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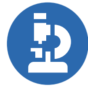
Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

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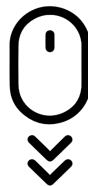
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

CellBrite® Cytoplasmic Membrane Dyes

Components

30021 CellBrite® Green Cytoplasmic Membrane Dye
1 mL Neuro-DiO 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

CellBrite® Green (Neuro-DiO): Ex/Em: 484/501 nm
CellBrite® Orange (DiI): Ex/Em: 549/565 nm
CellBrite® Red (DiD): Ex/Em: 644/665 nm
See Figure 1 for spectra

Product Description

The lipophilic carbocyanine dyes DiI, DiO and DiD label cytoplasmic membrane and intracellular membrane structures efficiently and stably (1). They have been used as tracers in 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 (8,9). 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 addition, CellBrite® Green Cytoplasmic Membrane Dye features NeuroDiO, a modified version of DiO with improved cytoplasmic membrane labeling.

CellBrite® Dyes are available in variety of fluorescent colors, including CellBrite® Blue, the first blue fluorescent lipophilic carbocyanine dye, and CellBrite® NIR Dyes with near-infrared fluorescence for microscopy or near-IR *in vivo* imaging (see Related Products) 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.

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).

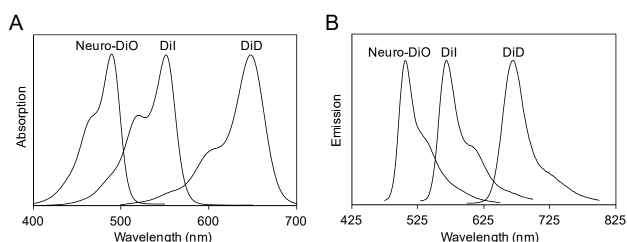

Figure 1. Absorption (A) and emission (B) of CellBrite® Green, Orange and Red dyes in liposomes.

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. Suspend cells at a density of 1×10^6/mL in normal growth medium.
2. Add 5 uL of the Cell Labeling Solution per 1 mL of cell suspension. Mix well by low-speed vortexing or flicking the tube.
3. 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.
4. Pellet the cells by centrifugation at 350 x g for 5 minutes.
5. Remove the supernatant and wash the cells by gently resuspending them in warm (37°C) medium.
6. Repeat the centrifugation and wash (Steps 4 and 5) two more times.
7. Image fluorescence. Cells can be imaged in culture medium.

Labeling Live Adherent Cells

1. Immediately before use, prepare staining medium by diluting Cell Labeling Solution in normal growth medium at 1:200 dilution (for example, add 5 uL Cell Labeling Solution to 1 mL of growth medium). Immediately vortex to mix well.

   Note: Proceed immediately to step 2. Within 1-2 minutes, the dye in the staining solution will begin adsorbing to the tube wall, which can result in weak and patchy staining.
2. Remove the growth medium from the cells and add the staining medium. Use enough medium to cover the cells (we usually use the same volume as used for normal culture medium).
3. 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.
4. Remove the staining medium.
5. Wash the cells by adding fresh warm growth medium and incubating at 37°C for 5 minutes. Repeat the wash step two more times.

   Note: Shorter wash steps may be performed to reduce dye internalization.
6. 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).

Note: Do not use mounting medium with glycerol, which can cause altered staining and high background. Organic mounding media are also not suitable. We recommend imaging in directly in PBS (or other aqueous buffers). Coverslips should be mounted using PBS and sealed with a suitable coverslip sealant such as CoverGrip™ or nail polish. Stained samples can be stored in PBS at 4°C for several weeks or longer.

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.

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 adding 5 uL of Cell Labeling Solution to 1 mL of PBS. Immediately vortex to mix well.

   Note: Proceed immediately to step 6. Within 1-2 minutes, the dye in the staining solution will begin adsorbing to the tube wall, which can result in weak and patchy staining.
6. Use enough solution to cover the cells (we usually use the same volume as used for culture medium).
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.

Related Products

Catalog number	Product
30024	CellBrite® Blue Cytoplasmic Membrane Labeling Kit
30070, 30077-30079	CellBrite® NIR Cytoplasmic Membrane Stains
60017	DiR, 25 mg
30105-30109	CellBrite® Steady Membrane Labeling Kits
30088-90	CellBrite® Fix Fixable Membrane Stains
30092-30099	MemBrite® Fix Fixable Cell Surface Staining Kits
40083	NucSpot® 470 Nuclear Stain for dead or fixed cells
40081	NucSpot® Live 488 Nuclear Stain for live or fixed cells
40082	NucSpot® Live 650 Nuclear Stain for live or fixed cells
40060	RedDot™1 Far-Red Nuclear Stain for live cells
40061	RedDot™2 Far-Red Nuclear Stain for dead or fixed cells
70054... 70075	MitoView™ Mitochondrial Stains
70082	MitoView™ Fix 640
70059... 70086	LysoView™ Lysosome Stains
70065	LipidSpot™ 488 Lipid Droplet Stain
70069	LipidSpot™ 610 Lipid Droplet Stain
40046	Hoechst 33342, 10 mg/mL in water
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative

Please visit our website at www.biotium.com for information on our life science research products, including our full selection of organelle stains for live or fixed cells, fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.

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Frequently Asked Questions (FAQs)

Question	Answer
I'm seeing weak, uneven, or non-uniform staining, what should I do?	CellBrite® Dyes are ready-to-use solutions of the lipophilic carbocyanine dyes DiI, DiO etc. It is challenging to get uniform labeling with CellBrite® Dyes due to their hydrophobicity. CellBrite® Red (Cat no. 30023), which is DiD, is particularly difficult as the hydrophobic dye tends to aggregate and give uneven staining. Warming the dye vial to ~50°C to dissolve any aggregates before use may improve staining. We do not recommend adding concentrated dye solution directly to the medium in a well of adherent cells, because this can result in uneven staining. Instead, prepare a diluted staining solution by adding dye to medium in a tube. Immediately vortex to mix well. It is important to add the staining solution to the cells as soon as possible after adding the dye, because the dye will begin to adsorb onto the tube, which may cause weak and uneven staining. Alternatively, for live cell staining, CellBrite® Steady or the reactive dyes CellBrite® Fix or MemBrite® Fix may be more suitable as they result in more even staining.
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. Immediately after staining cultured cells, the dyes primarily localize to the plasma membrane. **Note:** If cells are cultured after staining and washing, the labeled membranes will be internalized over time, and staining will gradually become mostly intracellular after 1-2 hours. For longer-term staining of plasma membranes in live cells, see our CellBrite® Steady Dyes.
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. 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. 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.
What is the difference between CellBrite® Dyes and PKH dyes?	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 subjects cells to an iso-osmotic mannitol loading medium that can negatively affect cell membrane integrity and viability.
What is the difference between CellBrite®, CellBrite® NIR, CellBrite® Fix, and MemBrite® Fix?	CellBrite® Cytoplasmic Membrane Stains are lipophilic dyes for simple, non-toxic, stable labeling of membranes in live or fixed cells. Cells can be fixed with formaldehyde before or after CellBrite® staining. But the staining has poor tolerance for permeabilization after fixation, and cannot be used with methanol fixation. The dyes also do not stain bacteria or yeast. CellBrite® NIR dyes are CellBrite® Dyes with near-infrared fluorescence compatible with small animal NIR imaging systems. CellBrite® Fix and MemBrite® Fix are novel covalent stains that can be fixed and permeabilized for IF staining. CellBrite® Fix Membrane Stains are fluorogenic reactive membrane dyes that rapidly accumulate at the plasma membrane and react covalently with membrane proteins for stable labeling. Staining takes only 15 minutes in a single step with no wash. CellBrite® Fix stains mammalian cells, yeast, and bacteria. MemBrite® Fix Cell Surface Stains do not bind lipids, but label cell surface proteins. MemBrite® Fix requires a two-step staining protocol with washing, but offers a more extensive choice of dye colors than CellBrite® Fix. 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.
Can CellBrite® or MemBrite® Dyes be used to stain exosomes/extracellular vesicles?	CellBrite® Cytoplasmic Membrane Dyes do not efficiently stain EVs, but CellBrite® Fix, MemBrite® Fix, CellBrite® Steady, and other stains have been used for this application. However, for optimal staining of exosome membranes we recommend our ExoBrite™ EV Membrane Stains.