GeneCopoeia™
Expressway to Discovery

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Product Information

Lipophilic Tracers-DiO, DiI, DiD, DiR

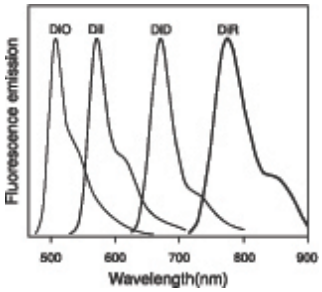
Catalog Number	Product Name	Unit Size
C016	DiO perchlorate	25 mg
C017	DiI perchlorate	25 mg
C018	DiD perchlorate	25 mg
C019	DiR iodide	10 mg

Storage upon receipt:
- -20°C
- Protect from light

Product Description

Long-chain dialkylcarbocyanines, in particular DiI, are widely used as anterograde and retrograde neuronal tracers in living and fixed tissues and cells. DiI labeling does not appreciably affect cell viability, development, or basic physiological properties. DiI-labeled motoneurons reportedly have remained viable for up to four weeks in culture and up to one year *in vivo*. The dyes uniformly label neurons via lateral diffusion in the plasma membrane at a rate of about 0.2–0.6 mm per day in fixed specimens; in living tissue labeling is more rapid (6 mm per day), due to active dye transport processes. In aldehyde-fixed tissue, diffusion of DiI can be followed for up to two years in some cases. In general, the dyes do not transfer from labeled to unlabeled cells, although some transfer may occur when the membrane is disrupted, as occurs when sectioning.

Spectral Characteristics



Normalized fluorescence emission spectra of DiO, DiI, DiD, and DiR bound to phospholipid bilayer membranes.

Experimental Protocols

Preparing Stock Solutions

Prepare stock solutions of lipophilic tracers in dimethyl formamide (DMF), dimethylsulfoxide (DMSO), or ethanol at 1 mM. DMF is preferable to ethanol as a solvent for DiO. Stock solutions can be stored for at least six months without deterioration under the same conditions as the undissolved product.

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 5 µL of the cell-labeling solution supplied per mL of cell suspension. Mix well by gentle pipetting.

1.3 Incubate for 1–20 minutes at 37°C. The optimal incubation time will vary depending on cell type. Typical incubation times required to produce uniform staining are shown in Table 1. 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 (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 by touching the edge of the coverslip with blotting paper. Place coverslip in a humidity chamber.

2.3 Prepare staining medium by adding 5 µL of the supplied dye labeling solution to 1 mL of normal growth medium.

2.4 Pipet 100 µL 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. Incubation times for selected cell types that have been tested in our laboratories are shown in Table 1. 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 for 10 minutes and then drain off the medium.

Table 1. *Optimal incubation times for cell staining*.

Cell Line	Optimal incubation time
Jurkat	2 minutes
HeLa	8 minutes
P3X	15 minutes
3T3	15 minutes

Table 2. *Spectral characteristics of DiO, DiI, DiD and DiR.*

Tracer	Ex (nm)	Em (nm)	Optical Filters	
			Omega	Chroma
DiO	484	501	XF23	31001
DiI	549	565	XF32	31002
DiD	644	665	XF47	31023
DiR	750	780	XF112	41009