ab287862 – LDL Uptake Assay Kit Cell-Based

For the quantitative measurement of LDL uptake.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287862

Storage and Stability

Store kit at -20°C and protect from light.

Materials Supplied

Item	Quantity	Storage Condition
Uptake Assay Buffer	50 ml	-20°C
Fluorophore-Labeled LDL	250 µl	-20°C
Unlabeled LDL	100 µl	-20°C
96 Well Poly-D-Lysine Cell Culture Plate	1 unit	-20°C

PLEASE NOTE: 96 Well Poly-D-Lysine Cell Culture Plate was previously labelled as Polylysine-Coated Cell Culture Plate.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Cell line for testing, appropriate cell culture medium and 5% CO2 cell culture incubator
- Multiwell fluorescence microplate reader (capable of bottom read)
- Optional: Lipoprotein-depleted serum for cholesterol-starvation

Reagent Preparation

- Briefly centrifuge all small vials prior to opening.
- Open all of the reagents under sterile conditions (e.g., a cell culture hood) only.
- Read entire protocol before performing the assay procedure.

Uptake Assay Buffer: Allow to thaw to room temperature and open under sterile conditions. Store at -20°C.

Fluorophore-Labeled LDL: Thaw at 4°C and open under sterile conditions, protected from light. Once thawed, both Fluorophore-Labeled and Unlabeled LDL may be stored at 4°C for 1 week, or aliquoted and stored at -20°C. If aliquoted and stored at -20°C, avoid additional freeze/thaw cycles.

96 Well Poly-D-Lysine Cell Culture Plate: Open and use under sterile conditions. Store at room temperature or -20°C.

Assay Protocol

- The procedure described below employs HepG2 cells (hepatocytes) as a model cell line for measuring LDL acid uptake. Other adherent cell lines known to import LDL may also be used if desired.

Cell Seeding and Cholesterol Starvation:

1. Seed approximately 3-4 x 10^4 cells/well in provided 96 Well Poly-D-Lysine Cell Culture Plate using 200 µl appropriate culture medium with serum per well. Incubate overnight in a 5% CO2 atmosphere 37°C incubator to allow cells to adhere (adherent cell monolayer should be ≈90% confluent for optimal assay).

Δ Note: *To ensure accuracy, we recommend that each treatment condition (including controls) be performed in duplicate or triplicate wells.*

Δ Note: *When planning the assay and plating cells, seed at least three additional wells (or more if performing duplicates/triplicates) for assay validation. These wells will be used for background, non-specific LDL binding and uptake competition controls.*

2. Prior to performing the assay, starve cells of exogenous cholesterol by incubating in serum-free or lipoprotein-deficient medium: gently aspirate growth medium from wells and replace with 200 µl serum- or lipoprotein-free culture medium. Incubate cholesterol-starved cells for 4-8 hours or overnight, depending upon cell type or established assay conditions (see note below).

Δ Note: *Overnight incubation in culture medium containing lipoprotein-deficient serum (≤ 2.5%) instead of standard serum may be used for cell lines that do not tolerate prolonged serum starvation.*

Δ Note: *If desired, test compounds (i.e., stimulants or inhibitors of LDL uptake) may be added during the cholesterol-starvation incubation period. If an organic solvent is used to dissolve test compound, we recommend also performing a vehicle condition (with the same final concentration of solvent) in order to control for possible effects of the solvent.*

Uptake Assay Reaction Preparation:

1. Warm Uptake Assay Buffer to 37°C. Following cholesterol starvation, remove microplate from incubator, gently aspirate culture medium, wash the cells once with 100 µl Uptake Assay Buffer to ensure complete removal of medium and add 100 µl fresh Uptake Assay Buffer to each well.
2. For each well, prepare 10 µl of a 1 mg/ml working solution of Fluorophore-Labeled LDL by diluting the 5 mg/ml stock solution in Uptake Assay Buffer at a 1:5 ratio. Make as much as needed depending on the number of wells to be assayed, including control condition wells for validating the specificity of labeled LDL uptake (an "uptake competition" control, in which an excess of Unlabeled LDL is used to compete out the Fluorophore-Labeled LDL uptake) and assessing non-specific binding (hydrophobic binding in the absence of uptake) of Fluorophore-Labeled LDL to cells or microplate well plastic (a "wash off" control) (see step 3).
3. Add 10 µl of diluted Fluorophore-Labeled LDL solution to each test well. For background control well(s) (cells only), add 10 µl Uptake Assay Buffer. For uptake competition control well(s), add 10 µl of Unlabeled LDL and 10 µl of diluted Fluorophore-Labeled LDL solution. To perform a Wash Off control for non-specific labeled LDL binding, add 10 µl Fluorophore-Labeled LDL to cells with diluted Fluorophore-Labeled LDL for only 2 min. Aspirate buffer containing Fluorophore-

Labeled LDL, wash with 100 µl of Uptake Assay Buffer and add 100 µl of fresh Uptake Assay Buffer.

4. Return microplate to 37°C incubator and incubate, protected from light, for 2-4 hours (or desired time depending upon established assay conditions). Following uptake incubation period, aspirate Fluorophore-Labeled LDL containing buffer, wash with 100 µl of Uptake Assay Buffer and add 100 µl fresh Uptake Assay Buffer to each well.

Standard Curve Preparation:

- Dilute the Fluorophore-Labeled LDL (5 mg/ml) stock solution at a 1:50 ratio by adding 5 µl of the stock to 245 µl Uptake Assay Buffer to obtain a 0.1 mg/ml working solution.
- Add 0, 2, 4, 6, 8 and 10 µl of the working solution into a series of wells in the provided Cell Culture Plate and adjust the volume of each well to 100 µl with Uptake Assay Buffer, yielding a standard curve of 0, 0.2, 0.4, 0.6, 0.8 and 1 µg/well Fluorophore-Labeled LDL.

Measurement

Measure the fluorescence (Ex/Em = 488/523 nm) of all wells (including standard curve, background, uptake competition and "wash off" control wells) in endpoint mode min using the 'bottom read' function.

Calculation:

- For the Fluorophore-Labeled LDL standard curve, subtract the zero standard (0 µg/well reagent blank) reading from all standard readings, plot the background-subtracted values and calculate the slope of the standard curve. For sample wells, subtract the background control (cells only) well RFU value from the sample reading (F = RFUSample – RFUBC) and apply the background-subtracted fluorescence (F) to the standard curve to get B µg of LDL taken up over the course of the incubation period.

$$\text{Sample LDL Uptake} = \boldsymbol{B}/(\boldsymbol{N}\times\boldsymbol{T}) = \mu g/10^4 \text{ cells/hr.}$$

- Where:
  - **B** is the amount of LDL, calculated from the standard curve (in µg)
  - **N** is the number of cells added to the well (N×10^4 cells)
  - **T** is the incubation time after addition of LDL (in hours)

Δ Note: LDL uptake can also be expressed in terms of protein per well (µg LDL/mg protein/hr). To measure protein concentration, lyse cells in 100 µl of cell lysis buffer and measure protein using a BCA protein assay or equivalent.

Technical Support

For all technical or commercial enquiries please go to:

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