

DII-AC-LDL

Name:	Dil-Ac-LDL
Cat. #:	10458
Size:	0.5 mg
Purity:	98% (Co-migrates with reference on agarose gel electrophoresis)
Concentration:	Minimum 1.2 mg/ml protein
Description:	<p>Human Dil-Labeled Acetylated Low Density Lipoprotein</p> <p>Dil-Ac-LDL, Acetylated 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. When cells are labeled with Dil-Ac-LDL, the lipoprotein is degraded by lysosomal enzymes and the Dil (fluorescent probe) accumulates in the intracellular membranes. Labeling cells with Dil-Ac-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 Dil-Ac-LDL. Contaminating cell types (fibroblasts, smooth muscle, pericytes, epithelial cells) are not labeled. Macrophages can be differentiated from mixed cell populations</p>
Background:	<p>(including endothelial cells) because they are more brightly labeled.</p> <p>Labeling endothelial cells with Dil-Ac-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 (Dil) 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 Dil-Ac-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. We also offer an "FITC-like" label DiO-Ac-LDL, which is useful for fixed wavelength FACS Cell sorters.</p>

Storage & Stability:

The labelled LDL is stable for 6 weeks after receipt when handled aseptically and stored at 2–8°C (**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.

Packaging:

The labelled LDL requires one week lead time. Please plan your experiments in advance and use the fresh material.

Typical Lipoprotein Labeling Protocol

1. Dilute Dil-Ac-LDL to 10–50 ug/ml in growth media.
2. Add to cells and incubate for 2–6 hours at 37°C.
3. Remove media.
4. Wash 3 times with probe-free media.
5. Visualize via Fluorescence Microscopy and/or trypsinize (or EDTA) for cell sorting.

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 positive culture must be stained for comparison purposes.

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