

Biomedical Technologies Inc.

378 Page Street • Stoughton, MA 02072 USA • Phone: (781) 344-9942 Fax: (781) 341-1451 **Web:** www.btiinc.com

DATA SHEET

DiO-LDL

Low Density Lipoprotein labeled with 3,3'-dioctadecyloxacarbocyanine, perchlorate

Catalog No: BT-916

Quantity: 1mg/vial

Lot No: 916K08

Absorbance Ratio: $\frac{\text{DiO}}{\text{Protein}} = \frac{507\text{nm}}{280\text{nm}} = 1.0$

Concentration: 1mg/ml

Product

Preparation: Purified Human Low Density Lipoprotein, LDL, (Catalog No. **BT-903**) is labeled with the fluorescent probe DiO. DiOLDL is reloaded by ultracentrifugation (1.019-1.063g/cc). The resultant product is exhaustively dialyzed against 0.15M NaCl, 0.05M Tris, pH 7.4, 0.3mM EDTA, sterilized by membrane filtration and then aseptically packaged. Sample lots of DiO-LDL are evaluated for the labeling of human skin fibroblasts or P-388D cells grown in Lipoprotein Deficient Serum (Catalog No. **BT-907**) and medium for 48 hours.

Storage & Stability:

DiO-LDL is stable for 5 months when kept sterile at 4°C. **NEVER FREEZE.**

Reference: 1) Tabas, I, et al. J.Cell Biology 111:929-940 (1990).

FOR RESEARCH USE ONLY (rev.4/06)

DiO-LDL Labeling Procedures

1. In order to visualize the maximum number of LDL receptors, preincubate 50-55% confluent 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 DiO-LDL to 10ug/ml in the preincubation media.
3. Add to live cells and incubate for five hours at 37°C.
4. Remove media containing DiO-LDL from your culture.
5. Wash cells several times with probe-free media.
6. **A. Fluorescence Microscopy:**

Visualize using standard fluorescein excitation: emission filters. If fixation is desired, use 3% formaldehyde in PBS. (Never use methanol or acetone fixation - DiO 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: 484nm
Emission: 507nm

C. Fixation and Mounting DiO 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.

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