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DiI-Labeled Acetylated Low Density Lipoprotein, Human

Cat No: H7970

Size: 500ug(micrograms)Protein/Vial

Concentration: >2mg/ml protein（Based on actual label concentration）.

Purity: 98%, is determined agarose gel electrophoresis.

Specifications: 0.22 micron membrane filtered, aseptically filled. Cell Culture Tested.

Storage:

This product is stable for 6 weeks after receipt when handled aseptically and stored at 2-8°C (Do not freeze). After prolonged storage, some precipitate may be observed. This is normal for this product. Clarify out the aggregates by spinning in centrifuge tube for 2 minutes.

Introduction

DiI-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 isolate these cells from mixed cell populations. When cells are labeled with DiI-Ac-LDL, the lipoprotein is degraded by lysosomal enzymes and the DiI (fluorescent probe) accumulates in the intracellular membranes. Labeling cells with DiI-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 DiI-Ac-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-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 (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-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.

Protocol

1. Dilute DiI-Ac-LDL to 20-50ug/ml in growth media.
2. Add to cells and incubate for 4 hours at 37ºC.
3. Remove media.
4. Wash cells several times with probe-free media.

5. Visualize via Fluorescence Microscopy and/or trypsinize (or EDTA) for cell sorting.

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. (Do not 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.

Special note:

1. Do not freeze.
2. Preparations of DiI-Ac-LDL are fairly unstable, prepare your experiments in advance and use fresh material.
3. For research use only.