Product Manual

OxiSelect™ Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)

Catalog Number

STA-389 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Lipoproteins are submicroscopic particles composed of lipid and protein held together by noncovalent forces. Their general structure is that of a putative spheroidal microemulsion formed from an outer layer of phospholipids, unesterified cholesterol, and proteins, with a core of neutral lipids, predominately cholesteryl esters and triacylglycerols (TAG). Low density lipoprotein (LDL) is the major transport protein for cholesterol in human plasma. LDL is a spherical particle with a diameter of 20-25 nm. Each LDL particle contains cholesteryl esters in its core which are surrounded by a hydrophilic coat composed of phospholipids, cholesterol, and one molecule of a hydrophobic protein known as apolipoprotein B-100 (Figure 1).

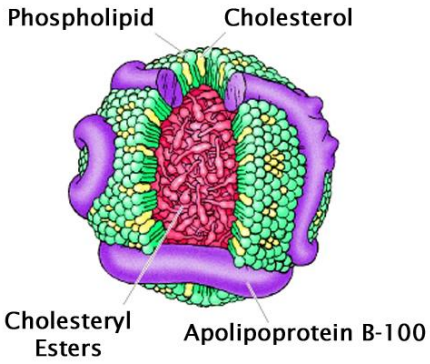
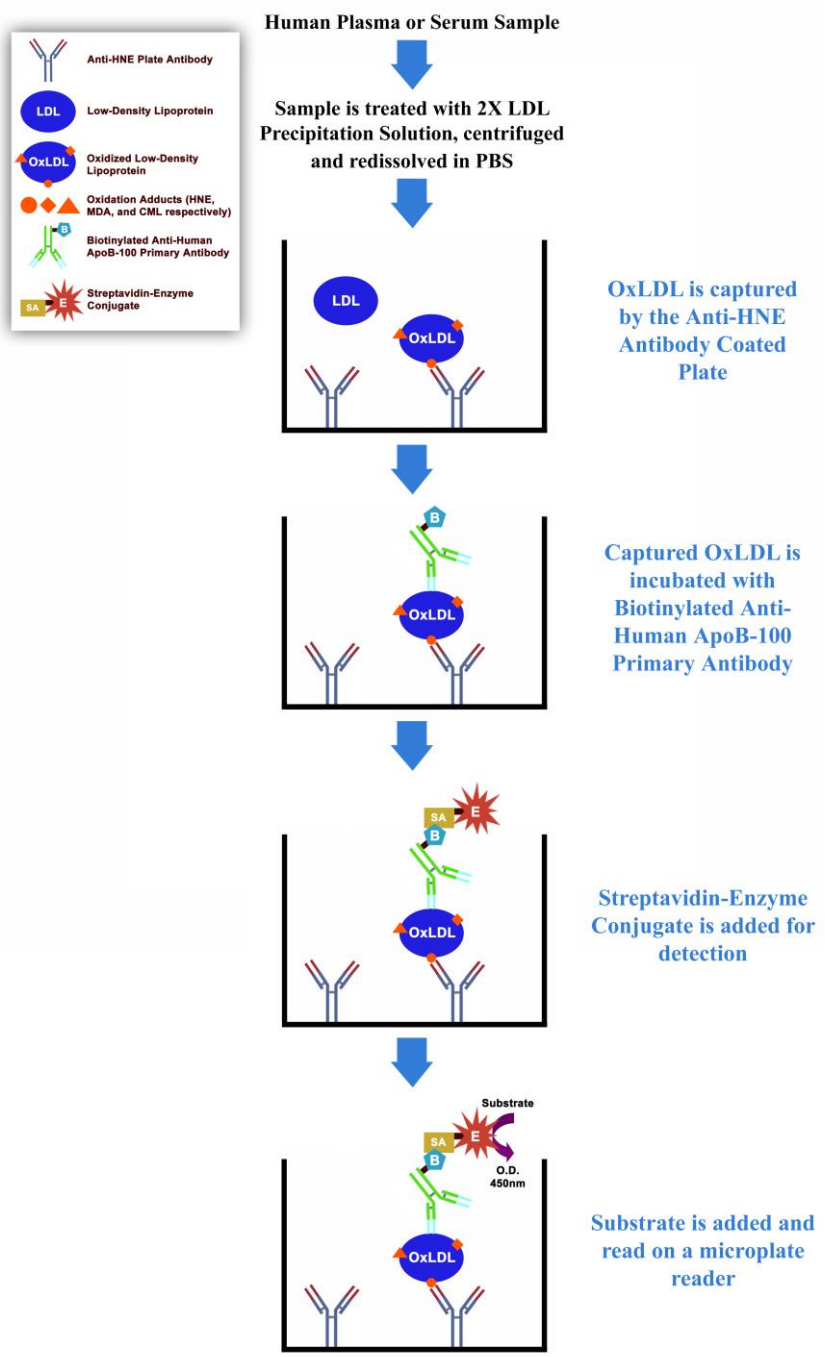


Figure 1: Structure of LDL.

LDL cholesterol, sometimes referred to as "bad" cholesterol, is even more dangerous when it becomes oxidized. Oxidized LDL (OxLDL) is more reactive with surrounding tissues and can collect within the inner-lining of arteries. Macrophages, cholesterol, and other lipids can accumulate at the site (atherosclerosis), ultimately forming a plaque that can lead to heart attack, stroke or death. LDL oxidation affects both the lipid and protein components of LDL. Reactive aldehyde products formed during the oxidation of polyunsaturated fatty acids, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), are capable of attaching covalently to the ε-amino groups of lysine residues of of ApoB-100 to form MDA-Lys and HNE-Lys adducts (MDA-LDL and HNE-LDL). Advanced glycosylation, such as the formation of CML-LDL and CEL-LDL, are also involved in LDL oxidation.

The OxiSelect™ Human Oxidized LDL ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human OxLDL in plasma, serum or other biological fluid samples. The kit contains an OxLDL Standard and has a detection sensitivity limit of <15 ng/mL. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Assay Principle



Related Products

1. STA-212: Malondialdehyde (MDA) Modified Human Low Density Lipoprotein (LDL)
2. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
3. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
4. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-HNE Antibody Coated Plate (Part No. 238901): One 96-well strip plate.
2. Biotinylated Anti-Human ApoB-100 Antibody (1000X) (Part No. 236906): One 20 µL vial.
3. LDL Precipitation Solution (2X) (Part No. 236904): One 20 mL bottle.
4. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
5. Assay Diluent (Part No. 310804): One 50 mL bottle.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Blocking Reagent (100X) (Part No. 238902): One 200 µL vial.
2. OxLDL Standard (Part No. 238803): One 25 µL vial of 0.5 mg/mL.

Materials Not Supplied

1. Human Plasma or Serum Samples
2. PBS
3. Microcentrifuge
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Blocking Reagent 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.
- Blocking Reagent: Immediately before use dilute the Blocking Reagent 1:100 with PBS. Do not store diluted solutions.

- Biotinylated Anti-Human ApoB-100 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-ApoB-100 antibody 1:1000 and Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of OxLDL Standard

Prepare a dilution series of OxLDL Standards in the concentration range of 0 to 1 µg/mL in Assay Diluent (Table 1).

Standard Tubes	0.5 mg/mL OxLDL Standard (µL)	Assay Diluent (µL)	Final OxLDL Standard (ng/mL)
1	2	998	1000
2	250 of Tube #1	250	500
3	250 of Tube #2	250	250
4	250 of Tube #3	250	125
5	250 of Tube #4	250	62.5
6	250 of Tube #5	250	31.25
7	250 of Tube #6	250	15.63
8	0	250	0

Table 1. Preparation of OxLDL 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 x *g* at 4ºC. Remove 200 µL of plasma and add 200 µL of LDL Precipitation Solution, mixing well. Incubate at room temperature for 5 minutes (precipitation will occur). Centrifuge for 20 minutes at 2000 x *g* (pellet should be visible). Carefully aspirate the supernatant and collect the pellet. Resuspend and dissolve the pellet in 1.6 mL of PBS, vortexing well. Further dilute the sample 1:25 to 1:200 in Assay Diluent before running the ELISA. Assay immediately and do not store solutions.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 x *g* at 4ºC. Remove 200 µL of serum and add 200 µL of LDL Precipitation Solution, mixing well. Incubate at room temperature for 5 minutes (precipitation will occur). Centrifuge for 20 minutes at 2000 x *g* (pellet should be visible). Carefully aspirate the supernatant and collect the pellet. Resuspend and dissolve the pellet in 1.6 mL of PBS, vortexing well. Further dilute the sample 1:25 to 1:200 in Assay Diluent before running the ELISA. Assay immediately and do not store solutions.

Assay Protocol

1. For plasma and serum samples, refer to the above Sample Preparation Section. These samples require LDL Precipitation Solution treatment immediately prior to running the assay.
2. Add 100 µL of OxLDL standard or unknown sample to the Anti-HNE Antibody Coated Plate. Each OxLDL standard, blank and unknown sample should be assayed in duplicate.

3. Cover with a plate cover and incubate at room temperature for 2 hours on an orbital shaker.
4. 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.
5. Add 100 µL of diluted Blocking Reagent to each well. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
6. Wash microwell strips 5 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.
7. Add 100 µL of the diluted Biotinylated Anti-Human ApoB-100 antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
8. Wash the strip wells 5 times according to step 6 above.
9. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
10. Wash the strip wells 5 times according to step 6 above. Proceed immediately to the next step.
11. 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.
12. 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).
13. 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 Oxidized LDL ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

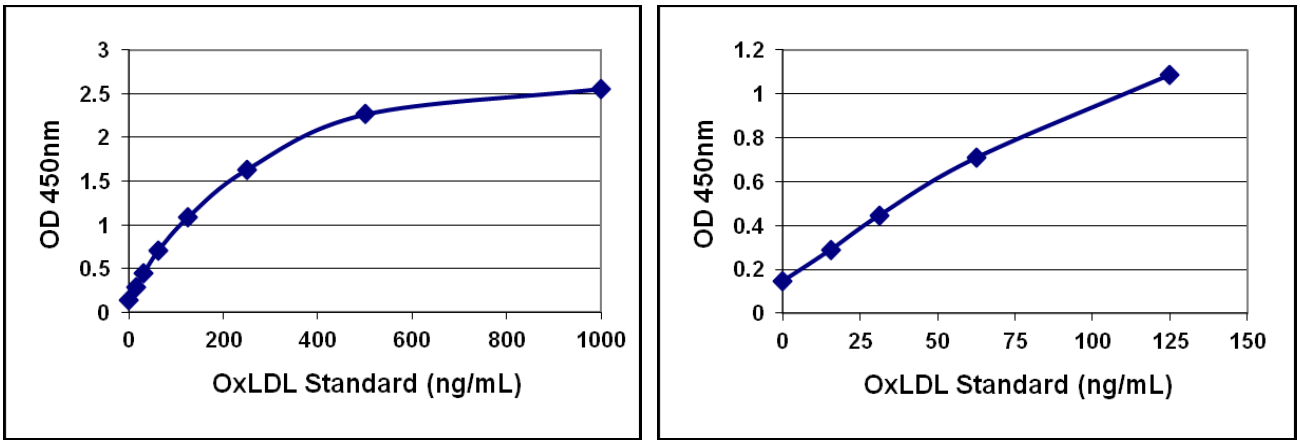


Figure 2: Human OxLDL Standard Curve.

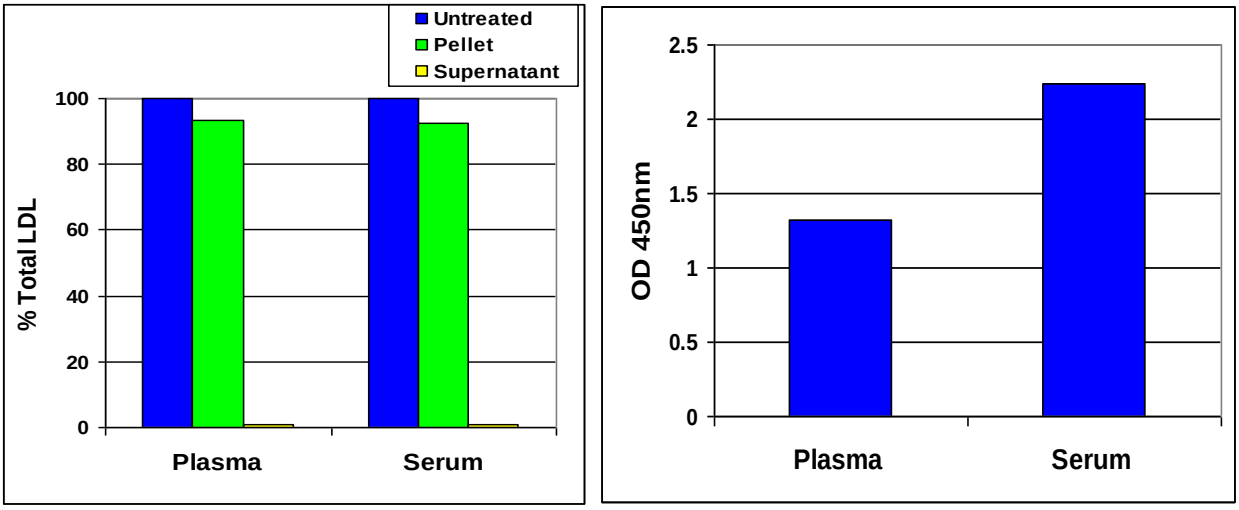


Figure 3: Quantitation of OxLDL in Serum and Plasma Samples. Left: LDL Recovery After Precipitation Solution. Serum and plasma samples were treated with LDL Precipitation Solution according to the Sample Preparation protocol. LDL recovery was determined by Human ApoB-100 ELISA (STA-368). **Right: OxLDL Determination of Serum and Plasma Samples.** Serum and plasma samples were treated with LDL Precipitation Solution according to the Sample Preparation Section. Precipitated LDL pellets were resuspended in 1.6 mL of PBS before further diluting 1:50 in Assay Diluent. Samples were tested according to the Assay Protocol.

References

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Recent Product Citations

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7. Wang, F. et al. (2015). Shift of the interconnection from the reaction system of paraoxonase 1 to the peroxidation reaction system of myeloperoxidase with HDL-C Levels: a marker of atherosclerosis in patients with normal cholesterol levels. *Clin Chim Acta*. **438**:370-375.

Warranty

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