DII-VLDL

Background:	Purified LDL is labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, and then isolated by ultracentrifugation. 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 0.2 mM EDTA. Each lot is evaluated on a murine macrophage cell line for fluorescence uptake.
Storage & Stability:	DiI-VLDL is stable for 6 weeks after receipt when handled aseptically and stored at 2-8ºC (**Don't Freeze**). Note: After prolonged storage, some precipitate may be observed. This is normal for the product. Spin in centrifugation at 5000×g for 10 minutes before using.
Tested Applications:	DiI-VLDL are evaluated for receptor binding to peritoneal macrophages in conjunction with VLDL and Ox-VLDL.

Typical Lipoprotein Labeling Protocol

1. Aseptically dilute the DiI-VLDL to 10-50 µg /ml in your culture media.
2. Add to live cells and incubate for 2-5 hours at 37ºC.
3. Remove media containing DiI-VLDL from your culture.
4. Wash cells several times with probe-free media.

    1. Fluorescence Microscopy:

Visualize using standard rhodamine excitation: emission filters (or suggested wavelengths

excitation:emission at 554nm:571nm or near). 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.

1. 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: 554 nm
Emission: 571 nm

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.