Fixation and Mounting of DiI 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.

DiI-LDL

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

Catalog No: L8930
Quantity: 500ug/vial
Concentration: **2. 0mg/ml (Protein)**

Absorbance Ratio: $\frac{\text{DiI}}{\text{Protein}} = \frac{555\text{nm}}{275\text{nm}} = 1.22$

Storage & Stability:

DiI -LDL should be kept sterile at 2-8°C. ***NEVER FREEZE***. The stability of this product is in the 6 week range after receipt. Clarify by centrifugation if needed.

Product Preparation:

Purified LDL is labeled with the fluorescent probe, DiI, and reisolated by ultracentrifugation (1.019-1.063). The resultant product is exhaustively dialyzed against phosphate buffered saline, (pH 7.4), sterilized by membrane filtration and then aseptically packaged in a solution containing phosphate-buffered saline at pH 7.4 and 0.02 mM EDTA. Each lot is evaluated on a murine macrophage cell line for fluorescence uptake.

Typical Lipoprotein Labeling Protocol

1. Aseptically dilute the DiI-LDL to 20-40μg/ml in your culture media.

2. Add to live cells and incubate for 4-5 hours at 37°C.

3. Remove media containing DiI-LDL from your culture.

4. Wash cells several times with probe-free media.

5. A. Fluorescence Microscopy:
   Visualize using standard rhodamine excitation: emission filters (or suggested wavelengths excitation:emission at 549nm:565nm). If fixation is desired use 3% formaldehyde in PBS. (Never use methanol or acetone fixation - DiI 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 Wavelengths for Cell Sorting: Excitation: 514/549nm
   Emission: 565nm