Product Manual

Human LDLR ELISA Kit

Catalog Number

STA-386 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Cholesterol is an essential component of cellular membranes, as well as an essential substrate for steroid hormones and bile acid synthesis. However, cholesterol is toxic when accumulated in excess in cellular membranes and elaborate pathways have evolved to control its synthesis, uptake and storage. Cells can obtain cholesterol either from de novo synthesis or uptake from circulating lipoproteins. The low-density lipoprotein (LDL) receptor (LDLR) is the primary pathway for removal of cholesterol from the circulation, and its activity is meticulously governed by intracellular cholesterol.

The LDLR is a transmembrane protein of 839 amino acids in length that can broadly be divided into 5 domains (Figure 1). The physiologic role of the LDLR is to transport cholesterol-carrying lipoprotein particles into cells. The primary ligand for the receptor is LDL, which contains a single copy of apolipoprotein B-100 (apoB); approximately 65-70% of plasma cholesterol in humans circulates in the form of LDL. The LDLR also binds tightly to beta-migrating forms of very low-density lipoprotein (b-VLDL), which contains multiple copies of apolipoprotein E (apoE). Receptor-ligand complexes enter the cell by endocytosis at clathrin-coated pits, where receptor molecules cluster on the cell surface. Bound lipoprotein particles are subsequently released in the low-pH setting of the endosome, and the receptors then return to the cell surface in a process called receptor recycling.

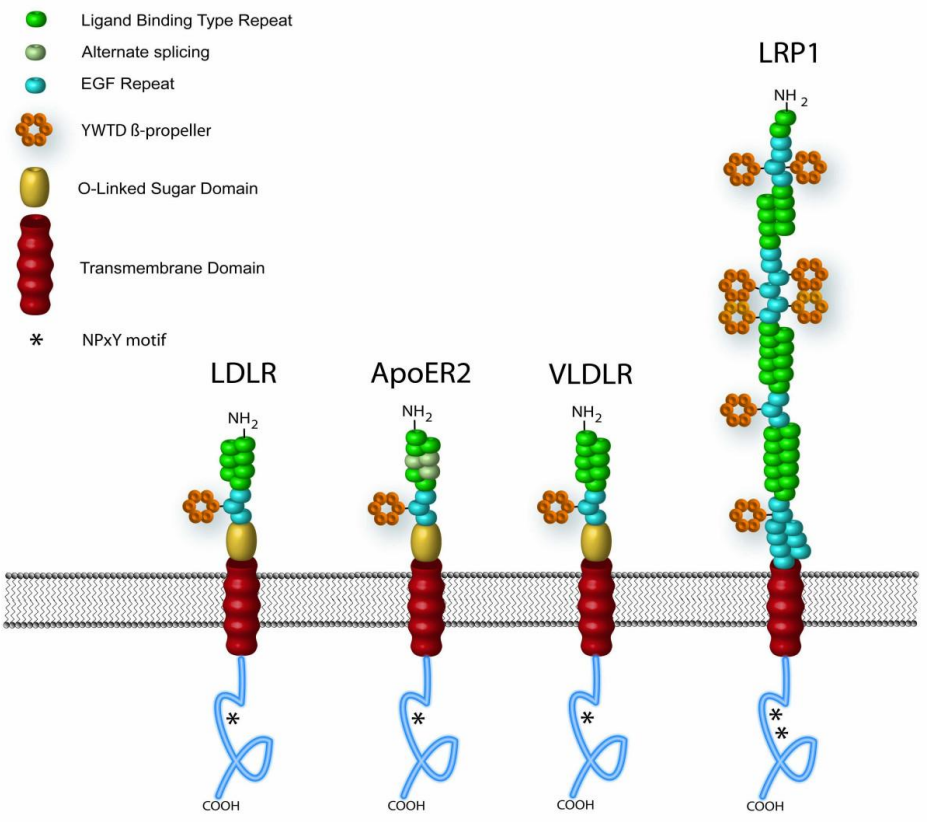

Figure 1. LDL receptor family members and structures. A schematic diagram illustrates the 5 domains of the low-density lipoprotein receptor (LDLR): 1) LDLR type A repeat domains; 2) epidermal growth factor (EGF) receptor homology domain containing the β-propeller subdomain; 3) O-linked glycosylation domain; 4) transmembrane domain; 5) cytoplasmic domain containing NPXY sequence.

Cell Biolabs' Human LDLR ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human LDLR in plasma, serum, cell or tissue lysate samples. The kit has a detection sensitivity limit of 50 pg/mL human LDLR. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
2. STA-368: Human ApoB ELISA Kit
3. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
4. STA-387: Human LOX-1 ELISA Kit
5. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
6. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Human LDLR Antibody Coated Plate (Part No. 238601): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Human LDLR Antibody (500X) (Part No. 238602): One 25 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human LDLR Standard (Part No. 238603): One 50 µL vial of 1 µg/mL Human LDLR in PBS plus BSA.

Materials Not Supplied

1. Plasma, Serum, Cell or Tissue Lysate
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Human LDLR Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Human LDLR Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Human LDLR antibody 1:500 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human LDLR Standard

Prepare a dilution series of human LDLR standards in the concentration range of 0 to 3200 pg/mL in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL Human LDLR Standard (µL)	Assay Diluent (µL)	Human LDLR (pg/mL)
1	4	1246	3200
2	400 of Tube #1	400	1600
3	400 of Tube #2	400	800
4	400 of Tube #3	400	400
5	400 of Tube #4	400	200
6	400 of Tube #5	400	100
7	400 of Tube #6	400	50
8	0	400	0

Table 1. Preparation of Human LDLR Standards

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold RIPA buffer and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of human LDLR unknown sample or standard to the Anti-Human LDLR Antibody Coated Plate. Each human LDLR unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-Human LDLR Antibody to each well. Incubate at room temperature for 2 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Human LDLR ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

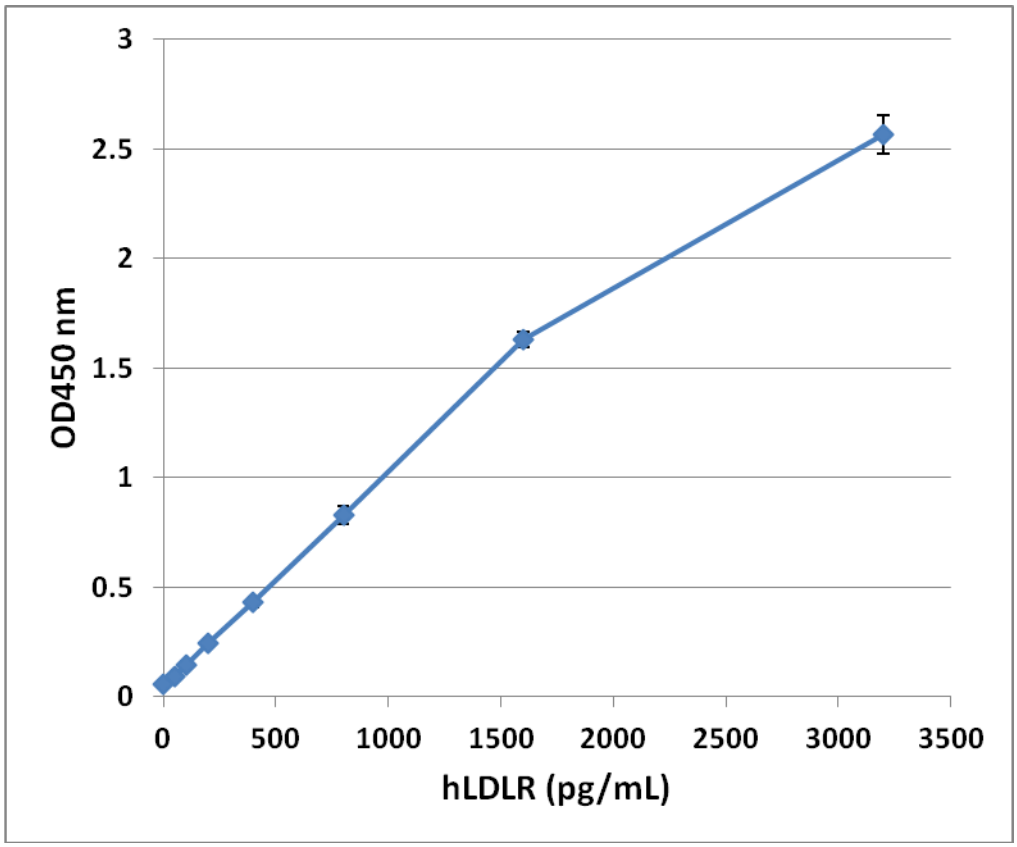

Figure 2: Human LDLR ELISA Standard Curve.

References

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Recent product citation

Alvarez, M. L. et al. (2015). MicroRNA-27a decreases the level and efficiency of the LDL receptor and contributes to the dysregulation of cholesterol homeostasis. *Atherosclerosis.* **242**:595-604.

Warranty

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