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DiA, DiD, DiI, DiO, DiR, and DiS for Labeling Cell Membranes

Introduction

DiA, DiD, DiI, DiO, DiR, and DiS 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.

Sample Protocol

1. Prepare DiA, DiD, DiI, DiO, DiR, or DiS 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 repeated 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, HBSS or PBS to make 1 to 5 μM working solutions.
Note: The final concentration of the working solution should be empirically determined for different cell types and/or experimental conditions. It is recommended to test at concentrations that are at least over a ten-fold range.

2. Stain the cells in suspension:

2.1 Suspend cells at a density of 1×10^6/mL in dye working solution (from Step 1.2).
2.2 Incubate at 37 °C for 2–20 minutes. The optimal incubation time varies depending on the cell type. 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 2.3 and 2.4.

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 at 37 °C for 2–20 minutes. The optimal incubation time varies depending on the cell type. 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 The selection of DiA, DiD, DiI, DiO, DiR, and DiS filter sets is summarized in Table 1.

4.2 For simultaneous detection of multiple dyes, multiband filter sets are available as follows:

a) DiI and DiO = Omega XF52, Chroma 51004

b) DiI and DiD = Omega XF92, Chroma 51007

c) DiI, DiO and DiD = Omega XF93, Chroma 61005

5. Flow Cytometry Detection:

Cells labeled with DiA, DiO, DiI, DiD, DiS and DiR can be analyzed using the conventional flow cytometer detection channels.

6. Fixation After Staining:

6.1 Cells stained with DiA, DiD, DiI, DiO, DiR, or DiS dyes can be fixed using formaldehyde-based fixatives such as paraformaldehyde (PFA). Avoid methanol or other organic solvent-based fixation methods, as they can extract membrane lipids and reduce staining quality.

6.2 Mild permeabilization (e.g., 0.1% Triton® X-100 or digitonin) is compatible but may increase intracellular signal due to dye redistribution.

6.3 Fixation prior to permeabilization and staining may help preserve plasma membrane localization.

7. Labeling Fixed Cells:

7.1 Wash fixed cells with PBS.

7.2 Optional: Permeabilize cells with 0.1% Triton® X-100 in PBS for 10 minutes at room temperature.

Note: This approach generally preserves membrane staining better than harsher detergents.

7.3 Wash cells three times with PBS to remove residual detergent.

7.4 Optional: Perform staining with antibodies or additional probes as needed.

Note: Do not include detergent in blocking or antibody incubation buffers.

7.5 Prepare staining solution by diluting dye stock into PBS or suitable buffer (1–10 µM).

7.6 Remove buffer and add staining solution.

7.7 Incubate for 10 minutes or longer at room temperature, protected from light.

7.8 Wash cells three times with PBS.

7.9 Image cells in PBS or another aqueous buffer.

8. Mounting Samples for Imaging:

8.1 Do not use mounting media containing glycerol or organic solvents, as they can solubilize membrane-bound dyes and increase background.

8.2 Image samples directly in PBS or another aqueous buffer.

8.3 Mount coverslips using PBS and seal with an appropriate sealant (e.g., nail polish).

8.4 Stained samples can be stored in PBS at 4 °C for extended periods.

References

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Appendix I: Product Ordering Information

Catalog Number	Product Name	Unit Size	MW	Excitation (nm)	Emission (nm)
22030	DiA [4-(4-(Dihexadecylamino)styryl)-N-methylpyridinium iodide]	25 mg	787.04	492	613
22033	DiD labeling solution [1,1-Dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine] *5 mM DMSO solution*	10 mL	959.91	646	663
22035	DiIC12(3) perchlorate [1,1-Didodecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate]	25 mg	765.55	550	564
22038	DiOC2(3) iodide [3,3-Diethyloxacarbocyanine iodide]	25 mg	460.31	483	501
22039	DiOC3(3) iodide [3,3-Dipropyloxacarbocyanine iodide]	25 mg	488.36	483	501
22040	DiOC7(3) iodide [3,3-Diheptyloxacarbocyanine iodide]	25 mg	600.57	483	501
22042	DiOC16(3) perchlorate [3,3-Dihexadecyloxacarbocyanine perchlorate]	25 mg	825.6	483	501
22044	DiIC16(3) perchlorate [1,1-Dihexadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate]	25 mg	877.76	550	564
22045	DiOC5(3) iodide [3,3-Dipentyloxacarbocyanine iodide]	25 mg	544.47	483	501
22046	DiOC6(3) iodide [3,3-Dihexyloxacarbocyanine iodide]	25 mg	572.52	483	501
22050	DiIC12(3)-DS [1,1-Diododecyl-3,3,3,3-tetramethylindocarbocyanine-5,5-disulfonic acid]	5 mg	825.21	550	564

Catalog Number	Product Name	Unit Size	MW	Excitation (nm)	Emission (nm)
22051	DiIC12(5)-DS [1,1-Diododecyl-3,3,3,3-tetramethylindodicarbocyanine-5,5-disulfonic acid]	5 mg	851.25	652	670
22052	DiIC18(3)-DS [1,1-Dioctadecyl-3,3,3,3-tetramethylindocarbocyanine-5,5-disulfonic acid]	5 mg	993.53	550	564
22054	DiIC18(5)-DS [1,1-Dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine-5,5-disulfonic acid]	5 mg	1019.57	652	668
22056	DiIC1(5) iodide [1,1,3,3,3,3-Hexamethylindodicarbocyanine iodide]	25 mg	510.45	640	657
22065	Neuro-DiO	5 mg	1043.55	483	501
22066	DiO perchlorate [3,3-Dioctadecyloxacarbocyanine perchlorate] *CAS#: 34215-57-1*	25 mg	881.7	483	501
22070	DiR iodide [1,1-dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine iodide]	25 mg	1013.39	754	778
22073	DiSC2(3) [3,3-Diethylthiacarbocyanine iodide]	25 mg	492.44		
22076	DiSC3(5) [3,3-Dipropylthiadicarbocyanine iodide]	25 mg	546.53		
22101	DiI iodide [1,1-Dioctadecyl-3,3,3,3- tetramethylindocarbocyanine iodide]	100 mg	961.32	550	564
22102	DiI perchlorate [1,1-Dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate] *CAS 41085-99-8*	100 mg	961.32	550	564
22103	DiI triflate [1,1-Dioctadecyl-3,3,3,3-tetramethylindocarbocyanine triflate]	100 mg	961.32	550	564