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Vybrant™ Cell-Labeling Solutions

Introduction

The highly lipophilic nature of the carbocyanine dyes DiI, CM-DiI, DiO and DiD has often posed an obstacle to uniform cellular labeling in aqueous culture media.[1] This technical difficulty has somewhat limited the use of these tracers in cell–cell fusion,[2,3] cellular adhesion [4,5] and migration [6] applications for which their properties of low cytotoxicity and high resistance to intercellular transfer [7] make them otherwise ideally suited. The structurally related PKH dyes have been developed and optimized for these applications.[8,9] However, PKH dye labeling protocols require suspension of cells in an iso-osmotic mannitol loading medium.[9] Molecular Probes' Vybrant™ DiI cell-labeling solution is a dye delivery solution that can be added directly to normal culture media to uniformly label suspended or attached culture cells. The complementary Vybrant DiO and DiD cell-labeling solutions allow cell populations to be marked in distinctive fluorescent colors for identification after mixing (Figure 1). Cells that have fused or formed stable clusters can be identified by double labeling (Figure 2).

Storage and Handling

Vybrant DiI, CM-DiI, DiO and DiD cell-labeling solutions are supplied in units of 1 mL. The solutions contain 1 mM DiI, DiO or DiD, and have been filtered through 0.2 µm polycarbonate filters. The DiI, CM-DiI and DiD solutions contain ethanol; the DiO solution contains dimethylformamide (DMF). Unused portions that are not required for immediate use should be stored tightly sealed and protected from light at room temperature (V-22885, V-22886, V-22887) or at -20°C (V-22888).

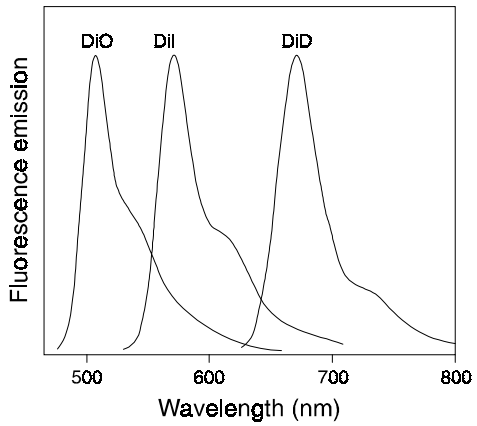

Figure 1. *Normalized fluorescence emission spectra of DiO, DiI and DiD bound to phospholipid bilayer membranes.*

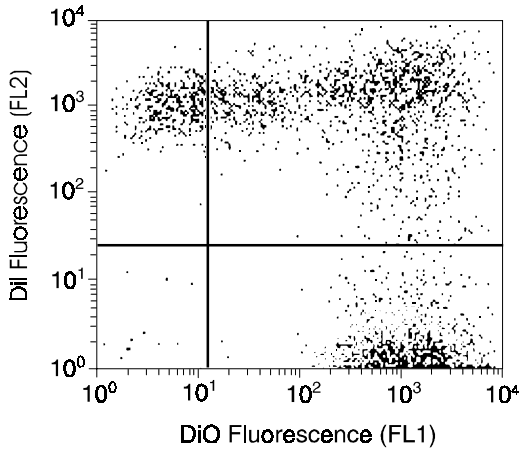

Figure 2. *Polyethylene glycol–induced fusion of Jurkat cells detected by flow cytometry. Two populations of Jurkat cells were separately labeled, one with Vybrant DiI cell-labeling solution and the other with Vybrant DiO cell-labeling solution, following the protocols described in this product information sheet. Equal portions (1 mL) of the labeled cell suspensions were combined and treated with polyethylene glycol for 45 seconds to induce fusion. The mixed cell population was analyzed by flow cytometry (Becton-Dickinson FACSVantage). Double-labeled fused cells appear in the upper right quadrant of this bivariate correlation plot.*

Experimental Protocols

Labeling of Cells in Suspension

1.1 Suspend cells at a density of 1×10^6/mL in any chosen serum-free culture medium (note **A**).

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 (note **B**). 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.

Notes

[A] Cell suspension densities >1 × 10^7/mL or <1 × 10^5/mL require much longer incubation times for uniform staining to be obtained.

[B] Uniform staining was not obtainable in our tests on certain cell types (e.g. mouse monocyte macrophages and MDCK cells).

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 (note **B**). For cell types other than those listed, start by incubating for 20 minutes and subsequently optimize as necessary to obtain uniform labeling.

Table 1. *Optimal incubation times for cell staining with Vybrant DiI cell-labeling solution.*

Cell line	Optimal incubation time (minutes) *
Jurkat (human T-cell leukemia)	2 minutes
HeLa (human cervical carcinoma)	8 minutes
P3X (mouse myeloma)	15 minutes
3T3 (mouse fibroblast)	15 minutes

* Cell suspensions (1 × 10^6/mL in DMEM or RPMI) were incubated at 37°C with Vybrant DiI cell-labeling solution (1:200 dilution). Optimal staining was qualified by flow cytometry.

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

Tracer (Catalog #)	Abs * (nm)	Em * (nm)	Optical Filters †	
			Omega	Chroma
DiI (V-22885)	549	565	XF32	31002
DiO (V-22886)	484	501	XF23	31001
DiD (V-22887)	644	665	XF47	31023
CM-DiI (V-22888)	553	570	XF32	31002

* **Abs**orption and fluorescence **Em**ission maxima determined in methanol. Values for membrane-bound tracers are similar. † Catalog numbers of bandpass 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).

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.

Detection Configurations

Microscopy

Filter sets for detection of DiI, DiO and DiD are selected based on their spectral characteristics, as summarized in Table 2. Multiband filter sets are available for simultaneous detection of multiple tracers as follows:

- DiI and DiO = Omega XF52, Chroma 51004
- DiI and DiD = Omega XF92, Chroma 51007
- DiI, DiO and DiD = Omega XF93, Chroma 61005

Omega® filters are supplied by Omega Optical, Inc. (www.omegafilters.com). Chroma filters are supplied by Chroma Technology Corp. (www. chroma.com).

Flow Cytometry

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

References

1. J Cell Biol 103, 171 (1986); **2.** J Cell Biol 135, 63 (1996); **3.** Cytometry 21, 160 (1995); **4.** J Biol Chem 273, 33354 (1998); **5.** J Cell Biol 136, 1109 (1997); **6.** Anticancer Res 18, 4181 (1998); **7.** J Immunol Methods 156, 179 (1992); **8.** Methods Cell Biol 33, 469 (1990); **9.** US Patent 4,783,401.

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
V-22888	Vybrant™ CM-DiI cell-labeling solution	1 mL
V-22885	Vybrant™ DiI cell-labeling solution	1 mL
V-22886	Vybrant™ DiO cell-labeling solution	1 mL
V-22887	Vybrant™ DiD cell-labeling solution	1 mL
V-22889	Vybrant™ Multicolor Cell-Labeling Kit *DiO, DiI, DiD solutions, 1 mL each	1 kit

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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