferation Kit Features

- Non-toxic dyes for covalent cytoplasm labeling
- Suitable for long-term imaging
- Tolerates fixation and permeabilization
- ViaFluor® 405 SE and ViaFluor® 488 SE offer better performance and less toxicity than CFSE

ViaFluor® SE Cell Proliferation Dyes

Cat. #	Product	Ex/Em (nm)
30068	ViaFluor® 405 SE Cell Proliferation Kit	408/452
30086	ViaFluor® 488 SE Cell Proliferation Kit	493/532
30050	ViaFluor® CFSE Cell Proliferation Kit	495/519

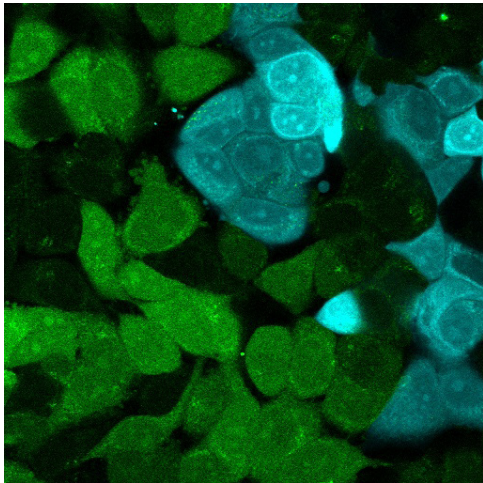


Figure 1. Adherent HeLa cells and MCF-7 cells were labeled with ViaFluor® 488 (green) and ViaFluor® 405 (blue), respectively. The cells were then trypsinized, mixed, plated, and grown in co-culture for four days.

Calcein AM for Cell Viability

Calcein AM is a widely used green fluorescent live cell stain. The compound is initially a non-fluorescent, membrane permeable compound. Once inside live cells, cytoplasmic esterase activity converts calcein AM to the green fluorescent, membrane-impermeant compound calcein that fills the entire cell (Fig. 2). We offer high-purity Calcein AM as a solid or as a ready-to-use solution in the Calcein AM Cell Viability Assay Kit. Because calcein is only retained in cells with intact plasma membranes, the Calcein AM Cell Viability Assay is a true live-cell endpoint assay that is compatible with microplate readers. Staining is fast and simple, requiring only 30 minutes. The Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells pairs calcein AM with the red dead cell stain Ethidium Homodimer III, to detect live and dead cells by microplate reader, flow cytometry, or microscopy (Fig. 3).

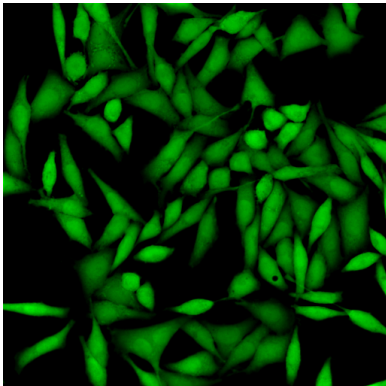


Figure 2. Live HeLa cells labeled with Calcein AM.

Cytosolic Tracers

Cat. #	Product	Ex/Em (nm)
80011	Calcein AM	494/517 (hydrolyzed product)
30026	Calcein AM Cell Viability Assay Kit	
30002	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells	494/517 (Calcein AM) 522/593 (EthD-III)

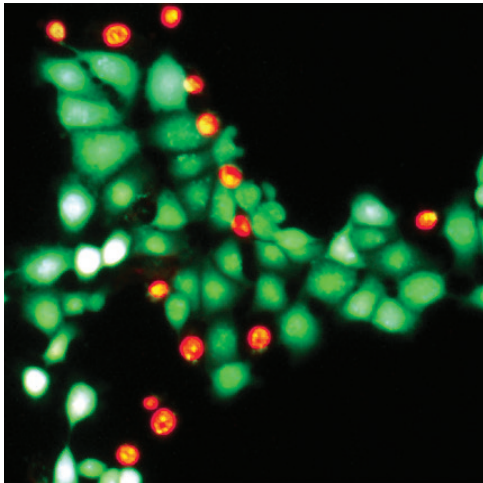


Figure 3. HeLa cells stained with the Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells. Live cells are stained green, dead cells are stained red.

Pacific Blue is a registered trademark of Thermo Fisher Scientific.

Membrane & Cell Surface Stains

Biotium offers a wide selection of highly fluorescent and photostable stains to visualize cell boundaries and morphology in multi-color staining experiments. Our novel CellBrite™ Fix and MemBrite™ Fix Membrane Stains are unique cell surface stains that can tolerate detergent permeabilization and methanol fixation. CellBrite™ Steady dyes are excellent for long-term imaging of cell surface membranes in live cells. Our original CellBrite™ Cytoplasmic Membrane Stains are lipophilic dyes for simple, non-toxic, and stable labeling of membranes in live or fixed cells. We also offer a several lectins conjugated to our bright and photostable CF® dyes for cell surface staining after fixation or permeabilization (see p. 6).

Membrane & Cell Surface Stains Comparison

Product Line	Colors	Protocol	Stain live cells	Stain fixed cells	Fixable Staining	Applications/Notes
CellBrite™ & CellBrite™ NIR Cytoplasmic Membrane Dyes	Blue to Near-IR	<ul style="list-style-type: none"> Stain in complete medium ~20 min Washing required Staining lost from cell surface over time by internalization 	Yes	Yes (PFA)	Yes (PFA) Does not tolerate detergent or MeOH fixation	<ul style="list-style-type: none"> Lipophilic carbocyanine dyes Non-toxic with minimal dye transfer between cells Suitable for live-cell surface staining, transplantation, or co-culture Can stain cell before or after PFA-fixation
CellBrite™ Fix Membrane Stains	Green, Red, Far-Red	<ul style="list-style-type: none"> Stain in buffer 15 min Washing optional¹ 	Yes	No	Yes Tolerates MeOH fixation & detergent	<ul style="list-style-type: none"> Fixable membrane stains that tolerate permeabilization For labeling cell surface before fixation & immunofluorescence Non-toxic, but covalent modification of cell surface may affect function
MemBrite™ Fix Cell Surface Stains	Blue to Near-IR	<ul style="list-style-type: none"> Treat with prestain solution 5 min Stain in buffer 5 min Washing required 	Yes	No	Yes Tolerates MeOH fixation & detergent	<ul style="list-style-type: none"> Fixable membrane stains that tolerate permeabilization For labeling cell surface before fixation & immunofluorescence STORM-compatible dye options Non-toxic, but covalent modification of cell surface may affect function
CF® Dye WGA & Other Lectin Conjugates (p. 6)	Blue to Near-IR	<ul style="list-style-type: none"> Stain in complete medium 10 min Washing recommended¹ 	Yes	Yes	Yes Tolerates MeOH fixation & detergent	<ul style="list-style-type: none"> Stain cells or tissue sections (staining is cell-type and tissue-dependent) Many dye options, including STORM-compatible dyes Binds to specific glycoproteins; staining may be toxic
CellBrite™ Steady Membrane Dyes	Blue to Near-IR	<ul style="list-style-type: none"> Stain in complete medium 30 min to several days No wash staining for confocal imaging¹ Equilibrates between cell surface and intracellular compartments 	Yes	No	Yes (PFA) ² Does not tolerate detergent or MeOH	<ul style="list-style-type: none"> Unique probes for stable multi-day imaging of the cell surface membrane Non-toxic, uniform, & even cell staining Optional enhancer masks intracellular signal STORM-compatible dye options

¹Washing optional for confocal imaging, required for epifluorescence.

²Staining is retained immediately after formaldehyde fixation, but dye redistributes over time; if cells must be fixed, image within 24 hours

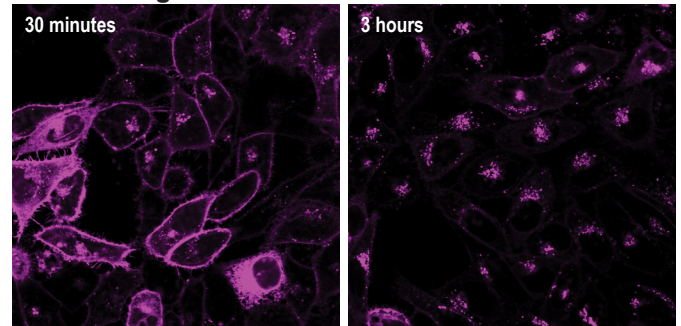
CellBrite™ Steady Membrane Staining Kits

CellBrite™ Steady Membrane Staining Kits are unique fluorescent membrane probes that allow fluorescence imaging of cell surface for up to several days in culture. Unlike non-covalent lipophilic membrane stains that are rapidly lost from the cell surface by endocytosis after labeling, CellBrite™ Steady Dyes equilibrate between intracellular compartments and the plasma membrane. Therefore, cells retain uniform cell surface and intracellular staining for 24 hours or longer (Fig. 1). CellBrite™ Steady Enhancer is an optional reagent included in the kits that can be used to mask intracellular fluorescence of CellBrite™ Steady Dyes, for more selective visualization of cell boundaries.

CellBrite™ Steady Features

- Rapid & uniform staining of live cell surface membranes for 24 hours or longer
- Optional Enhancer masks intracellular signal for selective imaging of cell surface
- Dye colors from blue to near-IR, with STORM-compatible options

DiD Staining



CellBrite™ Steady 650 + Enhancer

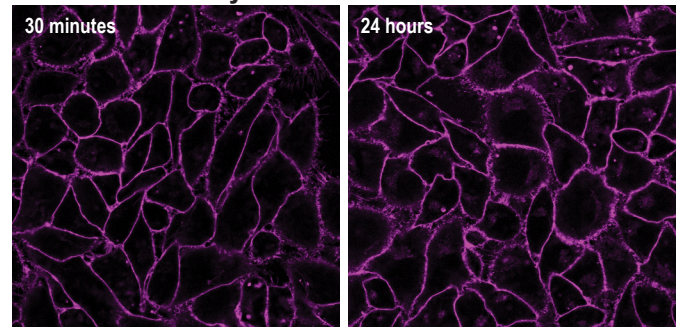


Figure 1. Comparison of lipophilic carbocyanine dye DiD and CellBrite™ Steady in live HeLa cells. DiD staining is heterogeneous and rapidly internalized into intracellular compartments. In contrast, CellBrite™ Steady 650 gives even staining of cell surface that can be clearly imaged after 24 hours in culture with the use of Enhancer.

CellBrite™ Fix and MemBrite™ Fix Membrane Dyes That Tolerate Permeabilization

While other lipophilic membrane dyes such as DiB, DiO and Dil can be fixed with formaldehyde, they are not compatible with detergent permeabilization or methanol fixation. To address these issues Biotium developed the novel CellBrite™ Fix and MemBrite™ Fix dyes. These are unique dyes that accumulate at the cell surface and bind covalently to membrane proteins for stable labeling. Both types of dyes can tolerate permeabilization and methanol fixation (Fig. 1), allowing membrane staining to be combined with intracellular labeling with antibodies. Moreover, CellBrite™ Fix and MemBrite™ Fix dyes offer greater water solubility compared to lipophilic carbocyanine dyes like DiO and Dil, resulting in much more uniform staining. The dyes are also non-cytotoxic and provide stable labeling that does not transfer readily between cells, allowing co-culture experiments.

CellBrite™ Fix and MemBrite™ Fix Features

- Tolerate permeabilization and fixation
- Stable and non-cytotoxic
- Simple and rapid staining protocols
- MemBrite™ Fix-ST dyes for STORM
- Suitable for mammalian cells, yeast, & bacteria

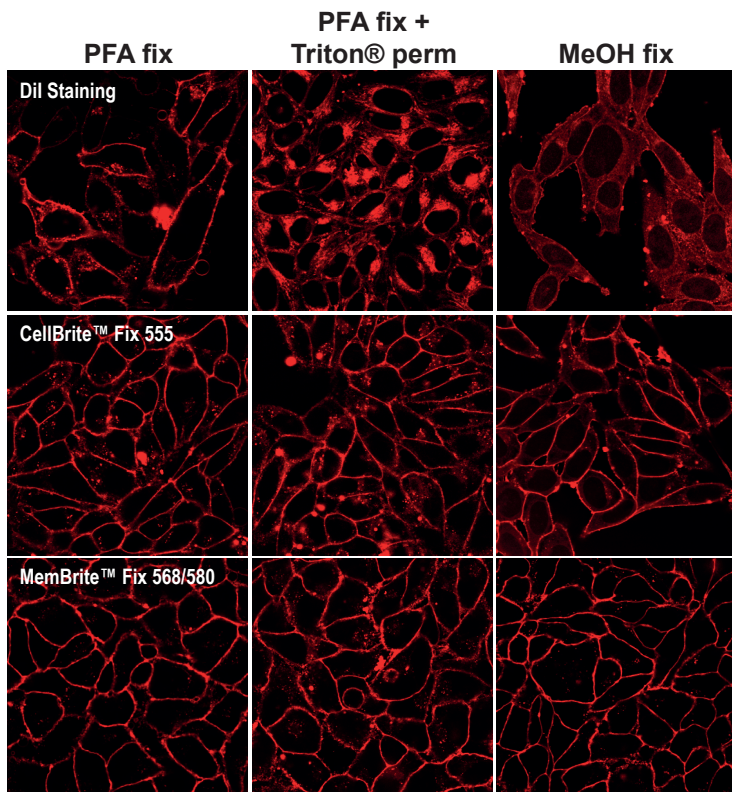


Figure 1. CellBrite™ Fix and MemBrite™ Fix label the cell surface more evenly than other lipophilic membrane stains, and tolerate fixation and permeabilization. Because CellBrite™ Fix and MemBrite™ Fix labeling is covalent, it does not redistribute after permeabilization, unlike lipophilic carbocyanine dye Dil. Live HeLa cells were labeled with Dil, CellBrite™ Fix 555, or MemBrite™ Fix 568/580. Cells were fixed with 4% paraformaldehyde (PFA) (left column), followed by permeabilization with 0.1% Triton® X-100 (center column), or fixed with methanol (MeOH) (right column).

CellBrite™ Cytoplasmic Membrane Dyes

CellBrite™ Cytoplasmic Membrane dyes are lipophilic carbocyanine dyes like Dil and DiO, which have been used extensively for cell tracing and tracking. Unlike other membrane stains, these dyes can be used for selective plasma membrane staining after cells are fixed and permeabilized. We offer a selection of dyes with fluorescence ranging from blue to near-infrared. We also offer CellBrite™ NIR dyes which are suitable for *in vivo* near-IR imaging in small animals. Please visit our website to view our full selection of stand-alone lipophilic carbocyanine dyes including DiO, Dil, and DiR.

CellBrite™ Steady Membrane Labeling Kits

Cat. #	Product	Ex/Em (nm)
30105	CellBrite™ Steady 405 Membrane Staining Kit	406/428
30106	CellBrite™ Steady 488 Membrane Staining Kit	505/529
30107	CellBrite™ Steady 550 Membrane Staining Kit	562/579
30108	CellBrite™ Steady 650 Membrane Staining Kit	656/676
30109	CellBrite™ Steady 685 Membrane Staining Kit	686/708

CellBrite™ Fix Membrane Labeling Kits

Cat. #	Product	Ex/Em (nm)
30090	CellBrite™ Fix 488 Membrane Stain	480/513
30088	CellBrite™ Fix 555 Membrane Stain	542/571
30089	CellBrite™ Fix 640 Membrane Stain	638/667

MemBrite™ Fix Cell Surface Labeling Kits

Cat. #	Product	Ex/Em (nm)
30092	MemBrite™ Fix 405/430 Cell Surface Staining Kit	405/430
30093	MemBrite™ Fix 488/515 Cell Surface Staining Kit	488/515
30094	MemBrite™ Fix 543/560 Cell Surface Staining Kit	543/560
30095	MemBrite™ Fix 568/580 Cell Surface Staining Kit	568/580
30096	MemBrite™ Fix 594/615 Cell Surface Staining Kit	594/615
30097	MemBrite™ Fix 640/660 Cell Surface Staining Kit	640/660
30098	MemBrite™ Fix 660/680 Cell Surface Staining Kit	660/680
30099	MemBrite™ Fix 680/700 Cell Surface Staining Kit	680/700
30101	MemBrite™ Fix-ST 650/665 Cell Surface Staining Kit	650/665
30102	MemBrite™ Fix-ST 667/685 Cell Surface Staining Kit	667/685
30103	MemBrite™ Fix-ST 681/698 Cell Surface Staining Kit	681/698
30104	MemBrite™ Fix-ST 755/777 Cell Surface Staining Kit	755/777

CellBrite™ & CellBrite™ NIR Cytoplasmic Membrane Dyes

Cat. #	Product	Ex/Em (nm)
30024	CellBrite™ Blue Cytoplasmic Membrane Staining Kit	366/441
30021	CellBrite™ Green Cytoplasmic Membrane Labeling Dye	484/501
30022	CellBrite™ Orange Cytoplasmic Membrane Labeling Dye	549/565
30023	CellBrite™ Red Cytoplasmic Membrane Labeling Dye	644/665
30070	CellBrite™ NIR680 Membrane Labeling Dye	683/724
30077	CellBrite™ NIR750 Membrane Labeling Dye	748/780
30078	CellBrite™ NIR770 Membrane Labeling Dye	767/806
30079	CellBrite™ NIR790 Membrane Labeling Dye	

Lectin Conjugates and Lipid Stains

CF® Dye Lectins

Lectins are carbohydrate binding proteins that recognize specific sugar moieties on cell surface glycoproteins. They can be used to stain the cell surface of live cells; and withstand fixation and permeabilization. When cells are fixed and permeabilized before staining, fluorescent lectins stain both cell surface and organelles in the secretory pathway. Biotium offers CF® dye lectin conjugates of wheat germ agglutinin (WGA) (Fig. 1), concanavalin A (Con A), and peanut lectin from *Arachis hypogaea* (PNA) in a variety of colors. Con A conjugates are used to stain the yeast cell wall and WGA conjugates are used to stain yeast bud scars. WGA may also be used as a live-cell Gram stain for bacteria.

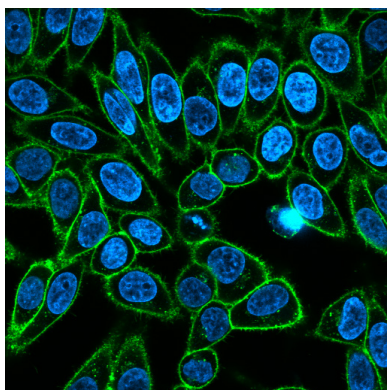


Figure 1. Live HeLa cells stained with CF@488A WGA (green) and Hoechst 33342 (blue).

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 with minimal background staining of cellular membranes or other organelles (Fig. 2). The protocol is simple and convenient, requiring as little as 30 minutes for incubation before or after fixation with no wash step required. LipidSpot™ dyes are available in green or far-red fluorescence with compatible detection channels for FITC, Texas Red®, or Cy®5. LipidSpot™ 488 has green fluorescence for the FITC channel and has been validated in super-resolution imaging by SIM. LipidSpot™ 610 has red/far-red fluorescence than can be detected in the Texas Red® or Cy® 5 channel.

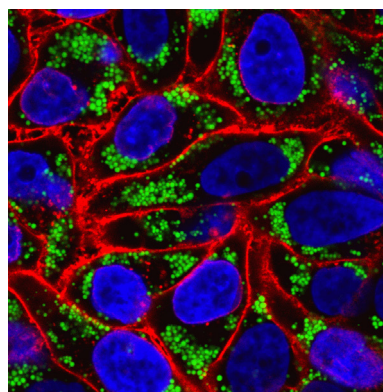


Figure 2. Oleic acid-treated HeLa cells were fixed with formaldehyde and stained with LipidSpot™ 488 (green), CF@594 WGA (red), and Hoechst (blue).

LipidSpot™ Features

- Rapid staining of lipid droplets
- Compatible with live or fixed cells
- Simple protocol with no wash step
- Minimal background staining
- Detect in FITC, Texas Red®, or Cy®5 channels

LipidSpot™ Lipid Droplet Stains

Cat. #	Product	Ex/Em (nm)
70065	LipidSpot™ 488 Lipid Droplet Stain, 1000X in DMSO	420/511
70069	LipidSpot™ 610 Lipid Droplet Stain, 1000X in DMSO	610/663

Texas Red is a registered trademark of Thermo Fisher Scientific. Cy dye is a registered trademark of GE Healthcare.

CF® Dye Labeled Lectins

Dye	Ex/Em (nm)	Con A	PNA	WGA
CF@350	347/448	29015	---	29021
CF@405S	404/431	29075	---	29027
CF@405M	408/452	29074	---	29028
CF@488A	490/515	29016	29060	29022
CF@532	527/558	---	---	29064
CF@555	555/565	---	---	29076
CF@568	562/583	---	29061	29077
CF@594	593/614	29017	29062	29023
CF@633	630/650	29018	---	29024
CF@640R	642/662	29019	29063	29026
CF@680	681/698	29020	---	29029
CF@680R	680/701	---	---	29025
CF@750	755/777	29080	---	---
CF@770	770/797	29058	---	29059

Labeled Phospholipids

These membrane probes are derived from natural phospholipids by modifying the head group with a fluorescent dye or biotin. The probes are useful for studying 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

Cat. #	Product	Ex/Em (nm)
60022	Biotin-DHPE	N/A
60023	Biotin-X-DHPE	N/A
60024	Fluorescein-DHPE	496/519
60025	NBD-PE	463/536
60028	TRITC-DHPE	540/566
60026	Rhodamine-DHPE	560/581
60027	Texas Red®-DHPE	582/601

NucSpot® Nuclear Stains for Live or Fixed Cells

NucSpot® Live Cell Nuclear Stains are cell-permeable DNA dyes that stain nuclei in live or fixed cells. Staining is specific, non-toxic, and does not require a wash step, so staining can be continuously monitored for several days. NucSpot® Live 488 has green fluorescence and NucSpot® Live 650 has far-red fluorescence for the Cy®5 channel (Fig. 1). NucSpot® Live 650 is also suitable for super-resolution imaging techniques such as STORM or SIM.

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 can also 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.

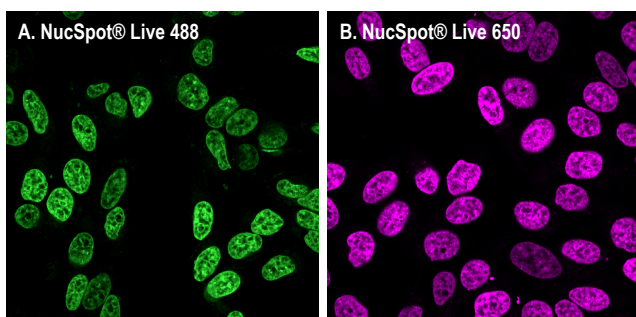


Figure 1. Live HeLa cells stained with 1X NucSpot® Live 488 (A) or 1X NucSpot® Live 650 (B).

NucSpot® Features

- Excellent nuclear specificity, no RNase or washing required
- Green or far-red fluorescence
- NucSpot® Live Stains have low toxicity and are suitable for long-term imaging
- NucSpot® 470 stains dead or fixed cells

RedDot™ 1 Features

- Cell-permeable, for staining live cells
- Can be used for cell cycle analysis by flow cytometry

RedDot™ 2 Features

- For fixed cells or tissues, or selective dead cell staining
- More nuclear-specific than Draq5™ or Draq7™, with no RNase or wash required

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, and can be used for cell cycle analysis by flow cytometry or for cell normalization by In-Cell Western™. It has also been used to stain nuclei in live flatworms.

RedDot™ 2 is membrane impermeant and can be used to selectively stain dead cells, or as a nuclear counterstain for fixed cells. RedDot™ 2 is functionally similar to Draq7™, but with better nuclear specificity in fixed cells and does not require a blocking step for nuclear-specific staining (Fig. 2).

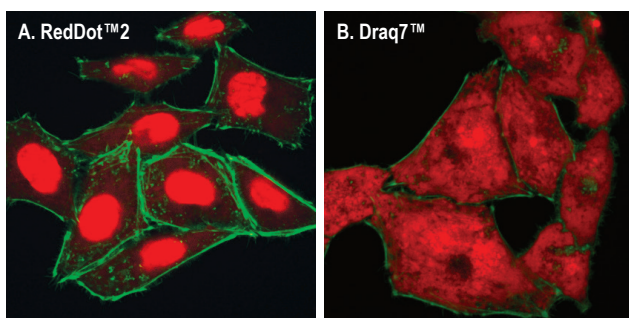


Figure 2. Formaldehyde fixed and detergent permeabilized HeLa cells stained with RedDot™ 2 (A) or Draq7™ (B). Actin is stained with CF®488A phalloidin (green).

Other Nucleic Acid Dyes

We offer a number of widely used nuclear specific dyes for microscopy analysis. This includes DAPI and Hoechst, commonly used for live or fixed cell nuclear staining. We also offer antibody conjugates that label nuclei, nuclear membranes, and nucleoli.

Visit our website at www.biotium.com to view our full selection of dead cell nucleic acid stains like propidium iodide (PI), 7-AAD, Ethidium Homodimer III, dimeric cyanine dyes (also known as TOTO® and TO-PRO®), as well as mounting media with DAPI.

Nuclear Stains

Cat. #	Product	Ex/Em (nm)
40081	NucSpot® Live 488 Nuclear Stain, 1000X in DMSO	500/515
40082	NucSpot® Live 650 Nuclear Stain, 1000X in DMSO	650/675
40083	NucSpot® 470 Nuclear Stain, 1000X in DMSO	460/546
40060	RedDot™ 1 Far-Red Nuclear Stain, 200X in Water	662/694*
40061	RedDot™ 2 Far-Red Nuclear Stain, 200X in DMSO	665/695*
40011	DAPI	358/461*
40044	Hoechst 33258, 10 mg/mL in H ₂ O	352/461
40046	Hoechst 33342, 10 mg/mL in H ₂ O	350/461

*With DNA

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.

Mitochondrial Stains

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 by microscopy or flow cytometry (Fig. 2). We also offer MitoView™ Green, a potential-independent mitochondrial dye that can be imaged following mitochondrial depolarization, or after fixation. MitoView™ dyes stain mitochondria in yeast, and also stain bacteria (gram-positive and gram-negative).

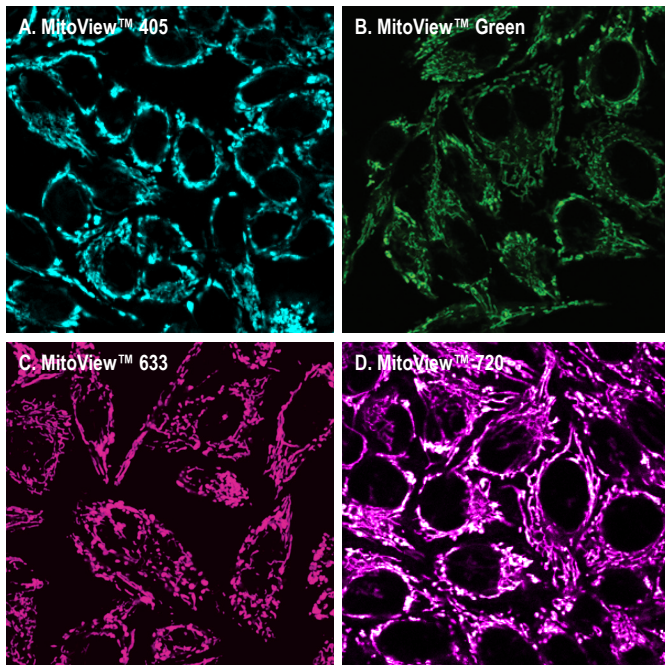


Figure 1. Live HeLa cells stained with MitoView™ 405 (A), MitoView™ Green (B), MitoView™ 633 (C), or MitoView™ 720 (D).

MitoView™ Dyes

Cat. #	Product	Ex/Em (nm)	Potential-dependent?
70070	MitoView™ 405	398/440	Partial ³
70054	MitoView™ Green	490/523	No
70055	MitoView™ 633	622/648 ¹	Yes
70075	MitoView™ 650	644/670	Partial ³
70068	MitoView™ 720	720/758 ²	Partial ³

¹ MitoView™ 633 also has red fluorescence in the Cy@3/rhodamine channel. It is not recommended for imaging with other red fluorescent probes.

² 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.

³ Dyes with partial mitochondrial membrane potential dependence localize to the cytoplasm after mitochondrial depolarization, but still retain fluorescence.

Assay Kits

Cat. #	Product	Features
30062	NucView® 488 and MitoView™ 633 Apoptosis Kit	Two color detection of caspase-3 activity and mitochondrial potential
30001	JC-1 Mitochondrial Membrane Potential Detection Kit	Two-color detection of mitochondria polarization/depolarization

Cy Dye is a registered trademark of GE Healthcare.

MitoView™ Features

- Rapid, no-wash, live cell mitochondria stains
- Bright, photostable, and non-toxic
- Available in five colors with blue, green, far-red, or near-IR emission
- Options for mitochondrial potential-dependent or -independent staining

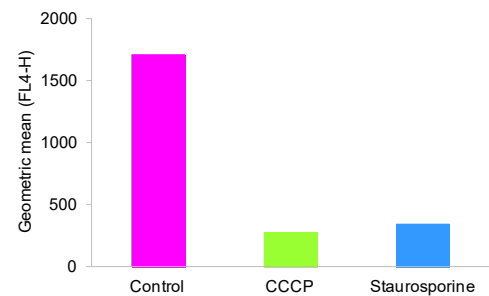


Figure 2. Flow cytometry analysis of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, resulting in decreased MitoView™ 633 staining.

Other Mitochondrial Dyes and Antibodies

We offer a wide selection of classic mitochondrial membrane-potential dyes including Rhodamine 123 and TMRE/TMRM. JC-1 dye can be used for ratiometric measurements of mitochondrial potential, while NAO is a green potential-independent dye that binds cardiolipin phosphoprotein in mitochondria.

For fixed cells and tissues we recommend using mitochondrial localized antibodies. We offer several mitochondrial localized primary antibodies conjugated to our bright and photostable CF® dyes. Visit our website at www.biotium.com to learn more.

More Mitochondrial Dyes

Cat. #	Product	Ex/Em (nm)	Potential-dependent?
70014	JC-1, Iodide Salt	510/527; 585/590*	Ratiometric*
70011	JC-1, Chloride Salt		
70010	Rhodamine 123	505/534	Yes
70016	TMRE	549/574	Yes
70005	TMRE, 2 mM in DMSO		
70017	TMRM	548/573	Yes
70018	DASPEI	461/589	Yes
70015	DiI _C (5)	638/658	Yes
70012	Nonyl Acridine Orange (NAO)	495/522	No

*JC-1 forms red fluorescent aggregates in polarized mitochondria, and green fluorescent monomers in cytoplasm

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 with weakly basic amine groups that accumulate in acidic organelles. LysoView™ dyes are available with blue, green, red, and far-red fluorescence (Fig. 1). Red fluorescent LysoView™ 540 and far-red fluorescent LysoView™ 633 dyes exhibit pH-dependent fluorescence (Fig. 2), resulting in specific lysosomal staining without a wash step. LysoView™ 488 has been validated for super-resolution imaging by SIM, while LysoView™ 650 is compatible with SIM and STED.

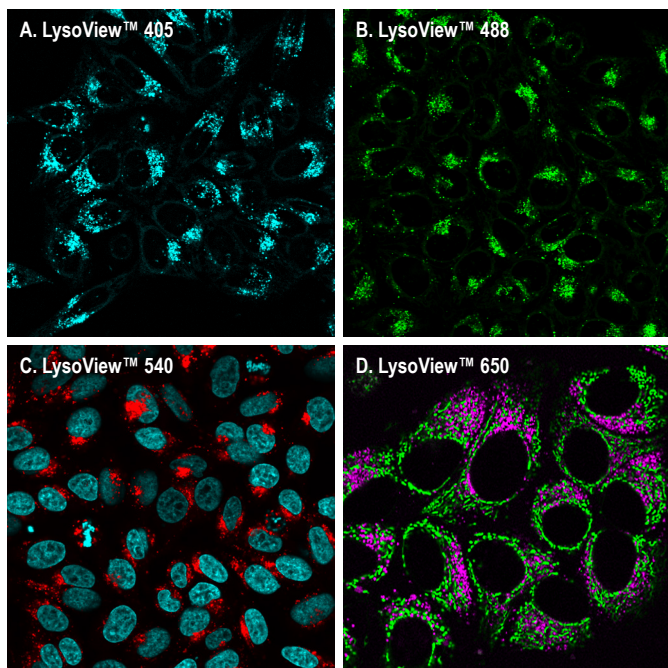


Figure 1. Live HeLa cells stained with LysoView™ 405 (A), LysoView™ 488 (B), LysoView™ 540 (red) and Hoechst 33342 (blue) (C), or LysoView™ 650 (magenta) and MitoView™ Green (D).

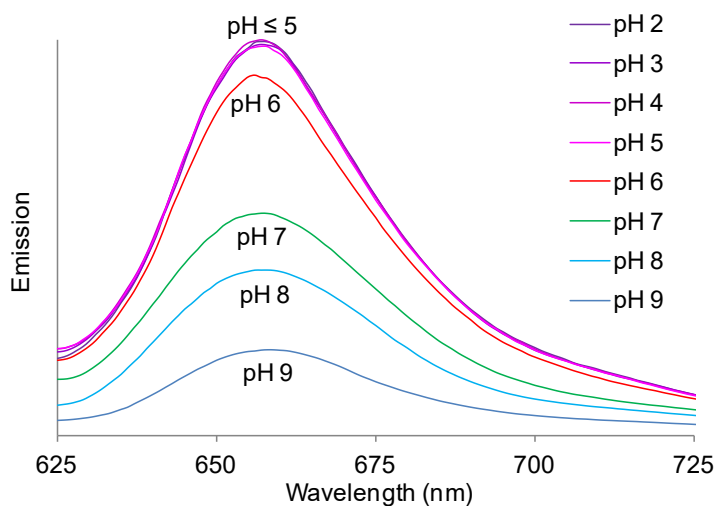


Figure 2. pH dependence of LysoView™ 633 fluorescence emission.

LysoView™ Features

- Fluorescent imaging of lysosomes in live cells
- Highly specific, no-wash staining of acidic organelles
- Dyes available for DAPI, FITC, Cy®3, and Cy®5 channels
- LysoView™ 650 compatible with SIM and STED imaging
- Options for yeast and bacteria staining
Visit website for details

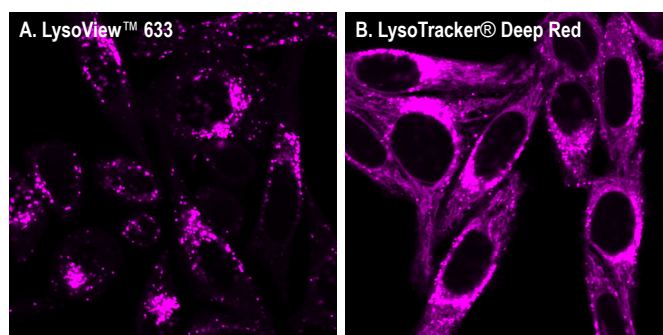


Figure 3. LysoView™ 633 compared to LysoTracker® Deep Red. Live HeLa cells were stained for 10 minutes at 37°C with 1X LysoView™ 633 or 50 nM LysoTracker® Deep Red (Thermo Fisher Scientific). LysoView™ 633 (A) showed more specific lysosomal staining with less cytoplasmic staining compared to LysoTracker®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

LysoView™ Dyes

Cat. #	Product	Ex/Em (nm)
70061	LysoView™ 405, 1000X in DMSO	318, 400/464
70061	LysoView™ 488, 1000X in DMSO	506/532
70061	LysoView™ 540, 1000X in DMSO	541/634*
70058	LysoView™ 633 (1000X)**	634/659*
70059	LysoView™ 650, 1000X in DMSO	650/675

*pH ≤ 5

**After reconstitution

Cytoskeleton Probes

ViaFluor® Live Cell Microtubule Stains

ViaFluor® Live Cell Microtubule Stains are simple, rapid, and sensitive stains for imaging the microtubule cytoskeleton in live cells (Fig.1). The stains are fluorescent, cell-permeable TAXOL® (paclitaxel) probes which label the cytoskeleton by binding to polymerized tubulin. Though TAXOL® is known for stabilizing microtubules and inhibiting mitosis, ViaFluor® Live Cell Microtubule Stains are less disruptive of microtubule dynamics and cell division. Presumably, this is because of the lower binding affinity of TAXOL® when conjugated to a fluorescent dye. The stains are supplied with a vial of verapamil, an efflux pump inhibitor that may improve probe retention and staining in certain cell types. ViaFluor® Live Cell Microtubule Stains are not suitable for fixation after staining and cannot be used with fixed cells or tissues.

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.

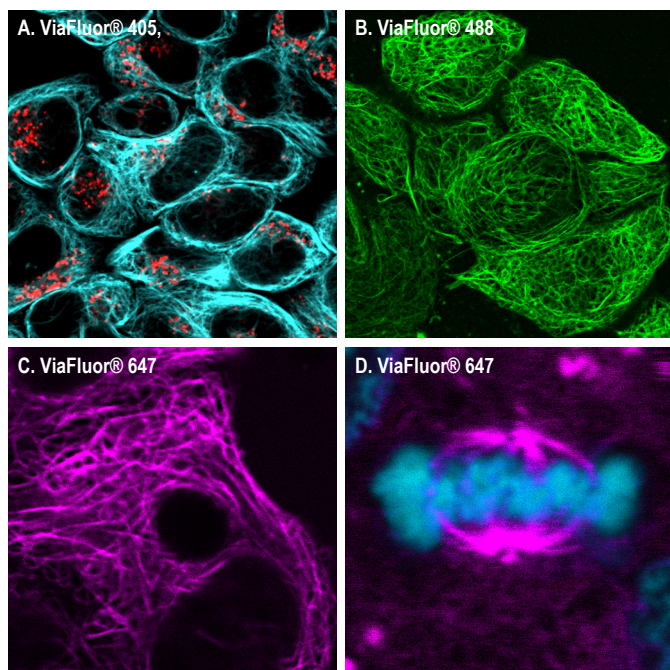


Figure 1. Live HeLa cells stained with ViaFluor® 405 microtubule stain (blue) and LysoView™ 540 (red) (A). HeLa cells stained with ViaFluor® 488 microtubule stain (B). Live MCF-7 cells stained with ViaFluor® 647 microtubule stain (C). Dividing HeLa cell stained with ViaFluor® 647 microtubule stain showing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

ViaFluor® Live Cell Microtubule Stains

Cat. #	Product	Ex/Em (nm)
70064-T 70064	ViaFluor® 405 Live Cell Microtubule Stain, 1000X in DMSO	408/452
70062-T 70062	ViaFluor® 488 Live Cell Microtubule Stain, 1000X in DMSO	500/515
70063-T 70063	ViaFluor® 647 Live Cell Microtubule Stain, 1000X in DMSO	650/675

Alexa Fluor, Texas Red, and DyLight are registered trademarks of Thermo Fisher Scientific. Cy Dye is a registered trademark of GE Healthcare. TAXOL is a registered trademark of Bristol-Myers Squibb.

Phalloidin conjugates

Phalloidin is a toxin isolated from the *Amanita phalloides* mushroom. It is a bicyclic peptide that binds specifically to F-actin (Fig. 2). 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 which are unrivaled in brightness, photostability, and water solubility compared to competing dyes such as Alexa Fluor®, DyLight®, and Cy® dyes. A number of our CF® dyes have also been validated for super-resolution imaging by STORM, STED, SIM, and other methods.

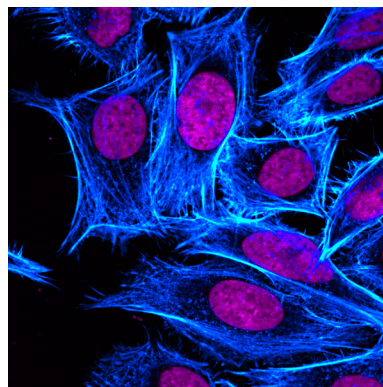


Figure 2. HeLa cells stained with CF@405M phalloidin (blue) and RedDot™2 Far-Red Nuclear Stain (magenta).

CF Dye® Phalloidin Conjugates

Cat #.	Product	Ex/Em (nm)
00049	CF@350 Phalloidin	347/448
00034	CF@405M Phalloidin	408/452
00054	CF@430 Phalloidin	426/498
00055	CF@440 Phalloidin	440/515
00042	CF@488A Phalloidin	490/515
00051	CF@532 Phalloidin	527/558
00043	CF@543 Phalloidin	541/560
00040	CF@555 Phalloidin	555/565
00044	CF@568 Phalloidin	562/583
00045	CF@594 Phalloidin	593/614
00046	CF@633 Phalloidin	630/650
00050	CF@640R Phalloidin	642/662
00041	CF@647 Phalloidin	650/665
00052	CF@660C Phalloidin	667/685
00047	CF@660R Phalloidin	663/682
00053	CF@680 Phalloidin	681/698
00048	CF@680R Phalloidin	680/701

Other Phalloidin Conjugates

Cat #.	Product	Ex/Em (nm)
00028	Phalloidin, Biotin-XX	N/A
00030	Phalloidin, Fluorescein	496/516
00032	Phalloidin, Rhodamine 110	502/524
00027	Phalloidin, Rhodamine	540/565
00033	Phalloidin, Sulforhodamine 101 (Texas Red®)	591/608

CF® Dye Dextran Conjugates

Dextran is a water soluble branched-chain polysaccharide. Fluorescently labeled dextrans are used as markers for trafficking of fluid phase endocytic cargo to lysosomes. In addition, dextrans may be used as tracers for epithelial and endothelial permeability as well as microinjected tracers for neuronal morphology. CF® Dye Dextrans are available with a variety of dye colors and molecular weights ranging from 3,500 to 250,000.

CF® Dye Transferrin Conjugates

Transferrin is a glycoprotein iron carrier that delivers iron to vertebrate cells via receptor-mediated endocytosis. Labeled human holo-transferrin is useful for microscopic studies of the endosomal pathway and/or transferrin uptake. After transferrin binds to its receptor on the cell surface, it is rapidly internalized by invagination of clathrin-coated pits. Inside endocytic vesicles, the acidic environment causes dissociation of iron from the transferrin-receptor complex. The apotransferrin is then recycled to the plasma membrane. We offer human transferrin labeled with a selection of our bright and photostable CF® dyes for imaging of recycling endosomes by microscopy.

CF® Dye Cholera Toxin Subunit B Conjugates

Cholera toxin is the symptom-causing toxin produced by the bacteria *Vibrio cholerae*. Cholera toxin subunit B (CT-B) is the non-toxic receptor binding subunit that binds to ganglioside GM1 in lipid rafts on the plasma membrane. Fluorescently labeled conjugates of CT-B are useful as endocytic tracers because the subunit is internalized by clathrin-dependent and -independent pathways. CT-B conjugates can also be used to label lipid rafts on the cell surface and as a retrograde neuronal tracer.

Bovine Serum Albumin CF® Dye Conjugates

Bovine serum albumin (BSA) conjugates are commonly used as protein tracers for studying the rate of receptor-mediated endocytosis and exocytosis during vesicle trafficking. Fluorescent BSA conjugates also have been used for quantitative studies of electroporation and measurement of plasma volume in rats. We offer BSA conjugates with four of our bright and photostable CF® dyes.

CF® Dye Labeled Endosomal Tracers

Dye	Ex/Em (nm)	BSA	Cholera Toxin B	Transferrin	Dextran 3.5K MW	Dextran 10K MW	Dextran 40K MW	Dextran 70K MW	Dextran 150K MW	Dextran 250K MW
CF@350	347/448	---	---	---	80137	---	---	---	---	---
CF@488A	490/515	20289	00070	00081	---	80110	80126	80117	80131	80134
CF@532	527/558	---	00074	---	---	---	---	---	---	---
CF@543	541/560	---	00075	00082	---	80111	---	---	---	---
CF@555	555/565	---	---	---	---	80112	---	---	---	---
CF@568	562/583	---	00071	00083	---	80113	---	---	---	---
CF@594	593/614	20290	00072	00084	---	80114	---	---	---	---
CF@620R	617/639	---	00076	---	---	---	---	---	---	---
CF@633	630/650	---	00077	---	---	---	---	---	---	---
CF@640R	642/662	20291	00073	00085	---	80115	---	---	---	---
CF@660R	663/682	---	00078	---	---	---	---	---	---	---
CF@680	681/698	20292	---	---	---	80118	80127	80129	80132	80135
CF@680R	680/701	---	00079	00086	---	80116	---	---	---	---
CF@750	755/777	---	---	00087	---	80119	80128	80130	80133	80136
CF@770	770/797	---	---	29059	---	80120	80122	80123	80124	80125
CF@790	784/806	---	---	---	---	80121	---	---	---	---

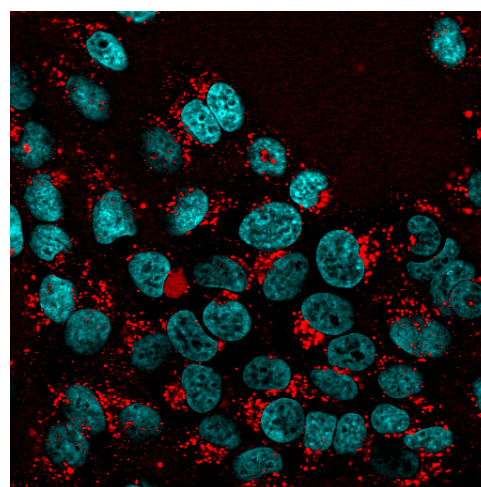


Figure 1. Live HeLa cells stained with CF@568 dextran (red) and counterstained with Hoechst (blue).

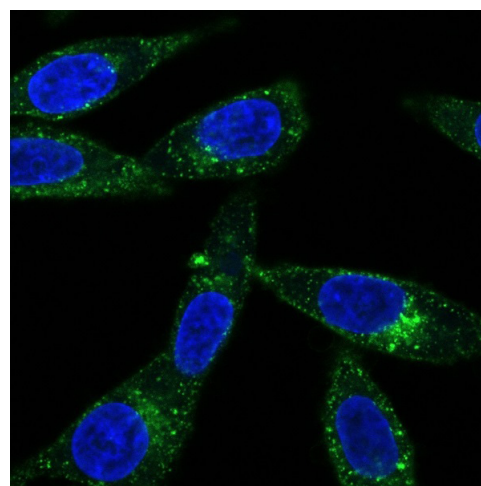


Figure 2. Live HeLa cells were stained with CF@488A transferrin (green) and DAPI (blue).