Product Manual

Human ApoAI ELISA Kit, Trial Size

Catalog Number

STA-362-T **32 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 (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.

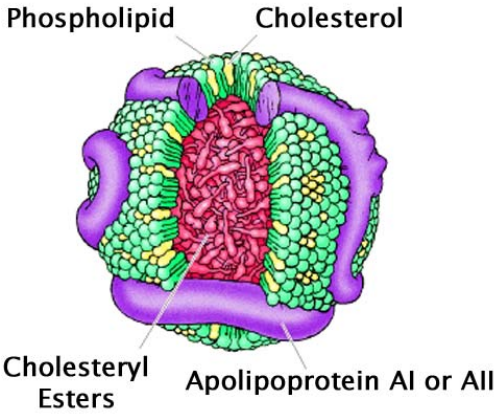

Figure 1: Structure of HDL.

Cell Biolabs' Human ApoAI ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human ApoAI in plasma, serum or other biological fluid samples. The kit has detection sensitivity limit of 50 pg/mL human ApoAI. This Trial Size kit provides sufficient reagents to perform up to 32 assays including standard curve and unknown samples.

Related Products

1. STA-132: Goat Anti-Human Apolipoprotein AI Polyclonal Antibody
2. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
3. STA-232: Human Apolipoprotein AI
4. STA-363: Human ApoAII ELISA Kit
5. STA-364: Human ApoCI ELISA Kit
6. STA-365: Human ApoCII ELISA Kit
7. STA-366: Human ApoCIII ELISA Kit
8. STA-367: Human ApoE ELISA Kit
9. STA-368: Human ApoB-100 ELISA Kit
10. STA-369: Human Oxidized LDL ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-ApoAI Antibody Coated Plate (Part No. 236201-T): One strip well plate containing 32 wells (8 x 4).
2. Biotinylated Anti-ApoAI Antibody (1000X) (Part No. 236202-T): One 10 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803-T): One 10 µL vial.
4. Assay Diluent (Part No. 310804-T): One 20 mL bottle.
5. 10X Wash Buffer (Part No. 310806-T): One 30 mL bottle.
6. Substrate Solution (Part No. 310807-T): One 4 mL amber bottle.
7. Stop Solution (Part. No. 310808-T): One 4 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human ApoAI Standard (Part No. 236203-T): One 20 µL vial of 1 µg/mL Human ApoAI 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 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-ApoAI Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-ApoAI antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human ApoAI Standard

Prepare a dilution series of human ApoAI standards in the concentration range of 0 to 2000 pg/mL in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL Human ApoAI Standard (µL)	Assay Diluent (µL)	Human ApoAI (pg/mL)
1	4	1998	2000
2	500 of Tube #1	500	1000
3	500 of Tube #2	500	500
4	500 of Tube #3	500	250
5	500 of Tube #4	500	125
6	500 of Tube #5	500	62.5
7	500 of Tube #6	500	31.3
8	0	500	0

Table 1. Preparation of Human ApoAI 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 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 ApoAI unknown sample or standard to the Anti-ApoAI Antibody Coated Plate. Each ApoAI 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 Biotinylated Anti-ApoAI 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 Streptavidin-Enzyme Conjugate 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 ApoAI ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

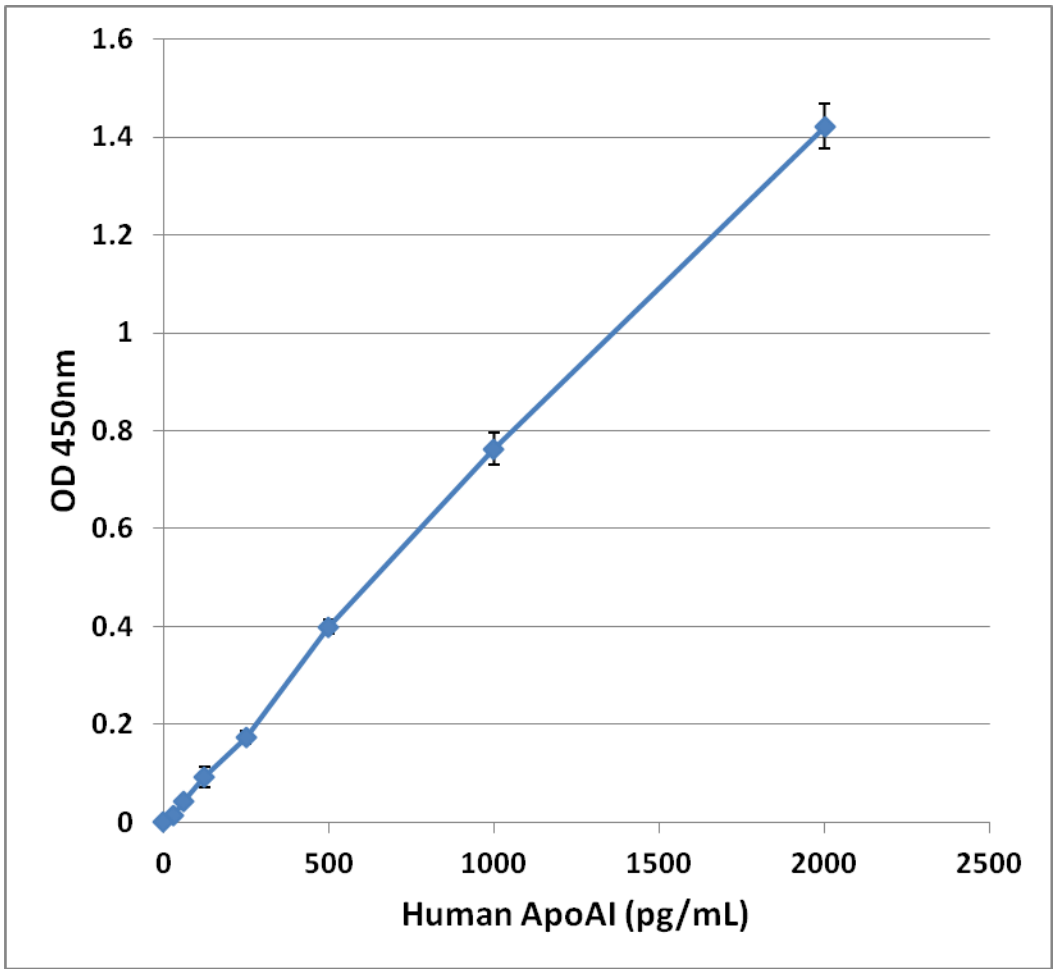

Figure 2: Human ApoAI ELISA Standard Curve.

References

1. Segrest J. P., Garber D. W., Brouillette C. G., Harvey S. C., and Anantharamaiah G. M. *Adv. Protein Chem.* **45**: 303–369, 1994.
2. Segrest J. P., Jones M. K., De Loof H., Brouillette C. G., Venkatachalapathi Y. V., and Anantharamaiah G. M. *J. Lipid Res.* **33**: 141–166, 1992.
3. Vaisar, T., Pennathur, S., Green, P. S., Gharib, S. A., Hoofnagle, A. N., Cheung, M. C., Byun, J., Vuletic, S., Kassim, S., Singh, P., Chea, H., Knopp, R. H., Brunzell, J., Geary, R., Chait, A., Zhao, X. Q., Elkon, K., Marcovina, S., Ridker, P., Oram, J. F., and Heinecke, J. W. *J. Clin. Investig.* **117**, 746–756, 2007.
4. Tailleux, A., Duriez, P., Fruchart, J. C., and Clavey, V. *Atherosclerosis* **164**, 1–13, 2003.

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 exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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