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Product Information

CellBrite® Blue Cytoplasmic Membrane Labeling Kit

Catalog Number: 30024

Unit Size: 50 assays

Kit Contents
30024A: 250 uL DiB Cell Labeling Solution, 200X in DMSO
30024B: 250 uL DiB Loading Buffer, 200X in DMSO

Absorption/Emission 366/441 nm in liposomes (Fig. 1)

Storage and Handling
Store vials at room temperature and protect from light. Centrifuge vials briefly before opening to collect solutions from the cap. Cap the vials tightly after each use to avoid evaporation. When stored as recommended, the kit components are stable for at least 12 months from date of receipt.

If precipitation of DiB Cell Labeling Solution (30024A) occurs, warm the vial at 37°C for 30 minutes or more with periodic vortexing until the dye has fully dissolved (the solution may be slightly turbid). DiB Loading Buffer (30024B) may solidify into a gel during storage. This is normal and does not affect the product, but the buffer must be in liquid form before use. Heat the solidified gel to 50-60°C for 5-10 minutes and vortex periodically until it has formed a clear liquid. When warming the vials in a water bath, make sure to completely submerge the upper conical portion of the vial that contains the liquid.

Product Description
The CellBrite® Blue Cytoplasmic Membrane Labeling Kit contains DiB, the first blue fluorescent lipophilic carbocyanine dye. Lipophilic carbocyanine dyes label the plasma membrane and intracellular membrane structures efficiently and stably (1). They have been used as tracers in cell fusion (2,3), adhesion (4,5), and migration (6) due to their low cytotoxicity and high resistance to intercellular transfer. They 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. See Related Products for other CellBrite® dye colors.

Unlike PKH dyes that require multiple steps and subject cells to an iso-osmotic mannitol loading medium (8,9) that can affect cell viability, CellBrite® Cytoplasmic Membrane Dyes are dye delivery solutions that can be added directly to normal culture media for non-toxic, uniform labeling of suspension or adherent cells.

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).

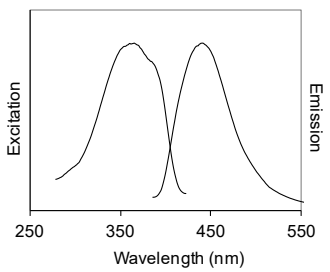

Figure 1. Normalized excitation and emission spectra of CellBrite® Blue in liposomes.

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 to achieve uniform labeling.

Preparation of Working Labeling Solution
Note: If DiB Loading Buffer or DiB Cell Labeling Solution have solidified or precipitated, heat to redissolve as described under Storage and Handling. DiB Loading Buffer is viscous, so pipet it slowly to ensure the correct volume is added. We recommend pipetting at least 5 uL for accurate pipetting.

Just before use, prepare 10 uL of working labeling solution per mL of desired staining solution by mixing 5 uL of DiB Cell Labeling Solution (30024A) with 5 uL of DiB Loading Buffer (30024B) in a clean tube. Pipette the mixture up and down to mix thoroughly. This is the working labeling solution.

Labeling Live Cells in Suspension
1. Suspend cells at a density of 1×10^6/mL in normal growth medium.
2. Prepare the working labeling solution as described above. Add 10 uL of the working labeling solution per 1 mL of cell suspension. Mix well by low-speed vortexing or flicking the tube.
3. Incubate the cells for 20 minutes at 37°C, in the dark. The optimal incubation time will vary depending on cell type. Start with 20 minutes and optimize as needed to get uniform staining.
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. Prepare the working labeling solution as described above. Make staining medium by adding 10 uL of working labeling solution to 1 mL of growth medium and mix well.
2. Remove medium from the cells and add enough staining medium to completely cover the cells. Alternatively, working solution (10 uL per mL) can be added directly to cells in growth medium, but this is not recommended as it may cause uneven staining.
3. Incubate the cells for 20 minutes at 37°C in the dark. The optimal incubation time will vary depending on the cell type. Start with 20 minutes and optimize as needed to improve uniformity of staining.
4. Remove the staining medium.
5. Wash the cells by adding warm growth medium and incubating at 37°C for 5 minutes. Repeat the wash step two more times.
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. For cell surface staining that is stable for several days in culture, check out our CellBrite® Steady Membrane Staining Kits (see Related Products).

Fixation After Staining
Live cells stained with CellBrite® Cytoplasmic Membrane 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 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 mounting 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.

For truly fixable staining, see our CellBrite® Fix and MemBrite® Fix Stains that covalently label cell membranes or cell surface under Related Products.

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 (22016) for 10 minutes at room temperature.

   Note: We have found these conditions 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 incubation, or washing.
5. Prepare working labeling solution as described on page 1. To make labeling buffer, add 10 uL working labeling solution to 1 mL PBS and mix well.
6. Remove PBS from the cells and add the labeling buffer from step 5.
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
30105-30109	CellBrite® Steady Membrane Staining Kits
30021	CellBrite® Green Cytoplasmic Membrane Dye
30022	CellBrite® Orange Cytoplasmic Membrane Dye
30023	CellBrite® Red Cytoplasmic Membrane Dye
30070-30079	CellBrite® NIR Cytoplasmic Membrane Dyes
30090	CellBrite® Fix 488 Fixable Membrane Stain
30088	CellBrite® Fix 555 Fixable Membrane Stain
30089	CellBrite® Fix 640 Fixable Membrane Stain
30092-30099	MemBrite® Fix Fixable Cell Surface Staining Kits
60010	DiI
60011	DiO
60014	DiD
60017	DiR
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22016	Permeabilization Buffer
40046	Hoechst 33342, 10 mg/mL in water
70065	LipidSpot™ 488 Lipid Droplet Stain
70069	LipidSpot™ 610 Lipid Droplet Stain

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.

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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. 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.
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.