# **Product Bulletin**



### **DATA SHEET**

#### DiO-LDL

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

Catalog No: J64267 (BT-916)

**Quantity:** 200ug/vial

Concentration: 200ug/ml

Absorbance Ratio: Dil = 507nm =

Protein 280nm

**Preparation:** Purified Low Density Lipoprotein is labeled with the fluorescent probe, DiO. DiO-LDL is

refloated 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 filtration and then aseptically packaged. Sample lots have been evaluated for the specific labeling of bovine aortic endothelium, HUVECS, murine macrophages and the current lot was

evaluated on P-388D cells to assure consistent results.

Storage &

Stability: DiO-Ac-LDL is stable 3 months when kept sterile at 4°C. **NEVER FREEZE**.

**NOTE:** BT also offers ECGS, Endothelial Mitogen (Cat. No. J64516, (BT-203)), which when

added as a media supplement, improves the viability of cultured (EC) endothelial cells (HUVEC, etc.). EC attachment may be enhanced by coating cultureware with Human Fibronectin (Cat. No. J64560,(BT-225)) or Bovine Fibronectin (Cat.No. J62380,

(BT-226)).

## FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS OR FOR IN-VITRO DIAGNOSTIC USE

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## **DiO-Ac-LDL** Labeling Procedures

- 1. Aseptically dilute the DilAcLDL to 10µg/ml in the preincubation media.
- Add to live cells and incubate for 4 hours at 37°C.
- 3. Remove media containing DiO-Ac-LDL from your culture.
- 4. Wash cells several times with probe-free media.

### A. Fluorescence Microscopy:

Visualize using standard FITC 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 1-4. 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. Negative cell types include human skin fibroblasts and bovine/smooth muscle cells.

Suggested Wave lengths for Cell Sorting: Excitation: 484nm

Emission: 507nm

## **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 bees wax. 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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