Yiyuan Biotechnologies. *Guangzhou • China*

•Phone: 020-38882231 •Mobile:13631373865 email. yuanyuanbiotech@126.com

DATA SHEET

DiI-HDL

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

Catalog No: YB-0017 **Concentration**: **>2.0mg/ml** (Corrected by Cholesterol in Lipoprotein, HDL-C:4.88mmol/L, Protein assayed by BCA:7.60mg/ml)

Quantity: >2.0mg(1ml)/vial **Lot No: lot specific**

Absorbance Ratio: $\frac{\text{DiI}}{\text{Protein}} = \frac{555\text{nm}}{275\text{nm}} = 1.82$

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, sterilized by membrane filtration and then aseptically packaged under nitrogen in a solution containing phosphate-buffered saline at pH 7.4 and 0.2 mM EDTA-Na_2.

Storage & Stability:

DiI-HDL should be kept sterile at 2-8°C. ***NEVER FREEZE***. The stability of this product is in the 6 week range after receipt. Clarify by centrifugation if needed.

Typical Lipoprotein Labeling Protocol

1. Aseptically dilute the DiI-HDL to 10-40μg/ml in your culture media.

2. Add to live cells and incubate for 4-6 hours at 37°C.

3. Remove media containing DiI-HDL 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). If fixation is desired use 3% formaldehyde in PBS. (Never 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: 554nm
   Emission: 571nm

Fixation and Mounting of DiI Labeled Cells

1. Wash 3 times in PBS.

2. Fix in 4% 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**: 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 a microfuge for 2 minutes.

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