

## DII-VLDL

<b>Background:</b>	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.
<b>Storage &amp; Stability:</b>	Dil-VLDL is stable for 6 weeks after receipt when handled aseptically and stored at 2-8°C ( <b>Don't Freeze</b> ). 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.
<b>Tested Applications:</b>	Dil-VLDL are evaluated for receptor binding to peritoneal macrophages in conjunction with VLDL and Ox-VLDL.

## Typical Lipoprotein Labeling Protocol

1. Aseptically dilute the Dil-VLDL to 10-50 µg /ml in your culture media.
2. Add to live cells and incubate for 2-5 hours at 37°C.
3. Remove media containing Dil-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 - Dil is soluble in organic solvents). Note: A

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positive culture must be stained for comparison purposes.

## 2. 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 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 Kroenigs wax, also known as cover glass cement (Pfaltz & Bauer, Waterbury, CT 06708). Do not use nail polish. Store at  $-20^{\circ}\text{C}$ .