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HDL Uptake Assay Kit (Fluorometric)

11/14

(Catalog # K586-100; 100 assays; Store at -20°C)

I. Introduction:

High-density lipoprotein (HDL) consists of a protein shell, containing apolipoproteins A-I and A-II, and a hydrophobic core consisting of cholesterol and triglycerides surrounded by phospholipids and cholesterol esters. HDL transports cholesterol to the liver or steroidogenic organs such as adrenals, ovary, and testes by both direct and indirect pathways. HDL-Cholesterol is removed by HDL receptors such as scavenger receptor BI (SR-BI), which mediate the selective uptake of cholesterol from HDL. Clinically, high levels of HDL has been associated with cardiovascular health. This is due to the ability of HDL particle to transport cholesterol from lipid-laden macrophages of atherosclerotic arteries to the liver for secretion into the bile by a process called as reverse cholesterol transport. BioVision's HDL Uptake Assay Kit contains a fluorescently-labeled HDL that can be measured when taken up by cells. Unlabeled-HDL is included in the kit for assay validation.

II. Application:

- Measure HDL uptake in mammalian cells
- Screen, study or characterize stimulators or inhibitors of HDL uptake

III. Kit Contents:

Components	K586-100	Cap Code	Part Number
Assay Buffer	100 ml	NM	K586-100-1
Fluorescently-labeled HDL (5 mg/ml)	1 ml	Green	K586-100-2
Unlabeled HDL (2X)	0.1 ml	Yellow	K586-100-3

IV. User Supplied Reagents and Equipment:

- 96-well white clear-bottom cell culture plate
- Multi-well fluorometer (fluorescence ELISA reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Upon opening, use within two months.

- **Assay Buffer:** Warm to room temperature before use. Assay Buffer can be stored at 4°C.
- **Fluorescently-labeled HDL and Unlabeled HDL:** Thaw on ice. Both Fluorescently-labeled and Unlabeled HDL can be stored at 4°C for 1 week. For long term storage, aliquot and store at -20°C. Keep protected from light and on ice while in use.

VI. HDL Uptake Assay Protocol:

1. **Sample Preparation:** Plate 1-2 x 10^4 cells*/well in a 96-well white clear-bottom cell culture plate and culture in desired media with serum overnight. Remove media and wash 3 times with 100 µl Assay Buffer. For each well, prepare 100 µl of diluted Fluorescently-labeled HDL by adding 2 µl (~10 µg) of Fluorescently-labeled HDL to 98 µl of serum-free media. Make as much as needed depending on the number of wells. Avoid light exposure. Add 100 µl of diluted Fluorescently-labeled HDL to desired wells. For background control (BC) well, add 100 µl serum-free media. To check the specificity of HDL uptake, compete out the signal by adding 10 µl of Unlabeled HDL to the diluted Fluorescently-labeled HDL in one of the wells. Incubate at 37°C for 2-24 hrs or as desired. Remove media and carefully wash 4 times with 100 µl Assay Buffer. After washing, add 100 µl Assay Buffer to each well.

   Notes:

   a. Optional: To increase the HDL uptake, cells can be starved in serum-free media for 4-16 hrs after overnight cell culture in media with serum.

   b. Concentration of the Fluorescently-labeled HDL may vary for different cell types. We recommend using 2 µl-10 µl (~10 µg-50 µg) of Fluorescently-labeled HDL depending on the cell type.

   c. To determine the non-specific Fluorescently-labeled HDL binding to the plate, perform a wash off step. Incubate cells with diluted Fluorescently-labeled HDL for 2 min. Remove media containing Fluorescently-labeled HDL and wash 4 times with 100 µl of Assay Buffer. Add 100 µl of Assay Buffer and measure.

   d. * For the suspension cells, centrifuge at 1,000 X g for 5 min. in a 96-well plate centrifuge before every media/buffer exchange.

   e. Stimulants and Inhibitors of HDL uptake can be preincubated with the cells 1-2 hrs before and during treatment with Fluorescently-labeled HDL.

2. **Standard Curve:** Make serial dilutions of the Fluorescently-labeled HDL in Assay Buffer. Dilute Fluorescently-labeled HDL 1:100 in Assay Buffer. Take 10 µl of diluted Fluorescently-labeled HDL into 190 µl of Assay Buffer in one of the wells (label as W8) of 96 well plate. Label next seven wells as W7 – W1. Aliquot 100 µl of Assay Buffer into each well (W7-W1). Add 100 µl from W8 into W7 and mix. Transfer 100 µl from W7 into W6 and mix, repeat for W6 – W2. W1 well (0 ng) is control well. The concentration of Fluorescently-labeled HDL in wells 1 through 8 will be 0, 3.9, 7.8, 15.6, 31.2, 62.5, 125, and 250 ng/well.
3. **Measurement:** Protect from light. Measure fluorescence (Ex/Em = 540/575 nm).
4. **Calculation:** Subtract 0 Standard reading from all the Standard Curve reading. Plot the Standard Curve of Fluorescently-labeled HDL Vs RFUs. Subtract sample background control reading from sample reading. Apply sample's corrected RFU to Standard Curve to get B ng of Fluorescently-labeled HDL uptake. Calculate HDL uptake using following equation:

Sample HDL Uptake (A) = B/P = ng/mg protein

Where: **B** is amount of Fluorescently-labeled HDL from Standard Curve (ng)
P is protein concentration (mg)

Notes:

a. HDL uptake can also be expressed as ng/number of cells.

b. To measure protein concentration, lyse cells in 100 µl of Cell Lysis Buffer (Cat. # 1067 or equivalent) and measure protein concentration using BCA Protein Assay Kit II (Cat. # K813 or equivalent).

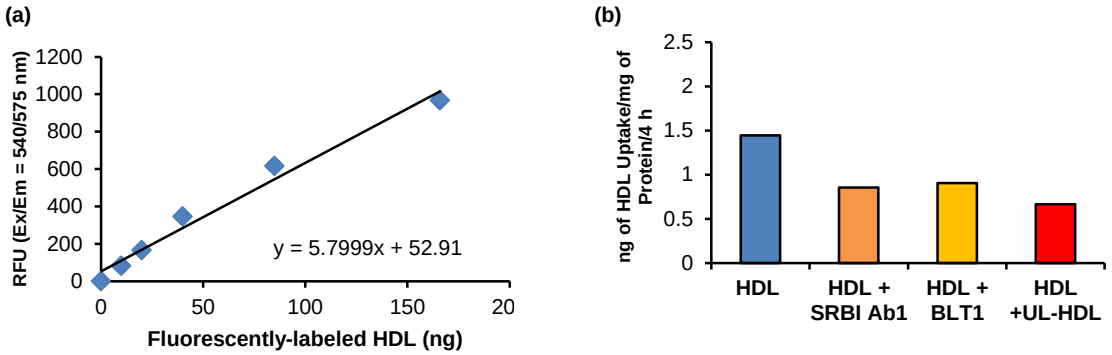

Figure: (a) Fluorescently-labeled HDL Standard curve. (b) Fluorescently-labeled HDL uptake in HepG2 (hepatocytes) cells. Cells were starved for 4 hrs and incubated with Fluorescently-labeled HDL (10 µg) for 4 hrs. Fluorescently-labeled HDL (HDL) uptake was inhibited by SR-BI (HDL receptor) antibody (HDL + SRB1 Ab1), BLT1 (SR-BI Inhibitor; HDL + BLT1) and outcompeted by Unlabeled-HDL (HDL+ UL-HDL [20 µg]). Background was subtracted from the data (Wash off as described in note 1c, was subtracted as background). Assay was performed following the kit protocol.

VII. RELATED PRODUCTS:

CETP Activity Fluorometric Assay Kit II (K595)
PLTP Activity Fluorometric Assay Kit II (K593)
Lipid (Oil Red O) Staining Kit (K580)
PCSK9 (mouse) ELISA Kit (K7266)
Free Fatty Acid Quantification Kit (K612)
Hepatic Steatosis Assay Kit (K584)
BCA Protein Assay Kit II (K813)
Lipoproteins, Human Plasma, Low Density (4931)
Lipoproteins, Human Plasma, Intermediate (4932)
HDLR Antibody (3839)
Human CellExp™ PCSK9, human recombinant (7265)
CETP Inhibitor Screening Kit (Fluorometric) (K594)
PLTP Inhibitor Drug Screening Kit (Fluorometric) (K605)
PCSK9 (human) ELISA Kit (K7265)
Cholesterol Efflux Fluorometric Assay Kit (Cell-based) (K582)
HDL and LDL/VLDL Quantification Kit (K613)
Cell Lysis Buffer (1067)
PCSK9 Antibody (5112)
Lipoproteins, Human Plasma, Very Low Density (4933)
Lipoproteins, Human Plasma, High Density (4934)
HDLR Blocking Peptide (3839BP)
Human CellExp™ HDLR, human recombinant (7472)

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