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Product Information

CellBrite™ NIR Cytoplasmic Membrane Dyes

Catalog number	Product description	Abs/Em*
30070	CellBrite™ NIR680 Cytoplasmic Membrane Dye	683/724 nm
30077	CellBrite™ NIR750 Cytoplasmic Membrane Dye	748/780 nm
30078	CellBrite™ NIR770 Cytoplasmic Membrane Dye	767/806 nm
30079	CellBrite™ NIR790 Cytoplasmic Membrane Dye	786/820 nm

* Absorption/emission maxima, measured in methanol (see Figure 1).

Unit Size: 100 uL

Concentration: 2 mM in DMSO

Storage and Handling
Store at room temperature, protected from light. Product is stable for at least one year from date of receipt when stored as recommended.

Product Description
Carbocyanine dyes label cytoplasmic membranes and intracellular membrane structures efficiently and stably (1). They have been used as tracers in cell–cell fusion (2,3), cellular adhesion (4,5), and migration (6) applications due to their properties of low cytotoxicity and high resistance to intercellular transfer. However, the lipophilic nature of these dyes can pose an obstacle to uniform cellular labeling. Although structurally related PKH dyes have been developed and optimized for cell labeling, the procedure requires multiple steps and subjects cells to an iso-osmotic mannitol loading medium that can affect cell viability (7,8). Biotium's CellBrite™ Cytoplasmic Membrane Dyes are ready-to-use dye delivery solutions that can be used in to normal culture media to uniformly label cells in suspension or adherent cultures. A variety of CellBrite™ dyes with different fluorescence colors are available, allowing cell populations to be marked with distinct fluorescent colors for identification after mixing (see Related Products). Double labeling can identify cells that have fused or formed stable clusters.

CellBrite™ NIR Cytoplasmic Membrane Dyes are novel near-infrared carbocyanine dyes for labeling the cytoplasmic membranes of living cells. Due to their long emission wavelengths (Figure 1), near-infrared cell membrane stains can be used to label cells for near-infrared small animal imaging studies for non-invasive imaging of cell migration and cell homing (9). The dyes have long 18-carbon hydrophobic tails and an additional water-soluble group. These unique chemical structure elements make the dyes easy to dissolve while providing highly stable cytoplasmic membrane staining, unlike traditional carbocyanine dyes like DiI, DiO, and DiR, which are often difficult to dissolve or prone to precipitation during cell staining.

CellBrite™ NIR680 Cytoplasmic Membrane Dye has emission at the far-red/near-infrared spectral boundary, and is compatible with both confocal microscopy and near-infrared imaging systems. CellBrite™ NIR750, CellBrite™ NIR770 and CellBrite™ NIR790 also can be imaged by confocal microscopy using 640 nm excitation for evaluation of cell labeling prior to small animal injection, however the sensitivity will be lower than with near-IR imaging.

Also see frequently asked questions (FAQs) for CellBrite™ dyes on the next page.

References
1. J Cell Biol 103, 171 (1986); 2. J Cell Biol 135, 63 (1996); 3. Cytometry 21, 160 (1995); 4. J Biol Chem 273, 33354 (1998); 5. J Cell Biol 136, 1109 (1997); 6. Anticancer Res 18, 4181 (1998); 7. J Immunol Methods 156, 179 (1992); 8. Methods Cell Biol 33, 469 (1990); 9. US Patent 4,783,401; 10. J Neurosci Methods 174, 71 (2008).

Staining Protocols
Note: It is recommended to optimize the staining procedure for each particular cell type. In some cases, it may be necessary to vary the staining volume and time.

Labeling Live Cells in Suspension
1. Prepare staining medium by diluting CellBrite™ NIR dye 1:2000 in culture medium for a final concentration of 1 uM.
2. Pellet cells by centrifugation at 350 x g for 5 minutes.
3. Remove supernatant and resuspend cells at a density of 1×10^6/mL in staining medium with dye.
4. Incubate for 20 minutes at 37°C. The optimal incubation time will vary depending on cell type. Start with 20 minutes and optimize as needed to get uniform labeling.
5. Pellet the cells by centrifugation at 350 x g for 5 minutes.
6. Remove the supernatant and wash the cells by gently resuspending them in warm (37°C) medium.
7. Repeat the centrifugation and wash (Steps 4 and 5) two more times.
8. Image fluorescence. Cells can be imaged in culture medium.

Labeling Live Adherent Cells
1. Prepare staining medium by diluting CellBrite™ NIR dye 1:2000 in culture medium for a final concentration of 1 uM.
2. Remove growth medium from the cells.
3. Add enough staining medium to completely cover the cells.
4. Incubate the cells at 37°C. The optimal incubation time will vary depending on the cell type. Start with 20 minutes and optimize as needed to get uniform labeling.
5. Remove the staining medium.
6. Wash the cells by adding fresh warm growth medium and incubating at 37°C for 5 minutes. Repeat the wash step two more times.
7. Image fluorescence. Cells can be imaged in culture medium.

Long Term Cell Staining
Lipophilic carbocyanine dyes like CellBrite™ are very stable, and have been reported to stain live cells for weeks in culture (1) or *in vivo* (6) with minimal transfer between cells. Immediately after labeling cells, the dyes primarily stain the plasma membrane, even in fixed cells. However, dye localization in live cells changes over time. If cells are cultured after staining, the labeled membrane will be internalized, so staining will gradually change from cell surface to intracellular vesicles, usually becoming mostly intracellular after about 24 hours in commonly used immortalized cell lines.

Fixation After Staining
Live cells stained with carbocyanine dyes can be fixed with formaldehyde (PFA), but not methanol or other solvents. Staining can withstand permeabilization with 0.1% Triton® X-100 or 0.1% digitonin (10). However, permeabilization can alter the dye localization, resulting in increased intracellular staining. Alternatively, we have seen good preservation of plasma membrane staining when cells are fixed with formaldehyde, then permeabilized before staining with CellBrite™ Dyes (see Labeling Fixed Cells).

Do not use mounting medium with glycerol, which can cause altered staining and high background. We recommend imaging in directly in buffer.

Also see our CellBrite™ Fix and MemBrite™ Fix Stains under Related Products. These are stains that covalently label cell membranes or cell surface for truly fixable staining.

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Labeling Fixed Cells

Note: cells should be fixed with formaldehyde (PFA). Fixation with methanol or other solvents extracts lipids and results in poor staining.

1. Wash cells with PBS after fixation.
2. Optional: permeabilize cells with 0.1% Triton® X-100 in PBS or Biotium's Permeabilization Buffer for 10 minutes at room temperature.

   Note: we have found this condition to preserve plasma membrane staining better than digitonin or saponin permeabilization.
3. Wash the cells 3 times with PBS to remove all traces of detergent.
4. Optional: perform staining with antibodies or other dyes. Do not use detergent in the buffers used for blocking, antibody dilution, or washing.
5. Prepare staining buffer by diluting CellBrite™ NIR dye 1:2000 in PBS for 1 uM final concentration.
6. Remove the buffer from the cells and add the staining solution.
7. Incubate 10 minutes or longer at RT, in the dark.
8. Wash the cells 3 times with PBS.
9. Do not use mounting medium with glycerol, which can cause altered staining and high background. We recommend imaging in PBS.

Frequently Asked Questions

Q. Do CellBrite™ dyes specifically stain the plasma membrane?

A. CellBrite™ cytoplasmic membrane stains are lipophilic carbocyanine dyes. These dyes undergo an increase in fluorescence when they insert into lipid bilayers. They stably label the plasma membrane and other intracellular membranes of cells. They also can be used to stain artificial lipid bilayers. Immediately after staining cultured cells, the dyes primarily localize to the plasma membrane. If cells are cultured over time after staining, the labeled membranes are internalized and staining gradually becomes mostly intracellular.

Q. How stable is CellBrite™ membrane staining? Are the dyes toxic to cells?

A. Lipophilic carbocyanine dyes have been used to stain neuronal cells in culture for several weeks, and in vivo for up to a year. The dyes do not appreciably affect cell viability, and do not readily transfer between cells, allowing cell migration and tracking studies in mixed populations. Stability of labeling may vary between cell types, depending on rates of membrane turnover or cell division.

Q. Can cells be fixed after CellBrite™ membrane staining? Can CellBrite™ membrane stains be used to stain cells or tissues after they are fixed?

A. Cells can be fixed with formaldehyde before or after labeling with CellBrite™ dyes. Permeabilization of cells with detergents or solvents, or mounting medium containing glycerol may adversely affect staining. Permeabilization with digitonin has been reported to be compatible with lipophilic carbocyanine dye staining. We've seen good results when fixed cells are permeabilized before staining (see Staining of Fixed Cells).

Q. What is the difference between CellBrite™ dyes and PKH dyes?

A. Biotium's CellBrite™ Cytoplasmic Membrane Dyes are dye delivery solutions that can be used in cell culture media to uniformly label suspended or adherent cells. The PKH dyes are structurally related dyes for cell membrane labeling. But unlike CellBrite™, labeling with PKH dyes requires multiple steps and the use of an iso-osmotic mannitol loading medium that can negatively affect cell viability.

Q. What is the difference between CellBrite™, CellBrite™ NIR, CellBrite™ Fix, and MemBrite™ Fix?

A. CellBrite™ Cytoplasmic Membrane Stains are lipophilic dyes for simple, non-toxic, stable labeling of membranes in live or fixed cells. Staining can be fixed with formaldehyde but has poor tolerance for permeabilization or methanol fixation. The dyes also do not stain bacteria or yeast. CellBrite™ NIR dyes have near-infrared fluorescence compatible with small animal NIR imaging systems.

CellBrite™ Fix and MemBrite™ Fix are covalent stains that react irreversibly with membrane proteins by different chemical mechanisms. Their staining can be fixed and permeabilized for IF staining. CellBrite™ Fix stains mammalian cells, yeast, and bacteria. MemBrite™ Fix also can be used to stain yeast. Unlike original CellBrite™ dyes and lectins, CellBrite™ Fix and MemBrite™ Fix cannot be used on cells that are already fixed.

To select a dye that's right for your application, visit www.biotium.com to download our Membrane & Surface Stains Brochure.

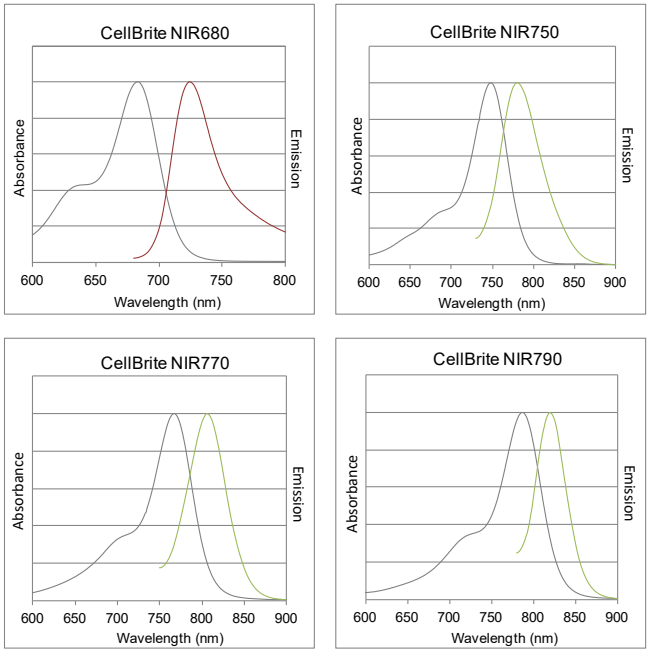

Figure 1. Absorbance and emission spectra of CellBrite™ NIR dyes in methanol.

Related Products

Cat. No.	Product
30024	CellBrite™ Blue Cytoplasmic Membrane Labeling Kit
30021	CellBrite™ Green Cytoplasmic Membrane Dye
30022	CellBrite™ Orange Cytoplasmic Membrane Dye
30023	CellBrite™ Red Cytoplasmic Membrane Dye
60013	DiA
60017	DiR
92160	VivoBrite™ Rapid Antibody Labeling Kit for Small Animal In Vivo Imaging, CF®680 SE
92161	VivoBrite™ Rapid Antibody Labeling Kit for Small Animal In Vivo Imaging, CF®750 SE
92162	VivoBrite™ Rapid Antibody Labeling Kit for Small Animal In Vivo Imaging, CF®770 SE
92163	VivoBrite™ Rapid Antibody Labeling Kit for Small Animal In Vivo Imaging, CF®790 SE
29007	CF®680 Annexin V, Lyophilized, 25 ug
29006	CF®750 Annexin V, Lyophilized, 25 ug
29046	CF®770 Annexin V, Lyophilized, 25 ug
29047	CF®790 Annexin V, Lyophilized, 25 ug
40060	RedDot™1 Far-Red Nuclear Stain for live cells
40061	RedDot™2 Far-Red Nuclear Stain for dead or fixed cells
22020	10X Phosphate Buffered Saline (PBS)
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22016	Permeabilization Buffer

Please visit our website at www.biotium.com for information on our life science research products, including near-infrared CF® dyes, conjugates and antibody labeling kits, other fluorescent CF® dye products, apoptosis reagents, bioluminescent substrates, and other probes and kits for life science research.