

Biomedical Technologies Inc.

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DATA SHEET DiI-LDL

Low Density Lipoprotein labeled with 1,1'-dioctadecyl-3,3',3'-tetramethyl-indocarbocyanine perchlorate

Catalog No: BT-904 **Concentration:** 200µg/ml

Quantity: 200µg /vial **Lot No:** 9040607

Absorbance Ratio: $\frac{\text{DiI}}{\text{Protein}} = \frac{555\text{nm}}{275\text{nm}} = 6.2$

Product Preparation:

Purified Low Density Lipoprotein is labeled with the fluorescent probe, DiI. DiILDL is refloated by ultracentrifugation. The resultant product is exhaustively dialyzed against 0.15M NaCl, 0.05M Tris, (pH 7.4), 0.3mM EDTA, sterilized by filtration and then aseptically packaged. Sample lots of DiI-LDL are individually evaluated for the labeling of Human Skin Fibroblasts or P-388D cells grown in lipoprotein deficient medium for 48 hours.

Storage & Stability:

DiI-LDL is stable for 3 months when kept sterile at 4°C. **NEVER FREEZE.**

References:

1. Pitas RE, et al. Arteriosclerosis 1: 177. 1983.
2. Pitas RE, et al. Arteriosclerosis 3: 1. 1983.
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5. Kingsley DM, Kreiger M. Proc. Natl. Acad. Sci. USA 81: 454. 1984.
6. Voyta JC, et al. J. Cell Biology 99: 2034. 1984.
7. Kreiger M, et al. J. Receptor Res. 3: 361. 1983.
8. Pita RE, et al. J. Cell Biology 100: 103. 1985.
9. Herman B, Albertini DF. J. Cell Biology 98: 565. 1984.
10. Stephan ZF and Yurachek EC. J. of Lipid Res. 34: 325. 1993
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FOR RESEARCH USE ONLY (Rev. 4/06)

Dil-LDL Labeling Procedures

1. In order to visualize the maximum number of LDL receptors, pre-incubate cells in medium containing 5-10% lipoprotein deficient serum or serum free medium containing 0.1-1% BSA for 24-48 hours.
2. Aseptically dilute the Dil-LDL to 10µg/ml in the preincubation media.
3. Add to live cells and incubate for five hours at 37°C.
4. Remove media containing Dil-LDL from your culture.
5. Wash cells several times with probe-free media.
6. A. Fluorescence Microscopy:

Visualize using standard rhodamine excitation: emission filters. If fixation is desired use 3% formaldehyde in PBS. (Never use methanol or acetone fixation - Dil is soluble in organic solvents). Note: A positive culture must be stained for comparison purposes.

B. Cell Sorting:

Label as in steps 1-5. Trypsinize or treat cultures with EDTA to produce a single cell suspension. Use labeled pure cultures of positive and negative cell types to set gates on the cell sorter.

Suggested Wave lengths for Cell Sorting: Excitation: 514nm
Emission: 550nm

Fixation and Mounting Dil Labeled Cells

1. Wash 3 times in PBS.
2. Fix in 3% formaldehyde/PBS for 20 minutes at room temperature.
3. Rinse 5 seconds in distilled water at room temperature.
4. Drain liquid onto chem-wipe.
5. Invert cover, slip on a drop of 90% Glycerol and 10% PBS onto a microscope slide.
6. Seal with Kroenigs wax, also known as cover glass cement (Pfaltz & Bauer, Waterbury, CT 06708). Do not use nail polish. Store at -20°C.

***Special Note:** LDL products have a natural tendency to aggregate. Aggregates of this product can interfere with its use. To clarify these aggregates out, simply spin in a microfuge for 2 minutes.