Vybrant™ DiI Cell-Labeling Solution

Cat. No. PA-3020

Introduction

The highly lipophilic nature of the carbocyanine dye DiI 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 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.

Instructions for Use

Storage and Handling

Vybrant DiI cell-labeling solution is supplied in units of 1 ml. The solution contains 1 mM DiI and has been filtered through 0.2 µm polycarbonate filter. The DiI solution contains ethanol.

Unused portions that are not required for immediate use should be stored tightly sealed and protected from light at room temperature.

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. 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.

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. 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.

Flow Cytometry

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

Cell line Optimal incubation time (minutes) *

Jurkat (human T-cell leukemia) 2 minutes

HeLa (human cervical carcinoma) 8 minutes

P3X (mouse myeloma) 15 minutes

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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.

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.

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