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

Catalog No: J65330 (BT-904)

Quantity: 200μg/vial

Concentration: 200μg/ml

Absorbance Ratio: \[
\text{Dil} = \frac{555\text{nm}}{275\text{nm}}
\]

Preparation: Purified Low Density Lipoprotein is labeled with the fluorescent probe, Dil. DilLDL 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 of Dil-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: Dil-LDL is stable at least three months when kept sterile at 4°C. NEVER FREEZE.

*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.

References:
10. Stephan ZF and Yurachek EC. J.of Lipid Res. 34: 325. 1993

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR FOR IN-VITRO DIAGNOSTIC USE
**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 10⁻⁹ g/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:**
   
<table>
<thead>
<tr>
<th>Excitation</th>
<th>Emission</th>
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<tbody>
<tr>
<td>514nm</td>
<td>550nm</td>
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**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 bees wax. Do not use nail polish. Store at -20°C.