Cytoplasmic Membrane Staining Kits

PromoKine

Instruction Manual

Product Name	Size	Catalog Number
Green-fluorescent Cytoplasmic Membrane Staining Kit	1 ml	PK-CA707-30021
Orange-fluorescent Cytoplasmic Membrane Staining Kit	1 ml	PK-CA707-30022
Red-fluorescent Cytoplasmic Membrane Staining Kit	1 ml	PK-CA707-30023
Blue-fluorescent Cytoplasmic Membrane Staining Kit	50 assays	PK-CA707-30024

Introduction

The carbocyanine dyes DiI, DiO, DiD and DiB 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]. PromoKine's Fluorescent 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. PromoKine also offers DiB, the first blue cytoplasmic membrane labeling dye. PromoKine's Fluorescent 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.

Kit Contents

Product Number	Product Name	Content
PK-CA707-30021	Green-fluorescent Cytoplasmic Membrane Staining Kit	1 ml NeuroDiO Cell Labeling Solution
PK-CA707-30022	Orange-fluorescent Cytoplasmic Membrane Staining Kit	1 ml DiI Cell Labeling Solution
PK-CA707-30023	Red-fluorescent Cytoplasmic Membrane Staining Kit	1 ml DiD Cell Labeling Solution
PK-CA707-30024	Blue-fluorescent Cytoplasmic Membrane Staining Kit	Reagent A: 0.25 ml DiB Cell Labeling Solution Reagent B: 0.25 ml DiB Loading Buffer

Storage and Stability

Store cell labeling solutions at 4°C and protected from light. If solutions appear cloudy or precipitation has occurred, warm the vials to 37°C and vortex periodically to dissolve completely. Use solutions only when they are clear. Centrifuge the vials before opening the caps and seal the vials quickly and tightly after each use to avoid evaporation. When stored properly, the kit components should remain stable for 6-12 months from date of receipt.

Experimental Procedures

1. *Labeling of Cells in Suspension*

1.1. Suspend cells at a density of 1×10^6/ml in normal cell culture medium.

1.2. Add 5 µl of the supplied *Cell Labeling Solution* per 1 ml of cell suspension. Mix well by flicking the tube.

Blue-fluorescent Cytoplasmic Membrane Staining Kit (PK-CA707-30024): Prepare a 1:1 mixture of DiB Cell Labeling Solution (Reagent A) and DiB Loading Buffer (Reagent B) in a clean tube (mix 5 µl of Reagent A with 5 µl of Reagent B per ml of staining medium required; this is your *Working Labeling Solution*). Add 10 µl of this *Working Labeling Solution* 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 on sterile glass coverslips 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 5 µl of the supplied *Cell Labeling Solution* to 1 ml of normal growth medium and mixing well.

Blue-fluorescent Cytoplasmic Membrane Staining Kit (PK-CA707-30024): Prepare staining medium by adding 10 µl of the *Working Labeling Solution* (1:1 mixture of DiB Cell Labeling Solution [Reagent A] and DiB Loading Buffer [Reagent B]) to 1 ml of normal growth medium and mixing well.

2.4. Pipet the staining medium onto the cells. Alternatively, *Cell Labeling Solution* can be added directly to the cell culture and mixed well by shaking or swirling the plate (add 5 µL of *Cell Labeling Solution* per ml of culture medium in the plate).

Blue-fluorescent Cytoplasmic Membrane Staining Kit (PK-CA707-30024): Pipet the staining medium onto the cells. Alternatively, 10 µl 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, 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 µg/ml-1 mg/ml) has been reported to be compatible with carbocyanine dye staining[10].

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.

Cells labelled with DiB can be analysed using the UV laser line.

Note: It is recommended to optimize the staining procedure for each individual 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.

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

Dye (Product Number)	Abs	Em	Optical Filters	
			Omega	Chroma
NeuroDiO (PK-CA707-30021)	484	501	XF23	31001 or 41001
DiI (PK-CA707-30022)	549	565	XF32	31002 or 41002
DiD (PK-CA707-30023)	644	665	XF47	31023 or 41008
DiB (PK-CA707-30024)	360	420	XF03	31000V2

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

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)

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