

## **DiI-LABELED OXIDIZED LOW DENSITY LIPOPROTEIN, HUMAN**

<b><u>Catalog No:</u></b>	<b>GDL008</b>
<b><u>Lot No:</u></b>	<b>lot specific</b>
<b><u>Quantity:</u></b>	<b>500ug(micrograms)Protein/vial</b>
<b><u>Concentration:</u></b>	<b>lot specific (mg/ml Protein)</b>

### **Introduction**

GenXion Biotechnologies DiI-Ox-LDL, oxidized Low Density Lipoprotein, labeled with 1,1'-dioctadecyl – 3,3,3',3'-tetramethyl-indocarbocyanine perchlorate, labels both vascular endothelial cells and macrophages. It can be used to identify and/or isolate these cells from mixed cell populations and investigate uptake of modified LDL by different cell types. When cells are labeled with DiI-Ox-LDL, the lipoprotein is degraded by lysosomal enzymes and the DiI (fluorescent probe) accumulates in the intracellular membranes. Labeling cells with DiI-Ox-LDL has no effect on cell viability. Pure cultures of vascular endothelial cells can be isolated from complex primary cultures using fluorescent activated cell sorting based on their increased metabolism of the DiI-Ox-LDL. Contaminating cell types (fibroblasts, smooth muscle, pericytes, epithelial cells) are not labeled. Macrophages can be differentiated from mixed cell populations (including endothelial cells) because they are more brightly labeled. Labeling endothelial cells with DiI-Ox-LDL has many advantages over labeling other endothelial cell associated antigens. The labeling procedure is one step, and once the cells are labeled, the fluorescent probe (DiI) is not removed by Trypsin. Both low density and confluent cultures of vascular endothelial cells are effectively labeled. No other cell type (other than macrophages) is labeled to the same level as vascular endothelial cells. Each lot of DiI-Ox-LDL is evaluated for the specific labeling of bovine aortic endothelial cells and murine macrophages to assure consistent results. A complete labeling protocol is included with each shipment.

### **Storage & Stability:**

This product is stable for **6 weeks** after receipt when handled aseptically and stored at 2-8°C. ***PROTECT FROM LIGHT AND NEVER FREEZE.***

***\*Special Note:*** *After prolonged storage, some precipitate may be observed. This is normal for this product. Clarify out the aggregates by spinning in a microfuge for 10 minutes over 5000g.*

***\*Preparations of DiI-Ox-LDL are fairly unstable; plan your experiments in advance and use fresh material. Before using, spin the vial in a microfuge for 3 minutes over 1500\*g.***

## Typical Lipoprotein Labeling Protocol

1. Aseptically dilute the Dil-OxLDL to 20-40 $\mu$ g/ml in your culture media.
2. Add to live cells and incubate for 3-6 hours at 37°C.
3. Remove media containing Dil-OxLDL 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 554nm:571nm or near).  
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: 554nm  
Emission: 571nm

**Special Note:** All our 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.

**NOTE: FOR RESEARCH USE ONLY**

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