Product Manual

Human Carbamylated LDL ELISA Kit (CBL-LDL Quantitation)

Catalog Number

MET-5032 **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).

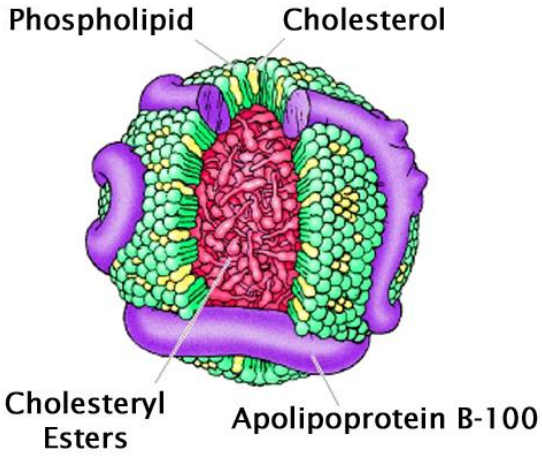


Figure 1: Structure of LDL.

Carbamylation is a post-translational modification which occurs throughout the lifespan of proteins in vivo. Carbamylation results from the binding of isocyanic acid, spontaneously derived from high concentrations of urea and leading to the formation of carbamyl-lysine (CBL) (Figure 2). The carbamylation of proteins is usually associated with a partial or complete loss of protein function. It is known that elevated urea directly induces the formation of potentially atherogenic carbamylated LDL (CBL-LDL). High blood concentrations of urea leading to the carbamylation process were detected in uremic patients and patients with end-stage renal disease.

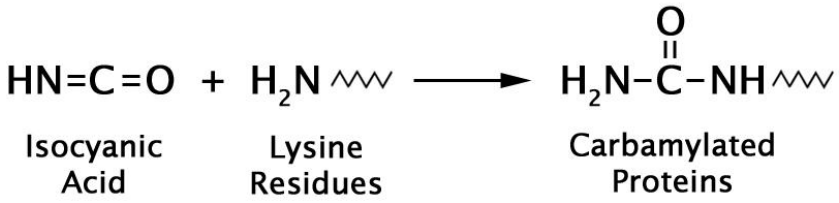
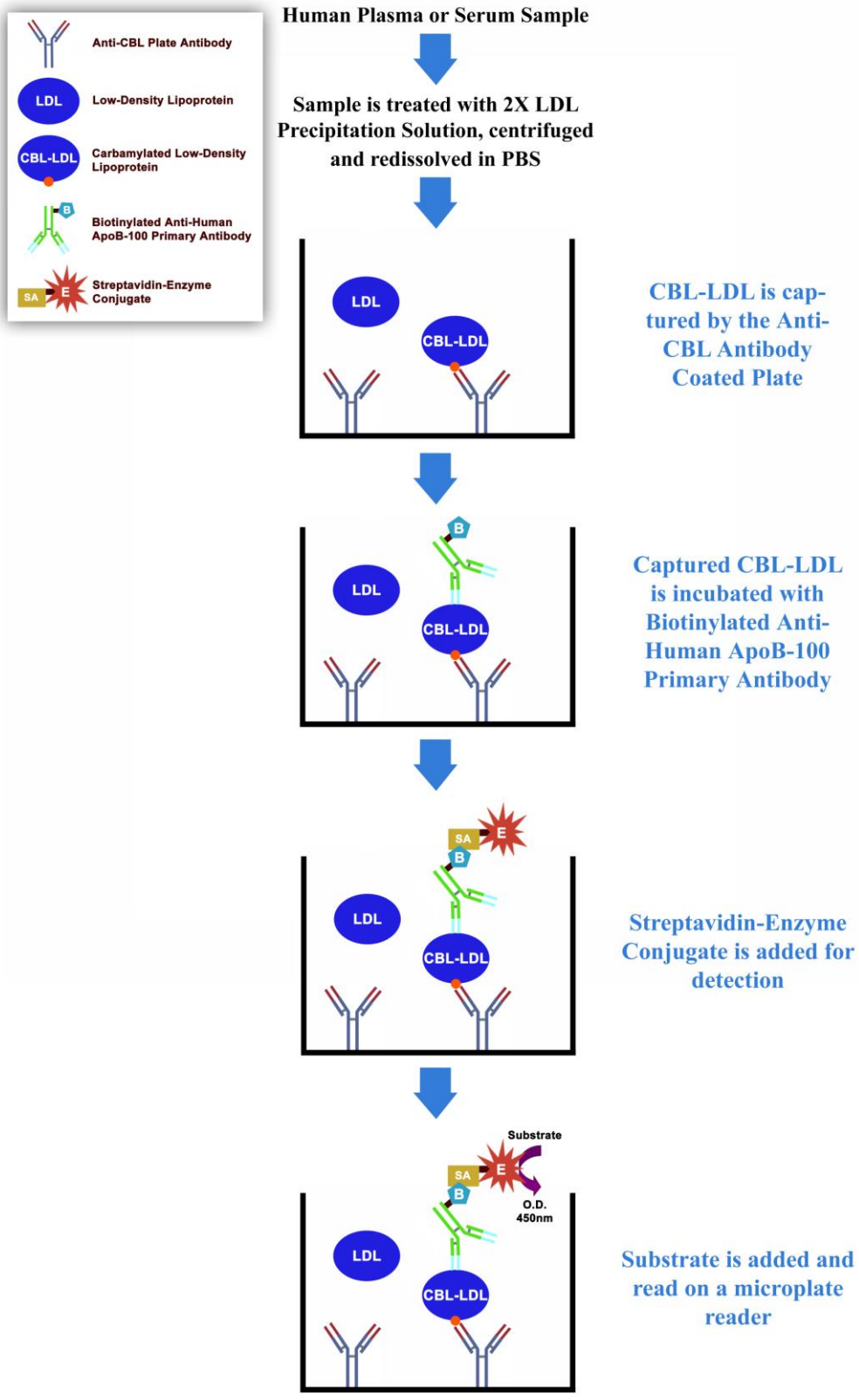


Figure 2: Formation of Carbamyl-Lysine (CBL) During Carbamylation of Proteins.

The Human Carbamylated LDL ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human CBL-LDL in plasma, serum or other biological fluid samples. The kit contains a CBL-LDL standard and has a detection sensitivity limit of 1 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-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
2. STA-241: Human Low Density Lipoprotein (LDL)
3. STA-358: Human Oxidized LDL ELISA Kit (OxPL-LDL Quantitation)
4. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
5. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)
6. STA-877: OxiselectTM Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)

Kit Components

Box 1 (shipped at room temperature)

1. 96-Well Anti-CBL Antibody Coated Plate (Part No. 287701): One strip well 96-well plate.
2. Biotinylated Anti-Human ApoB-100 Antibody (1000X) (Part No. 236902): 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. CBL-LDL Standard (Part No. 50321D): One 25 µL vial of 4 µg/mL Carbamylated, Purified Human LDL.

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 CBL-LDL at -80°C to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Human ApoB-100 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Anti-ApoB-100 antibody 1:1000 and Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of CBL-LDL Standard

Prepare a dilution series of CBL-LDL Standards in the concentration range of 0 to 40 ng/mL in Assay Diluent (Table 1).

Standard Tubes	4 µg/mL CBL-LDL Standard (µL)	Assay Diluent (µL)	CBL-LDL Standard (ng/mL)
1	5	495	40
2	250 of Tube #1	250	20
3	250 of Tube #2	250	10
4	250 of Tube #3	250	5
5	250 of Tube #4	250	2.5
6	250 of Tube #5	250	1.25
7	250 of Tube #6	250	0.625
8	0	250	0

Table 1. Preparation of CBL-LDL 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 (2X), 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 1:50 to 1:200 in Assay Diluent before running the ELISA. Assay immediately and do not store solutions.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Remove 200 µL of serum and add 200 µL of LDL Precipitation Solution (2X), 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 1:50 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 CBL-LDL standard or unknown sample to the Anti-CBL Antibody Coated Plate. Each CBL-LDL standard, blank and unknown sample should be assayed in duplicate.
3. Cover with a plate cover and incubate at 37°C for 2 hours without shaking (or incubate at 4°C overnight).
4. 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.
5. 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.
6. Wash the strip wells 5 times according to step 4 above.
7. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate 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. Proceed immediately to the next step.
9. 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 2-20 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
10. 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).
11. 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 Carbamylated LDL ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

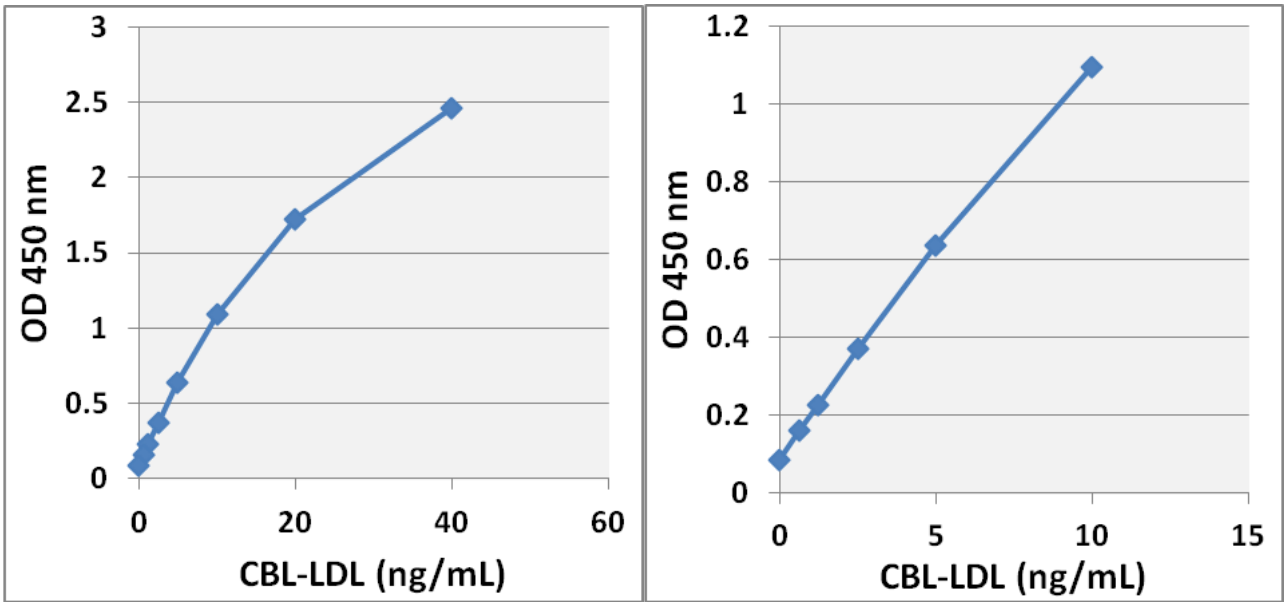


Figure 3: Human CBL-LDL Standard Curve.

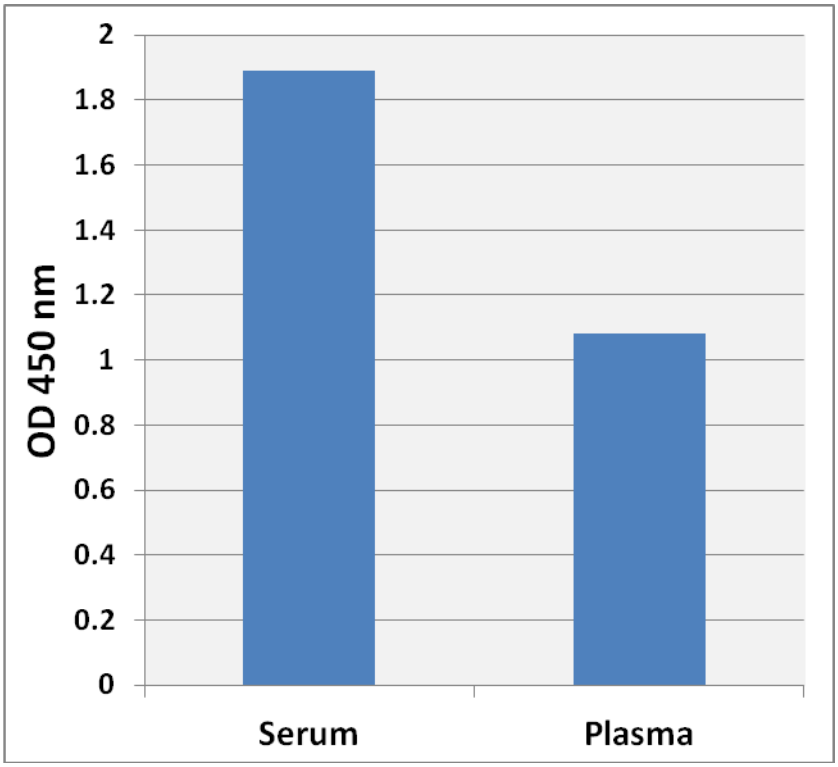


Figure 4: CBL-LDL Determination of Human Serum and Plasma Samples. Human serum and plasma samples were treated with LDL Precipitation Solution (2X) according to the Sample Preparation Section. Precipitated LDL pellets were resuspended in 1.6 mL of PBS before further diluting 1:80 into Assay Diluent. Samples were tested according to the Assay Protocol.

References

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Warranty

In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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