# LDL Uptake Assay Kit (Cell-Based)



**(Catalog # K436-100; 100 Reactions; Store at -20°C)**

## I. Introduction:

Low-density lipoprotein (LDL) is a large spherical macromolecule that serves as a 'shuttle' to transport hydrophobic molecules (lipids and cholesterol) throughout the body via the aqueous environment of the bloodstream. An LDL particle consists of a hydrophobic core of cholesterol esters (with long-chain fatty acids linked to the hydroxyl group) and triglycerides surrounded by an amphipathic shell containing the 510 kDa protein Apolipoprotein B-100, various phospholipids and non-esterified cholesterol molecules. LDL is taken up by cells such as hepatocytes, macrophages, and endothelial cells by specific receptor-mediated endocytosis. High levels of LDL have been associated with arterial inflammation, which leads to cardiovascular diseases (CVD) like atherosclerosis, ischemic stroke and myocardial infarction. LDL particles can migrate through inflamed vascular endothelial tight junctions, where they become oxidized and attract circulating monocytes. Monocytes differentiate into activated macrophages, which further accumulate LDL and become foam cells, leading to arterial wall thickening/scaring and formation of fibrous fatty plaques. Current CVD preventative therapies include decreasing LDL with statins, which prevent endogenous cholesterol biosynthesis by inhibiting HMG-CoA reductase. However, drugs that inhibit LDL uptake by macrophages or enhance LDL clearance by increasing hepatic LDL reuptake are also being investigated. BioVision's LDL Uptake Assay Kit enables measurement of receptor-mediated LDL uptake by various cell types. The kit contains fluorescent human LDL, which is covalently-labeled with a bright, highly photostable fluorophore that can be detected when taken up by cells (Ex/Em = 488/523 nm). Unlabeled human LDL is included in the kit for validation of specific labeled LDL uptake. The kit contains a complete set of reagents sufficient for performing 100 reactions and a 96-well polylysine-coated black cell culture plate with a thin, microscopy-grade clear bottom.

## II. Applications:

- Characterization of LDL uptake in different cell types.
- Screening and characterization of drugs or treatment conditions that inhibit or stimulate LDL uptake.

## III. Sample Type:

- Adherent cell line known to uptake LDL (*e.g.* HepG2 or Huh-7 cells)

## IV. Kit Contents:

| Components | K436-100 | Cap Code | Part Number |
|---|---|---|---|
| Uptake Assay Buffer | 50 ml | NM | K436-100-1 |
| Fluorophore-Labeled LDL (5 mg/ml) | 250 µl | Red | K436-100-2 |
| Unlabeled LDL | 100 µl | White | K436-100-3 |
| Polylysine-Coated Cell Culture Plate | 1 plate | N/A | K436-100-4 |

## V. User Supplied Reagents and Equipment:

- Cell line for testing, appropriate cell culture medium and 5% $CO_2$ cell culture incubator
- Multiwell fluorescence microplate reader (capable of bottom read)
- **Optional:** Lipoprotein-depleted serum for cholesterol-starvation

## VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. 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 4°C.
- **Fluorophore-Labeled LDL (5 mg/ml)** and **Unlabeled 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.
- **Polylysine-Coated Cell Culture Plate:** Open and use under sterile conditions. Store at room temperature or 4°C.

## VII. LDL Uptake 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.

### 1. Cell Seeding and Cholesterol Starvation:

**a.** Seed approximately 3-4 x $10^4$ cells/well in provided Polylysine-Coated Cell Culture Plate using 200 µl appropriate culture medium with serum per well. Incubate overnight in a 5% $CO_2$ atmosphere 37°C incubator to allow cells to adhere (adherent cell monolayer should be ≈90% confluent for optimal assay).

**Notes:**

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

**b.** 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).

**Notes:**

- 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.
- 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.

**2. Uptake Assay Reaction Preparation:**

**a.** 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.

**b.** 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 competitition" 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 c).

**c.** 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.

**d.** 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.

**3. 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.

**4. 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.**

**4. 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 ($F = RFU_{Sample} - RFU_{BC}$) 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.

$$\textbf{Sample LDL Uptake} = \frac{B}{N \times T} = \textbf{µg/10}^4\textbf{ cells/hr}$$

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

**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 (Cat. #1067 or equivalent) and measure protein using BCA Protein Assay (Cat. #K813) or equivalent.

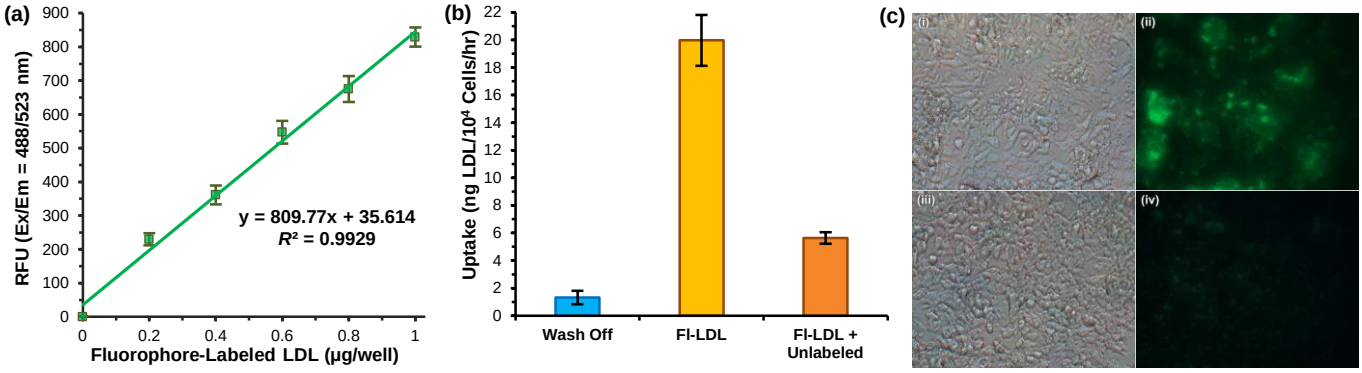


**Figure: (a)** Fluorophore-Labeled LDL Standard curve. **(b)** Labeled LDL uptake in HepG2 cells. Cells were cholesterol-starved overnight in culture medium containing 1% lipoprotein-deficient serum, then incubated with Fluorophore-Labeled LDL (10 µg/well) for 4 hours. Specificity of labeled (Fl-LDL) uptake was determined by competition with excess unlabeled LDL (Fl-LDL + Unlabeled). To account for any potential non-specific binding of labeled LDL (in the absence of functional uptake), cells were incubated with Fluorophore-Labeled LDL (10 µg/well) for 2 min, followed by aspiration and rinsing with Assay Buffer (Wash Off). Uptake data are presented as mean LDL mass/hour per $10^4$ cells ± SD, with each data point representing the mean of ≥5 wells. **(c)** Fluorescence imaging showing the extent of fluorescent LDL uptake in HepG2 cells after 4 hours. **(i-ii)** phase contrast and fluorescence images of cells treated with labeled LDL (10 µg), **(iii-iv)** phase contrast and fluorescence images of cells treated with Fluorophore-Labeled LDL (10 µg) and an excess of unlabeled LDL to outcompete labeled uptake by saturating LDL receptors. Cells were grown on the included polylysine-coated 96-well plate; fluorescence images were obtained with a Nikon TE2000 inverted microscope using a 20X Plan Fluor objective and a FITC filter cube.

## VIII. RELATED PRODUCTS:

Total Cholesterol/Cholesteryl Ester Assay Kit (K603)
HDL and LDL/VLDL Quantification Kit (K613)
Cholesterol Detection Kit (Cell-based) (K587)
Cholesterol Efflux Assay Kit (Cell-based) (K582)
PLTP Activity Assay Kit II (K593)
CETP Activity Assay Kit II (K595)
HMG-CoA Reductase Activity/Inhibitor Screening Kit (K588)
CETP Inhibitor Screening Kit II (K594)

***FOR RESEARCH USE ONLY! Not to be used on humans.***