Biotium, Inc.
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CellBrite™ Cytoplasmic Membrane Staining Kit

Catalog Number: 30021, 30022, and 30023

Contact Information

www.interchim.com
interchim

211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com

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 Staining Kits are ready-to-use dye delivery solutions that can be added directly to normal culture media to uniformly label suspended or attached culture cells. In addition, NeuroDiO, an improved version of DiO, further improves cytoplasmic membrane labeling by a green fluorescent carbocyanine dye. Biotium also offers DiB, the first blue cytoplasmic membrane labeling dye. Biotium's CellBrite™ Cytoplasmic Membrane Staining Kits include cytoplasmic membrane orange labeling (DiI), cytoplasmic membrane green labeling (NeuroDiO), cytoplasmic membrane red labeling (DiD), and cytoplasmic membrane blue labeling (DiB). 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.

Product Components

CellBrite™ Green Cytoplasmic Membrane Staining Kit (Cat.30021)
1mL NeuroDiO cell labeling solution
CellBrite™ Orange Cytoplasmic Membrane Staining Kit (Cat.30022)
1mL DiI cell labeling solution
CellBrite™ Red Cytoplasmic Membrane Staining Kit (Cat.30023)
1mL DiD cell labeling solution

Storage Conditions

Store cell labeling solutions at 4°C and protected from light. Seal the vials of cell labeling solutions tightly after each use to avoid evaporation. When stored properly, the kit components should remain stable for 12 months from date of receipt.

Experimental Protocols

Labeling of Cells in Suspension

1.1 Suspend cells at a density of 1×10^6/mL in any chosen serum-free culture medium.
1.2 Add 5µL of the cell-labeling solution supplied 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. For cell types other than those listed, 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, 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 Allow 10 minutes recovery time before proceeding with fluorescence measurements.

Labeling of Adherent Cells

2.1 Culture adherent cells on sterile glass coverslips as either confluent or subconfluent monolayers.
2.2 Remove coverslips from growth medium and gently drain off excess medium through aspiration. Then place coverslips in a humidity chamber.
2.3 Prepare staining medium by adding 5µL of the supplied dye labeling solution to 1mL of normal growth medium with serum.

2.4 Pipet 200µL of the staining medium onto the corner of a coverslip and gently agitate until all cells are covered.
2.5 Incubate the coverslip at 37°C. The optimal incubation time will vary depending on the cell type. For cell types other than those listed, start by incubating for 20 minutes and subsequently optimize as necessary to obtain uniform labeling.
2.6 Drain off the staining medium and wash the coverslips three times. For each wash cycle, cover the cells with fresh, warmed growth medium, incubate at 37°C for 10 minutes, and then drain off the medium.

Detection Configurations

Microscopy

Filter sets for detection of NeuroDiO, DiI, DiD and DiB are selected based on their spectral characteristics, as summarized in Table 1. Multiband filter sets are available for simultaneous detection of multiple tracers as follows:

- DiI and NeuroDiO = Omega XF52, Chroma 51004
- DiI and DiD = Omega XF92, Chroma 51007
- DiI, NeuroDiO and DiD = Omega XF93, Chroma 61005
- DiB, NeuroDiO and DiI = Chroma 61000V2

Omega® filters are supplied by Omega Optical, Inc. (www.omegafilters.com). Chroma filters are supplied by Chroma Technology Corp. (www.chroma.com).

Flow Cytometry

Cells labeled with DiI, NeuroDiO and DiD can be analyzed using the conventional FL2, FL1 and FL3 flow cytometer detection channels, respectively.

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

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

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.