Product Information

CellBrite™ Cytoplasmic Membrane Dyes

Components

30021 CellBrite™ Green Cytoplasmic Membrane Dye
1 mL NeuroDiO cell labeling solution

30022 CellBrite™ Orange Cytoplasmic Membrane Dye
1 mL DiI cell labeling solution

30023 CellBrite™ Red Cytoplasmic Membrane Dye
1 mL DiD cell labeling solution

Storage and Handling

Store cell labeling solutions at 4°C, protected from light. Cap the vials tightly after each use to avoid evaporation. When stored as recommended, the cell labeling solutions are stable for at least 12 months from date of receipt.

Spectral Properties

NeuroDiO (30021) Ex/Em: 484/501 nm
DiI (30022) Ex/Em: 549/565 nm
DiD (30023) Ex/Em: 644/665 nm
See Fig. 1 for spectra

Product Description

The carbocyanine dyes DiI, DiO and DiD label cytoplasmic membrane 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 (8,9). Biotium's CellBrite™ Cytoplasmic Membrane Dyes are dye delivery solutions that can be added directly to normal culture media to uniformly label suspended or adherent cells in culture. In addition, CellBrite™ Green Cytoplasmic Membrane Dye contains NeuroDiO, a modified version of DiO with improved cytoplasmic membrane labeling.

Biotium also offers the CellBrite™ Blue Cytoplasmic Membrane Labeling Kit featuring DiB, the first blue carbocyanine dye (Ex/Em 360/420 nm), and CellBrite™ NIR Cytoplasmic Membrane Dyes that have emission in the far-red/near-infrared region for imaging by either microscopy or near-infrared imaging devices (see Related Products, next page) CellBrite™ dyes allow cell populations to be marked in distinctive fluorescent colors for identification after mixing. Double labeling can identify cells that have fused or formed stable clusters.

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

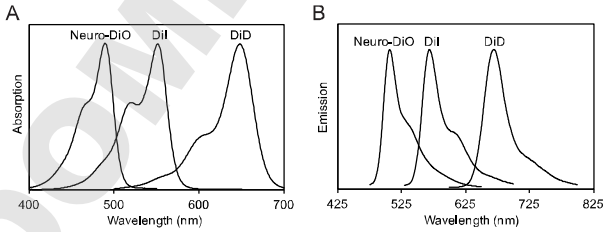

Figure 1. Absorption (A) and emission (B) of CellBrite Green, Orange and Red dyes in liposomes.

Staining Protocols

1. Labeling of Cells in Suspension

1.1 Suspend cells at a density of 1×10^6/mL in normal growth medium.
1.2 Add 5 uL of the cell labeling solution per 1 mL of cell suspension. Mix well by flicking the tube.
1.3 Incubate for 1–20 minutes at 37°C. The optimal incubation time will vary depending on cell type. Start by incubating for 20 minutes and subsequently optimize as necessary to obtain uniform labeling.
1.4 Centrifuge the labeled suspension tubes at 1500 rpm for 5 minutes at 37°C.
1.5 Remove the supernatant and gently resuspend the cells in warm (37°C) medium.
1.6 Repeat the wash procedure (Steps 1.4 and 1.5) two more times.
1.7 Proceed with fluorescence observation.

2. Labeling of Adherent Cells

2.1 Culture adherent cells in sterile glass coverslips or chamber slides as either confluent or subconfluent monolayers.
2.2 Remove coverslips from growth medium and gently drain off or aspirate excess medium. Then place coverslips in a humidity chamber.
2.3 Prepare staining medium by adding 5 uL of the cell labeling solution to 1 mL of normal growth medium and mixing well.
2.4 Pipet the staining medium onto the cells. Alternatively, cell labeling solution can be added directly to the cell culture and mixed well by shaking or swirling the plate. Add 5 uL of cell labeling solution per mL of culture medium in the plate.
2.5 Incubate the cells at 37°C. The optimal incubation time will vary depending on the cell type. Start by incubating for 20 minutes and subsequently optimize as necessary to obtain uniform labeling.
2.6 Aspirate the staining medium and wash the cells three times. For each wash cycle, cover the cells with fresh, warmed growth medium, and incubate at 37°C for 5 minutes.
2.7 Proceed with fluorescence observation.

Notes:

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.

Cells stained with carbocyanine dyes can be fixed with formaldehyde. Detergent permeabilization may adversely affect staining. Digitonin permeabilization (10 ug/mL-1 mg/mL) has been reported to be compatible with carbocyanine dye staining (10). Avoid mounting medium containing glycerol.

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).

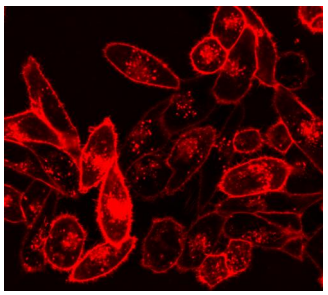

Figure 2. HeLa cells were stained for 20 minutes with CellBrite Orange using the adherent cell labeling protocol and imaged immediately after labeling.

Frequently asked questions (FAQs)

Question	Answer
Do CellBrite dyes specifically stain the plasma membrane?	CellBrite cytoplasmic membrane stains are lipophilic carbocyanine dyes. These dyes undergo an increase in fluorescence when they insert into lipid bilayers. Lipophilic carbocyanine dyes stably label the plasma membrane and other intracellular membranes of cells. They also can be used to stain artificial lipid bilayers.
How stable is CellBrite membrane staining? Are the dyes toxic to cells?	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 with intact membranes, 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.
Can cells be fixed after CellBrite membrane staining? Can CellBrite membrane stains be used to stain cells or tissues after they are fixed?	Cells can be fixed with formaldehyde after labeling with CellBrite dyes. Lipophilic carbocyanine dyes like the CellBrite dyes have also been used to stain cells or tissues after formaldehyde fixation. Permeabilization of cells with detergents or solvents, or mounting medium containing glycerol may adversely affect staining. Permeabilization with digitonin (10 ug/mL to 1 mg/mL) has been reported to be compatible with lipophilic carbocyanine dye staining.

Related Products

Catalog number	Product
30024	CellBrite™ Blue Cytoplasmic Membrane Labeling Kit, 50 assays
30070	CellBrite™ NIR680 Cytoplasmic Membrane Dye, 100 uL
30077	CellBrite™ NIR750 Cytoplasmic Membrane Dye, 100 uL
30078	CellBrite™ NIR770 Cytoplasmic Membrane Dye, 100 uL
30079	CellBrite™ NIR790 Cytoplasmic Membrane Dye, 100 uL
60013	DiA, 50 mg
60014	DiD, 50 mg
60034	Dilinoleyl DiI (Fast DiI™), 5 mg
60010	DiI, 50 mg
60018	DiI in vegetable oil, 0.5 mL
60035	Dilinoleyl DiO (Fast DiO_1™), 5 mg
60011	DiO, 50 mg
60012	$DiOC_{14}(3)$ hexanethiosulfonate, 50 mg
60038	$DiOC_{16}(3)$, 25 mg
60017	DiR, 25 mg
60016	Neuro-DiI, 25 mg
60015	Neuro-DiO, 25 mg
60019	Neuro-DiO in vegetable oil, 0.2 mL

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