Product Manual

Human ApoB ELISA Kit

Catalog Number

STA-368 **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). Plasma apolipoproteins can be grouped into two classes: the nonexchangeable apolipoproteins (ApoB-100 and ApoB-48), and the exchangeable apolipoproteins (ApoA-I, ApoA-II, ApoA-IV, ApoC-I, ApoC-II, ApoC-III, and ApoE). ApoB-100 is found in lipoproteins originating from the liver (VLDL, IDL, and LDL). 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). ApoB-48 is synthesized exclusively by the small intestine. As a result of the RNA editing, ApoB-48 and ApoB-100 share a common N-terminal sequence, but ApoB-48 lacks ApoB-100's C-terminal LDL receptor binding region. ApoB-48 is so called because it constitutes 48% of the sequence for ApoB-100. ApoB-48 is a unique protein to chylomicrons from the small intestine.

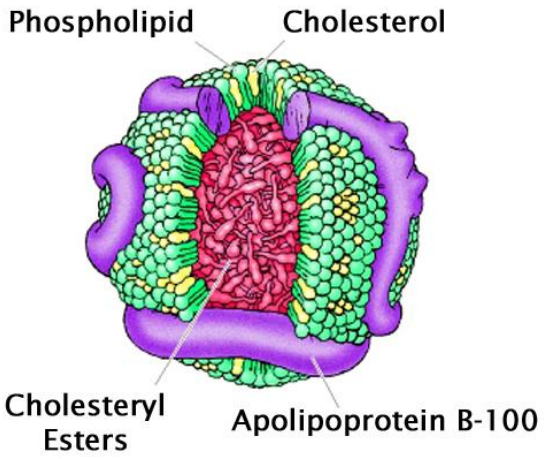

Figure 1: Structure of LDL.

Cell Biolabs' Human ApoB ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human ApoB (ApoB-100/48) in plasma, serum or other biological fluid samples. The kit has detection sensitivity limit of 1 ng/mL human ApoB. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-134: Goat Anti-Human Apolipoprotein B-100/48 Polyclonal Antibody
2. STA-211: Malondialdehyde (MDA) Modified Human Apolipoprotein B-100
3. STA-212: Malondialdehyde (MDA) Modified Human Low Density Lipoprotein (LDL)
4. STA-213: Nitrated Human Low Density Lipoprotein (LDL)

5. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
6. STA-234: Human Apolipoprotein B-100
7. STA-241: Human Low Density Lipoprotein (LDL)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-ApoB Antibody Coated Plate (Part No. 236801): One 96-well strip plate (8 x 12).
2. Anti-ApoB Antibody (1000X) (Part No. 236802): One 20 µL vial of anti-ApoB Mouse IgG.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 10902): One 50 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 ApoB-100 Standard (Part No. 236803): One 50 µL vial of 50 µg/mL Human ApoB-100 in PBS plus BSA.

Materials Not Supplied

1. Plasma, Serum or Other Biological Fluids
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 ApoB-100 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.
- Anti-ApoB Antibody and Secondary Antibody: Immediately before use dilute the Anti-ApoB antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human ApoB-100 Standard

Prepare a dilution series of human ApoB-100 standards in the concentration range of 0 to 50 ng/mL in Assay Diluent (Table 1).

Standard Tubes	50 µg/mL Human ApoB-100 Standard (µL)	Assay Diluent (µL)	Human ApoB-100 (ng/mL)
1	2	1998	50
2	500 of Tube #1	500	25
3	500 of Tube #2	500	12.5
4	500 of Tube #3	500	6.25
5	500 of Tube #4	500	3.12
6	500 of Tube #5	500	1.56
7	500 of Tube #6	500	0.78
8	0	500	0

Table 1. Preparation of Human ApoB-100 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 up to three months. Normal plasma sample requires about 20,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC up to three months. Normal serum sample requires about 20,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC up to three months.

Assay Protocol

1. Prepare dilutions of plasma, serum or other biological fluid samples in PBS containing 0.1% BSA.
2. Add 100 µL of ApoB unknown sample or standard to the Anti-ApoB Antibody Coated Plate. Each ApoB unknown sample, standard and blank should be assayed in duplicate.
3. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
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 the diluted anti-ApoB antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
6. Wash the strip wells 3 times according to step 4 above.
7. Add 100 µL of the diluted secondary antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
8. Wash the strip wells 3 times according to step 4 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 2-30 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 ApoB ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

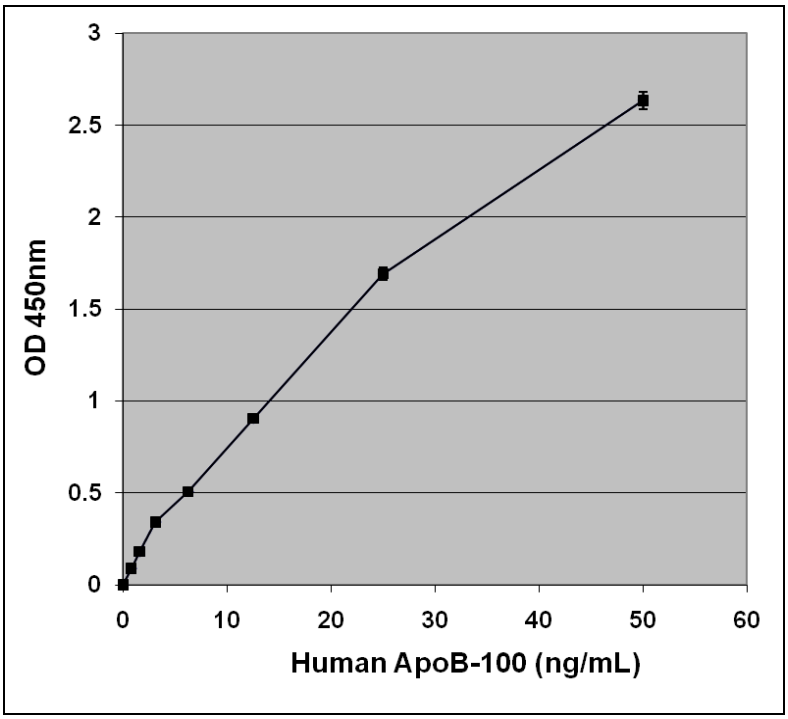

Figure 2: Human ApoB ELISA Standard Curve.

References

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Recent Product Citation

Sukhanov, S. et al. (2015). Insulin-like growth factor I reduces lipid oxidation and foam cell formation via downregulation of 12/15-lipoxygenase. *Atherosclerosis.* **238**:313-320.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com