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

Ordering Information:	*Storage Conditions:*
Product Number: DiA (22030) DiD (22033, 22051, 22054, 22056) DiO (22038, 22039, 22040, 22042, 22045, 22046, 22066) DiI (22035, 22044, 22050, 22052, 22101, 22102, 22103) DiR (22070) DiS (22073, 22076)	Keep at -20 °C Protect from moisture and light

Introduction

DiA, 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, DiR, DiS

Catalog #	Molecular Weight	Ex/Em	Solvent	Recommended Optical Filter*
DiA				
22030	787.04	491/613	DMSO	XF21-Omega, 31024-Chroma
DiD				
22033	959.91	644/663 nm	DMSO	XF47-Omega, 31023-Chroma
22051	851.25	650/670 nm	DMSO	XF47-Omega, 31023-Chroma
22054	1019.57	650/670 nm	DMSO	XF47-Omega, 31023-Chroma
22056	510.45	638/658 nm	DMSO	XF47-Omega, 31023-Chroma
DiO				
22038	881.7	484/501 nm	DMSO	XF23-Omega, 31001-Chroma
22039	488.32	482/497 nm	DMSO	XF23-Omega, 31001-Chroma
22040	600.57	482/504 nm	DMSO	XF23-Omega, 31001-Chroma
22042	825.6	484/501 nm	DMSO	XF23-Omega, 31001-Chroma
22045	544.47	482/504 nm	DMSO	XF23-Omega, 31001-Chroma
22046	572.52	482/504 nm	DMSO	XF23-Omega, 31001-Chroma
22066	881.7	484/501nm	DMSO	XF23-Omega, 31001-Chroma
DiI				
22035	765.55	549/565 nm	DMSO	XF32-Omega, 31002-Chroma
22044	877.76	549/565 nm	DMSO	XF32-Omega, 31002-Chroma
22050	825.21	555/570 nm	DMSO	XF32-Omega, 31002-Chroma
22052	993.53	555/570 nm	DMSO	XF32-Omega, 31002-Chroma
22101	961.32	549/565 nm	DMSO	XF32-Omega, 31002-Chroma
22102	933.87	549/565 nm	DMSO	XF32-Omega, 31002-Chroma
22103	983.48	549/565 nm	DMSO	XF32-Omega, 31002-Chroma
DiR**				
22070	1013.39	748/780 nm	DMSO	XF112-Omega, 41009-Chroma
DiS				
22073	492.44	560/571 nm	DMSO	XF32-Omega, 31002-Chroma
22076	546.53	660/675 nm	DMSO	XF47-Omega, 31023-Chroma

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** 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 TechnologyCorp. (www.chroma.com). **Fluorescence emission of this dye is invisible to human eyes and must be detected by using a CCD camera or other infrared-sensitive detector.*

Sample Protocol

1. Prepare DiO, DiI DiD, DiS 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 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 the concentrations that are at least over a tenfold 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 DiD, DiO, DiI, DiS and DiR's 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 DiO, DiI, DiD, DiS and DiR can be analyzed using the conventional FL1, FL2, FL3 and FL4 flow cytometer detection channels, respectively.

References

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2. Higashide T, Kawaguchi I, Ohkubo S, Takeda H, Sugiyama K. (2006) In vivo imaging and counting of rat retinal ganglion cells using a scanning laser ophthalmoscope. Invest Ophthalmol Vis Sci, 47, 2943.
3. Kalchenko V, Shivtiel S, Malina V, Lapid K, Haramati S, Lapidot T, Brill A, Harmelin A. (2006) Use of lipophilic near-infrared dye in whole-body optical imaging of hematopoietic cell homing. J Biomed Opt, 11, 050507.
4. Wang G, Anrather J, Glass MJ, Tarsitano MJ, Zhou P, Frys KA, Pickel VM, Iadecola C. (2006) Nox2, Ca2+, and protein kinase C play a role in angiotensin II-induced free radical production in nucleus tractus solitarius. Hypertension, 48, 482.