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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 permanently (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 posed 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 (7,8). Biotium's CellBrite™ Cytoplasmic Membrane Dyes are ready-to-use dye delivery solutions that can be added directly to normal culture media to uniformly label cells in suspension or adherent cultures. A variety of carbocyanine dyes with different optical properties 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 (Figure 2) for evaluation of cell labeling prior to small animal injection, however the sensitivity of detection using confocal microscopy will be lower compared to near-infrared imaging because 640 nm excitation is sub-optimal for these dyes, especially for CellBrite™ NIR790.

Staining Protocols

Labeling of Cells in Suspension

1. Prepare staining medium by diluting dye 1:2000 in culture medium for a final dye concentration of 1 uM (see notes below).
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.
4. Incubate cells for 20 minutes at 37°C (see notes below).
5. Pellet the labeled cells at 350 x*g* for 5 minutes, preferably at 37°C.
6. Remove supernatant and resuspend the cells in warm (37°C) medium.
7. Repeat wash steps (5 and 6) two more times.
8. Observe fluorescence by confocal microscopy, or proceed with experiment.

Labeling of Adherent Cells

1. Prepare staining medium by diluting dye 1:2000 in culture medium for a final dye concentration of 1 uM (see notes below).
2. Aspirate culture medium and add sufficient staining medium to completely cover the cells.
3. Incubate cells for 20 minutes at 37°C (see notes below).
4. Aspirate the staining medium and wash the cells three times. For each wash, cover the cells with fresh, warm growth medium, and incubate at 37°C for 5 minutes.
5. Observe fluorescence by confocal microscopy, or proceed with experiment.

Notes:
We recommend optimizing the staining procedure for each particular cell type. In some cases, it may be necessary to vary dye concentration (recommended range 1-5 uM) or staining time to obtain optimal labeling. Higher dye concentrations may be required to detect cell staining by confocal microscopy using 640 nm excitation, especially for CellBrite NIR790.

Cells stained with carbocyanine dyes can be fixed with formaldehyde. Detergent permeabilization may adversely affect staining. Permeabilization with digitonin (10-1000 ug/mL) has been reported to be compatible with carbocyanine dye staining (10).

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. Methods Cell Biol. 33, 469 (1990).
8. U.S. Patent No. 4,783,401.
9. J. Biomed. Optics 11(5):050507 (2006).
10. J. Neurosci. Methods. 174, 71 (2008).

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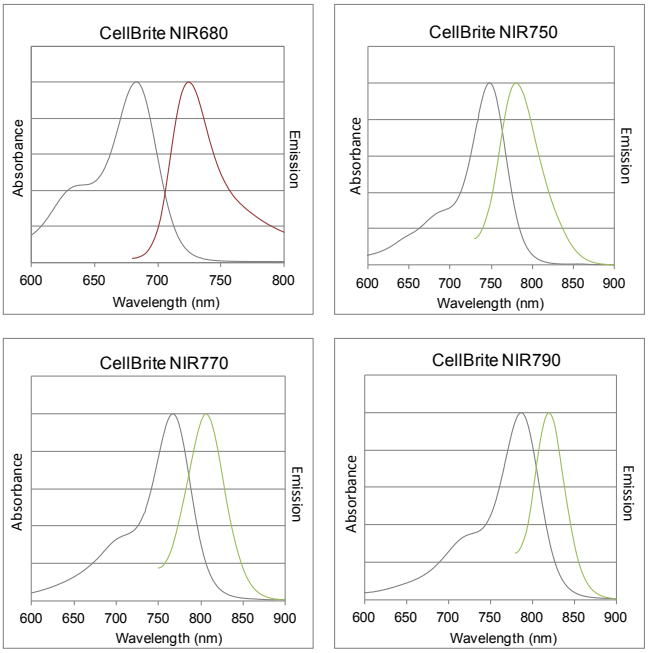

Figure 1. Absorbance and emission spectra of CellBrite™ NIR dyes in methanol.

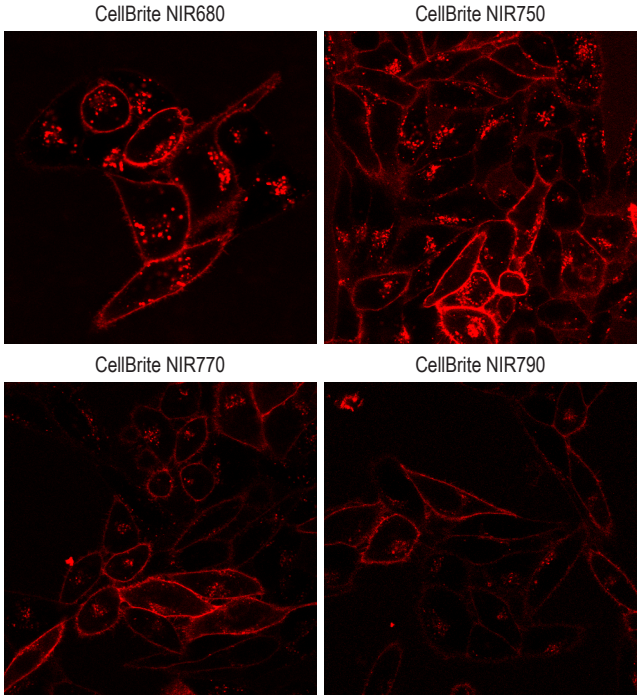

Figure 2. HeLa cells were stained with CellBrite™ NIR dyes and imaged on a Zeiss LSM 700 confocal microscope with 639 nm excitation laser. CellBrite™ NIR680 was imaged using filter settings for AlexaFluor® 680; CellBrite™ NIR750, CellBrite™ NIR770, and CellBrite™ NIR790 were imaged using filter settings for Cy®7. Note that image brightness has been increased for CellBrite™ NIR790 relative to the other images to show staining pattern, but fluorescence intensity is lower for CellBrite™ NIR 790 compared to CellBrite™ NIR 750 and CellBrite™ NIR790 when imaged by confocal microscopy using 639 nm excitation.

Related Products

Cat. No.	Product
30024	CellBrite™ Blue Cytoplasmic Membrane Labeling Kit, 1 kit
30021	CellBrite™ Green Cytoplasmic Membrane Dye, 1 mL
30022	CellBrite™ Orange Cytoplasmic Membrane Dye, 1 mL
30023	CellBrite™ Red Cytoplasmic Membrane Dye, 1 mL
60013	DiA, 50 mg
60017	DiR, 25 mg
92160	VivoBrite™ Rapid Antibody Labeling Kit for Small Animal In Vivo Imaging, CF680 SE, 3 labelings
92161	VivoBrite™ Rapid Antibody Labeling Kit for Small Animal In Vivo Imaging, CF750 SE, 3 labelings
92162	VivoBrite™ Rapid Antibody Labeling Kit for Small Animal In Vivo Imaging, CF770 SE, 3 labelings
92163	VivoBrite™ Rapid Antibody Labeling Kit for Small Animal In Vivo Imaging, CF790 SE, 3 labelings
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

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.