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**Product Information**

**Lipophilic Tracers-DiO, DiI, DiD, DiR**

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| **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**

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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 × 106/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*.

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| --- | --- |
| **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.*

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| **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 |