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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
30024B: 250 uL DiB loading buffer

Spectral Properties
366/441 nm in liposomes (Fig. 1)

Storage and Handling
Store vials at room temperature and protect from light. If solutions appear cloudy or precipitation has occurred, warm the vial(s) to 37°C and vortex periodically to dissolve completely. Use solutions only when they are clear. 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 6 months from date of receipt.

Product Description
Biotium's CellBrite™ Cytoplasmic Membrane Labeling Dyes are dye delivery solutions that can be added directly to normal culture media to label suspended or adherent cells in culture. The CellBrite™ Blue Cytoplasmic Membrane Labeing Kit features DiB, the first blue carbocyanine dye. Other CellBrite™ Cytoplasmic Membrane Dyes include CellBrite™ Green (NeuroDiO), CellBrite™ Orange (DiI), and CellBrite™ Red (DiD), and CellBrite™ IR680 (see related products). 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.

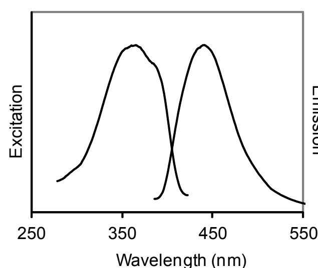

Figure 1. Normalized excitation and emission spectra of CellBrite Blue in liposomes.

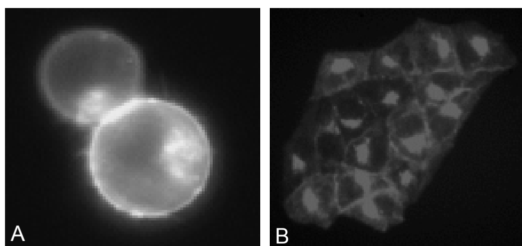

Figure 2. A. Jurkat cells were stained in suspension with CellBrite Blue. B. HeLa cell cluster stained with CellBrite Blue. Images were captured on an Olympus mercury arc lamp microscope using a DAPI filter set.

Staining Protocols

Preparation of Working Labeling Solution
Prepare a 1:1 mixture of Reagent A and Reagent B in a clean tube. Mix 5 uL of Reagent A with 5 uL of Reagent B per mL of staining medium required. This is the working labeling solution. Add 10 uL of the working labeling solution (Reagent A+B) per mL of cell suspension or staining medium as described below.

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 10 uL of the working labeling solution (Reagent A+B) 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 10 uL of the working labeling solution (Reagent A+B) to 1 mL of normal growth medium and mixing well.
2.4 Pipet the staining medium onto the cells. Alternatively, 10 uL of working labeling solution can be added directly to the cell culture and mixed well by shaking or swirling 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).

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. Anti-cancer 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).

Related Products

Catalog number	Product
30021	CellBrite™ Green Cytoplasmic Membrane Dye, 1 mL
30022	CellBrite™ Orange Cytoplasmic Membrane Dye, 1 mL
30023	CellBrite™ Red Cytoplasmic Membrane Dye, 1 mL
30070	CellBrite™ IR680 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

Please visit our website at **www.biotium.com** to view our selection of exceptionally bright and photostable CF™ dye bioconjugates, including antibodies, antibody labeling kits, phalloidin, annexin V and α-bungarotoxin, as wells as classic fluorescent nucleic acid dyes and hundreds of other products for life science research.

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