Biotium
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Product Information

CellBrite™ Blue Cytoplasmic Membrane Staining Kit

Catalog Number: 30024

Unit Size: 50 assays

Kit Contents
30024A: 250 uL DiB cell labeling solution
30024B: 250 uL DiB loading buffer

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 before opening the cap. Cap the vials quickly and tightly after each use to avoid evaporation. When stored properly, the kit components should remain stable for 6 months from date of receipt.

Product Description
Biotium's CellBrite™ Cytoplasmic Membrane Staining Kits are ready-to-use dye delivery solutions that can be added directly to normal culture media to label suspended or attached culture cells. Biotium offers DiB, the first blue cytoplasmic membrane labeling dye. Biotium's CellBrite™ Cytoplasmic Membrane Staining Kits also include cytoplasmic membrane orange labeling (DiI), cytoplasmic membrane green labeling (NeuroDiO), and cytoplasmic membrane red labeling (DiD). 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.

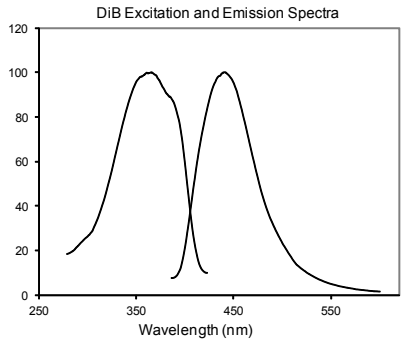

Figure 1. Normalized excitation and emission spectra of CellBrite Blue in liposomes.

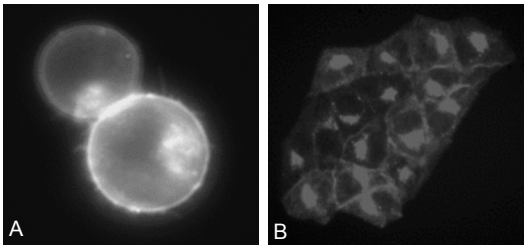

Figure 2. A. Jurkat cells were stained in suspension with CellBrite Blue. B. HeLa cell cluster stained with CellBrite Blue. Images were captured on an Olympus mercury arc lamp microscope using a DAPI filter set.

Experimental Protocols

Preparation of Staining Solution
Prepare a 1:1 mixture of Reagent A and Reagent B in a clean tube. Mix together 5 µL of each component for each mL of cells to be stained. This is the working labeling solution. Thus, 10 uL of the final mixture (Reagent A+B) should be used for each mL of cells.

1. Labeling of Cells in Suspension
1.1 Suspend cells at a density of 1×10^6/mL in any chosen culture medium.
1.2 Add 10µL of the working cell-labeling solution (Reagent A + B) per 1mL of cell suspension. Mix well by tapping 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 more uniform labeling.
1.4 Centrifuge the labeled suspension tubes at 1500 rpm for 5 minutes, preferably 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 10µL of the working cell-labeling solution (Reagent A + B) to 1mL of normal growth medium.
2.4 Pipet the staining medium onto the cells. Alternatively, 10uL of working labeling solution can be added directly to the cell culture.
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 more 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.

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. Cells stained with DiB can be fixed with formaldehyde but further permeabilization steps adversely affect the dye.

Detection

Table 1. Spectral characteristics of DiI, DiO, DiD and DiB.

Dye (Catalog #)	Abs	Em	Optical Filters	
			Omega	Chroma
NeuroDiO (30021)	484	501	XF23	31001 or 41001
DiI (30022)	549	565	XF32	31002 or 41002
DiD (30023)	644	665	XF47	31023 or 41008
DiB (30024)	360	440	XF03	31000V2

Flow Cytometry
Cells labeled with DiB can be analyzed using the UV laser line.

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.

Related Products

Catalog number	Product
30021	CellBrite™ Green Cytoplasmic Membrane-Labeling Kit, 1 mL
30022	CellBrite™ Orange Cytoplasmic Membrane-Labeling Kit, 1 mL
30023	CellBrite™ Red Cytoplasmic Membrane-Labeling Kit, 1 mL
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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