Product Manual

Human ApoAI and ApoB Duplex ELISA Kit

Catalog Number

STA-361 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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 (ApoAI, ApoAII, ApoAIV, ApoCI, ApoCII, ApoCIII, and ApoE).

ApoAI comprises approximately 70% of the protein moiety in HDL (Figure 1). It is a single polypeptide chain consisting of 223 amino acid residues without disulfide bond and with aspartic acid as the the N-terminal residue and glutamic acid as the C-terminal residue. It has an approximate molecular weight 28 kDa. ApoAI activates lecithin-chlesterol (LCAT) acyltransferase, which is responsible for cholesterol esterification in plasma.

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.

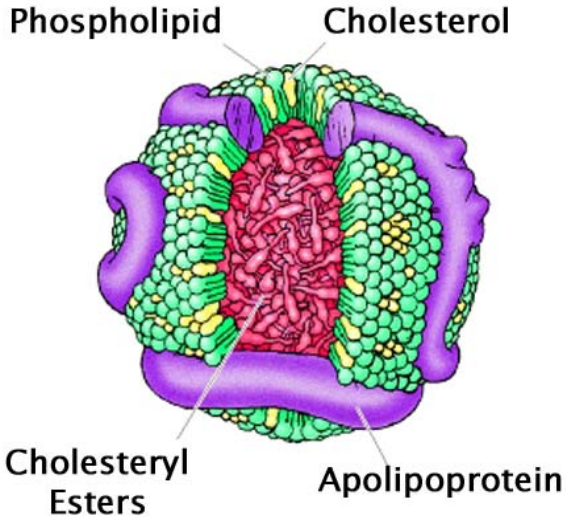
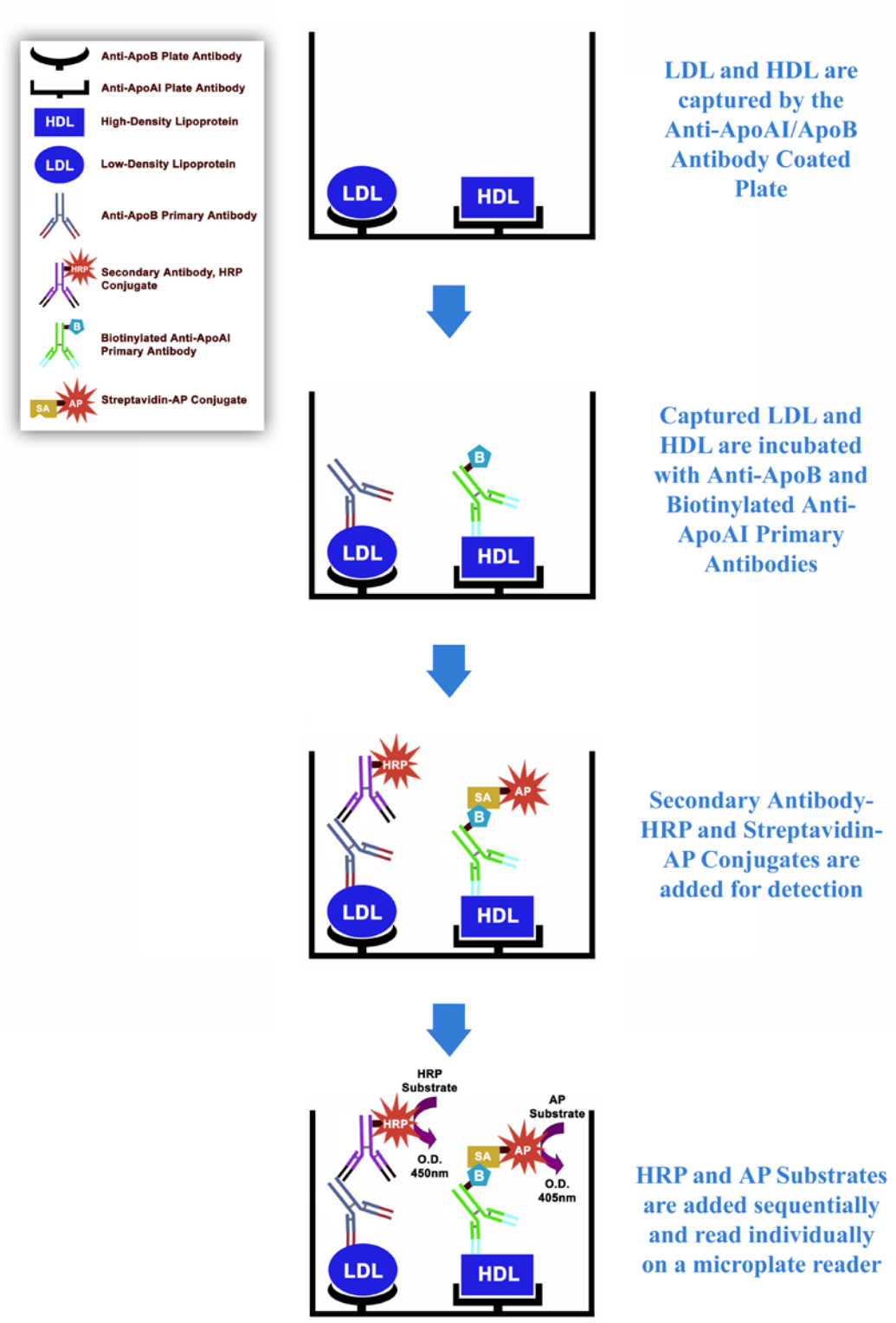


Figure 1: Structure of HDL and LDL

Cell Biolabs' Human ApoAI and ApoB Duplex ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of both human ApoAI and ApoB (ApoB-100/48) in the same sample of plasma, serum or other biological fluids. The kit has detection sensitivity limit of 0.1 ng/mL (human ApoAI) or 1 ng/mL (human ApoB). 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-232: Human Apolipoprotein AI
3. STA-234: Human Apolipoprotein B-100
4. STA-362: Human ApoAI ELISA Kit
5. STA-363: Human ApoAII ELISA Kit
6. STA-364: Human ApoCI ELISA Kit
7. STA-365: Human ApoCII ELISA Kit
8. STA-366: Human ApoCIII ELISA Kit
9. STA-367: Human ApoE ELISA Kit
10. STA-368: Human ApoB ELISA Kit
11. STA-369: Human Oxidized LDL ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-ApoAI and Anti-ApoB Antibody Coated Plate (Part No. 236101): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-ApoAI Antibody (1000X) (Part No. 236202): One 20 µL vial.
3. Anti-ApoB Antibody (1000X) (Part No. 236802): One 20 µL vial of anti-ApoB Mouse IgG.
4. Streptavidin-AP Conjugate (1000X) (Part No. 236102): One 20 µL vial.
5. Secondary Antibody, HRP Conjugate (1000X) (Part No. 10902): One 50 µL vial.
6. Assay Diluent (Part No. 310804): One 50 mL bottle.
7. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
8. AP Substrate (Part No. 236103): One 12 mL amber bottle.
9. HRP Substrate (Part No. 236104): One 12 mL amber bottle.
10. Stop Solution (Part. No. 211003): One 20 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human ApoAI Standard (Part No. 236203): One 50 µL vial of 1 µg/mL Human ApoAI in PBS plus BSA.
2. 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 ApoAI and ApoB 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 to 1X with deionized water. Stir to homogeneity.
- Primary Antibody Mixture: Immediately before use mix 1 part each of both the Biotinylated Anti-ApoAI antibody and the Anti-ApoB Antibody with 1000 parts of Assay Diluent. For example, add 5 µL of Biotinylated Anti-ApoAI Antibody and 5 µL of Anti-ApoB Antibody to 5 mL of Assay Diluent. Do not store diluted solutions.
- Enzyme Conjugate Mixture: Immediately before use mix 1 part each of both the Streptavidin-AP Conjugate and the Secondary Antibody, HRP Conjugate with 1000 parts of Assay Diluent. For example, add 5 µL of Streptavidin-AP Conjugate and 5 µL of Secondary Antibody, HRP Conjugate to 5 mL of Assay Diluent. Do not store diluted solutions.

Preparation of Human ApoAI and ApoB Standards

Prepare a dilution series of human ApoAI and ApoB standards in the concentration range of 0 to 10 ng/mL for human ApoAI and 0 to 100 ng/mL for human ApoB in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL Human ApoAI Standard and 50 µg/mL Human ApoB Standard (µL)	Assay Diluent (µL)	Human ApoAI (ng/mL)	Human ApoB (ng/mL)
1	10 of ApoAI + 2 of ApoB	988	10	100
2	500 of Tube #1	500	5	50
3	500 of Tube #2	500	2.5	25
4	500 of Tube #3	500	1.25	12.5
5	500 of Tube #4	500	0.625	6.25
6	500 of Tube #5	500	0.312	3.12
7	500 of Tube #6	500	0.156	1.56
8	0	500	0	0

Table 1. Preparation of Human ApoAI and ApoB Standards.

Preparation of Sample

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 samples require about 20,000 to 40,000 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 samples require about 20,000 to 40,000 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 unknown sample or ApoAI/B standard to the Anti-ApoAI and Anti-ApoB Antibody Coated Plate. Each 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 Primary Antibody Mixture 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 Enzyme Conjugate Mixture 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 AP and HRP Substrates to room temperature. Add 100 µL of AP Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker for 5 to 20 minutes. Read absorbance of each microwell on a spectrophotometer using 405 nm as the primary wave length.
10. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.

11. Add 100 µL of HRP Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 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 ApoAI and ApoB Duplex ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

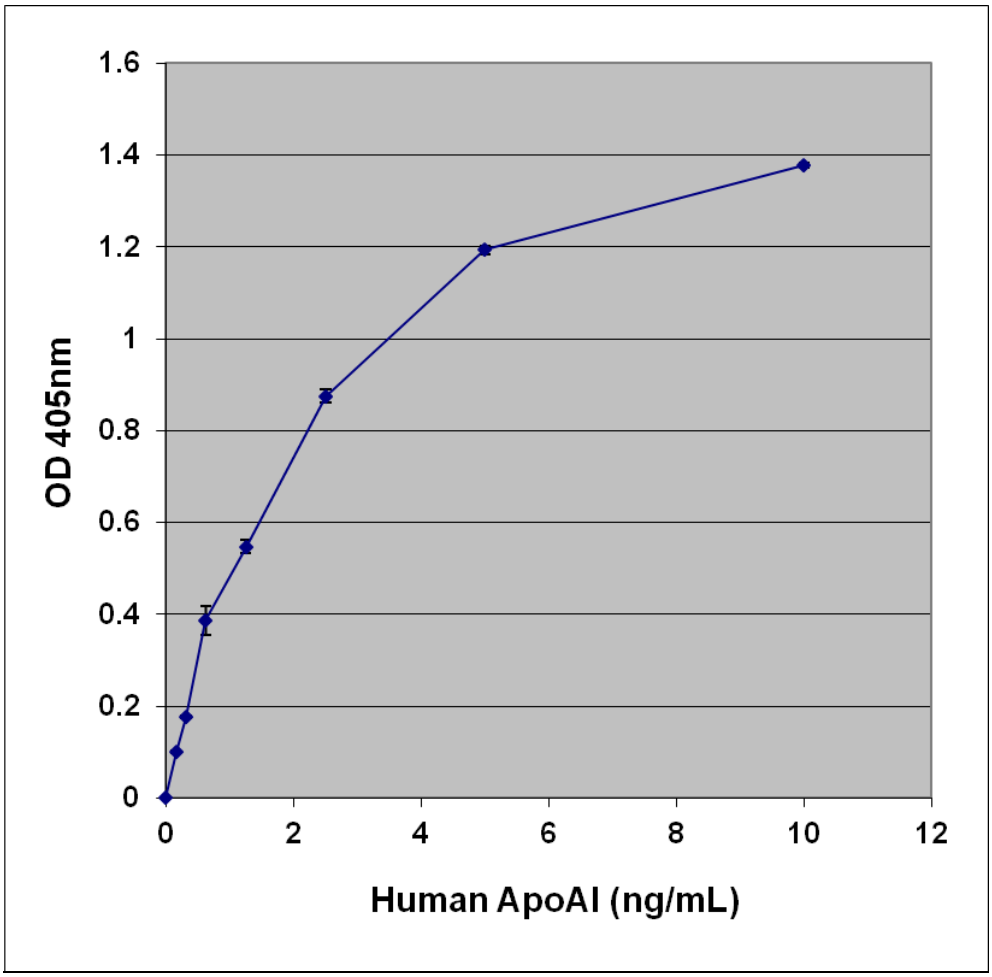


Figure 2: Human ApoAI Standard Curve.

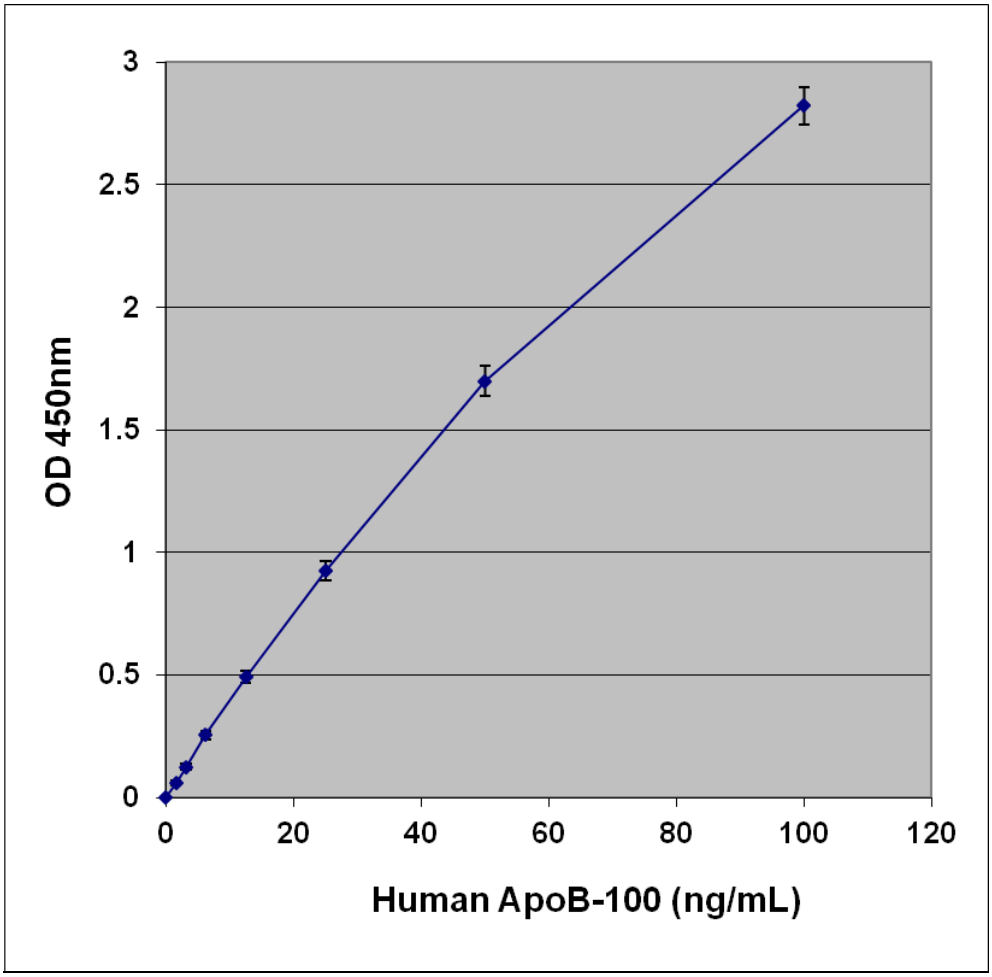


Figure 3: Human ApoB Standard Curve.

References

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