DiI-High Density Lipoprotein DiI 标记高密度脂蛋白

High Density Lipoprotein labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate.

Cat No: IL2170

Size: 500μg/vial

Concentration: Based on actual label concentration. Buffer containing phosphate-buffered saline at pH 7.4 and 0.01 mM EDTA.

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

Absorbance Ratio: DiI/Protein=484nm/275nm=1.21

Storage:

This product is stable for 6 weeks after receipt when handled aseptically and stored at 2-8°C (Do not free). HDL products have a natural tendency to aggregate. Aggregates of this product can interfere with its use. To clarify these aggregates out, simply spin in centrifuge tube for 2 minutes.

Product Preparation:

Purified HDL is labeled with the fluorescent probe, DiI, and reisolated by ultracentrifugation (1.063-1.21g/mL). 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 μM EDTA. Each lot is evaluated on a murine macrophage cell line for fluorescence uptake.

Typical Lipoprotein Labeling Protocol

1. Aseptically dilute the DiI-HDL to 20-40 μg/mL in growth media.
2. Add to live cells and incubate for 4-5 hours at 37°C.
3. Remove media containing DiI-HDL from your culture.
4. Wash cells several times with probe-free media.

A. Fluorescence Microscopy:

Visualize using standard rhodamine excitation: emission filters (or suggested wavelengths excitation: emission at 484nm:501nm). 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 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: 484nm. Emission: 501nm.

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.

Special note:

1. Do not freeze.
2. 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.
3. For research use only.