DiD, DiI, DiO and DiR for Labeling Cell Membranes

Introduction

DiI, DiO, DiD and DiR dyes are a family of lipophilic fluorescent stains for labeling cell membranes and other hydrophobic structures. The fluorescence of these environment-sensitive dyes is greatly enhanced when incorporated into membranes or bound to lipophilic biomolecules such as proteins although they are weakly fluorescent in water. They have high extinction coefficients, polarity-dependent fluorescence and short excited-state lifetimes. Once applied to cells, these dyes diffuse laterally within the cellular plasma membranes, resulting in even staining of the entire cell at their optimal concentrations. The distinct fluorescence colors of DiI (orange fluorescence), DiO (green fluorescence), DiD (red fluorescence) and DiR (deep red fluorescent) provide a convenient tool for multicolor imaging and flow cytometric analysis of live cells. DiO and DiI can be used with standard FITC and TRITC filters respectively. Among them DiD is well excited by the 633 nm He–Ne laser, and has much longer excitation and emission wavelengths than those of DiI, providing a valuable alternative for labeling cells and tissues that have significant intrinsic fluorescence. DiR might be useful for in vivo imaging or tracing due to the effective transmission of infrared light through cells and tissues and low level of autofluorescence in the infrared range.

Chemical Properties

Table 1: Chemical Properties of DiD, DiO, DiI and DiR

<table>
<thead>
<tr>
<th>Product</th>
<th>DiD</th>
<th>DiO</th>
<th>DiI</th>
<th>DiR***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight</td>
<td>959.91</td>
<td>881.7</td>
<td>961.32</td>
<td>1013.39</td>
</tr>
<tr>
<td>Ex/Em</td>
<td>644/663 nm</td>
<td>484/501 nm</td>
<td>549/565 nm</td>
<td>748/780 nm</td>
</tr>
<tr>
<td>Solvent</td>
<td>DMSO</td>
<td>DMSO</td>
<td>DMSO</td>
<td>DMSO</td>
</tr>
<tr>
<td>Recommended Optical Filter*</td>
<td>XF47-Omega 31023-Chroma</td>
<td>XF23-Omega 31001-Chroma</td>
<td>XF32-Omega 31002-Chroma</td>
<td>XF112-Omega 41009-Chroma</td>
</tr>
</tbody>
</table>

* Catalog numbers of optical filter sets recommended for fluorescence imaging. Omega filters are supplied by Omega Optical, Inc. (www.omegafilters.com). Chroma filters are supplied by Chroma Technology Corp. (www.chroma.com); **Fluorescence emission of this dye is invisible to the human eye and must be detected using a CCD camera or other infrared-sensitive detector.

Sample Protocol

1. Prepare DiO, DiI DiD or DiR membrane stain solutions:
   1.1 Prepare DMSO or EtOH stock solutions: The stock solutions should be prepared in DMSO or EtOH at 1-5 mM. Note: The unused portion of the stock solution should be stored at -20°C. Avoid freeze/thaw cycles.
   1.2 Prepare working solutions: Dilute the stock solutions (from step 1.1) into a suitable buffer such as serum-free culture medium, or HBSS or PBS to make 1 to 5 μM working solutions. Note: The final concentration of the working solution will need be empirically determined for different cell types and/or experimental conditions. Testing at least a tenfold range of concentrations is recommended.

2. Stain the cells in Suspension
   2.1 Suspend cells at a density of 1 × 10⁶/mL in dye working solution (from step 1.2).
2.2 Incubate for 2–20 minutes at 37°C. The optimal incubation time will vary depending on cell type. Typical incubation times required to produce uniform staining, start by incubating for 20 minutes and subsequently optimize as necessary to obtain uniform labeling.

2.3 Centrifuge the labeled suspension tubes at 1000 to 1500 rpm for 5 minutes.

2.4 Remove the supernatant and gently resuspend the cells in pre-warmed (37°C) growth medium.

2.5 Wash two more times as steps 1.3 and 1.4.

3. Stain adherent cells
   3.1 Grow adherent cells on sterile glass coverslips.
   3.2 Remove coverslips from growth medium and gently drain off excess medium. Place coverslip in a humidity chamber.
   3.3 Pipet 100 μL of the dye working solution (from step 1.2) onto the corner of a coverslip and gently agitate until all cells are covered.
   3.4 Incubate the coverslip for 2–20 minutes at 37°C. The optimal incubation time will vary depending on cell type. Typical incubation times required to produce uniform staining, start by incubating for 20 minutes and subsequently optimize as necessary to obtain uniform labeling.
   3.5 Drain off the dye working solution and wash the coverslips two to three times with growth medium. For each wash cycle, cover the cells with pre-warmed growth medium, incubate for 5-10 minutes and then drain off the medium.

4. Microscopy Detection
   4.1 Selection of DiD, DiO, DiI and DiR’s filter sets are summarized in Table 1.
   4.2 For simultaneous detection of multiple dyes, multiband filter sets are available as follows:
      a) Dil and DiO = Omega XF52, Chroma 51004
      b) Dil and DiD = Omega XF92, Chroma 51007
      c) Dil, DiO and DiD = Omega XF93, Chroma 61005

5. Flow Cytometry Detection
   Cells labeled with DiD, DiO, DiI and DiR can be analyzed using the conventional FL3, FL1 and FL2 flow cytometer detection channels, respectively.

References