Product Manual

Human ApoE ELISA Kit

Catalog Number

STA-367 **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).

ApoE is a 34-37 kDa glycosylated protein containing 299 amino acid residues. ApoE is involved with trglycreride, phospholipid, cholesteryl ester, and cholesterol transport in and out of cells and is a ligand for LDL receptors. ApoE has also been implicated in immune and nerve degeneration. ApoE4 isoform has been detected in senile plaques and neurofibrillary tangles of Alzheimer disease patients.

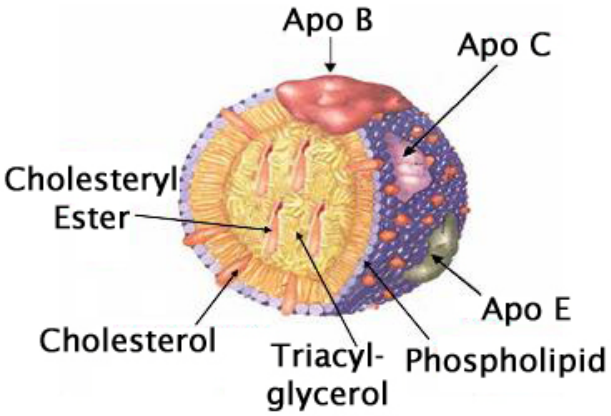

Figure 1: Structure of VLDL.

Cell Biolabs' Human ApoE ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human ApoE in plasma, serum or other biological fluid samples. The kit has detection sensitivity limit of 0.2 ng/mL human ApoE. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-138: Goat Anti-Human Apolipoprotein E Polyclonal Antibody
2. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
3. STA-237: Human Apolipoprotein CIII
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-368: Human ApoB-100 ELISA Kit
10. STA-369: Human Oxidized LDL ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-ApoE Antibody Coated Plate (Part No. 236701): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-ApoE Antibody (1000X) (Part No. 236702): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 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 ApoE Standard (Part No. 236703): One 50 µL vial of 10 µg/mL Human ApoE 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 ApoE 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-ApoE Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-ApoE antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human ApoE Standard

Prepare a dilution series of human ApoE standards in the concentration range of 0 to 10,000 pg/mL in Assay Diluent (Table 1).

Standard Tubes	10 µg/mL Human ApoE Standard (µL)	Assay Diluent (µL)	Human ApoE (pg/mL)
1	2	1998	10,000
2	500 of Tube #1	500	5,000
3	500 of Tube #2	500	2,500
4	500 of Tube #3	500	1,250
5	500 of Tube #4	500	625
6	500 of Tube #5	500	313
7	500 of Tube #6	500	156
8	0	500	0

Table 1. Preparation of Human ApoE 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 2,000 to 5,000 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 2,000 to 5,000 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 ApoE unknown sample or standard to the Anti-ApoE Antibody Coated Plate. Each ApoE 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 Biotinylated diluted Anti-ApoE 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 ApoE ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

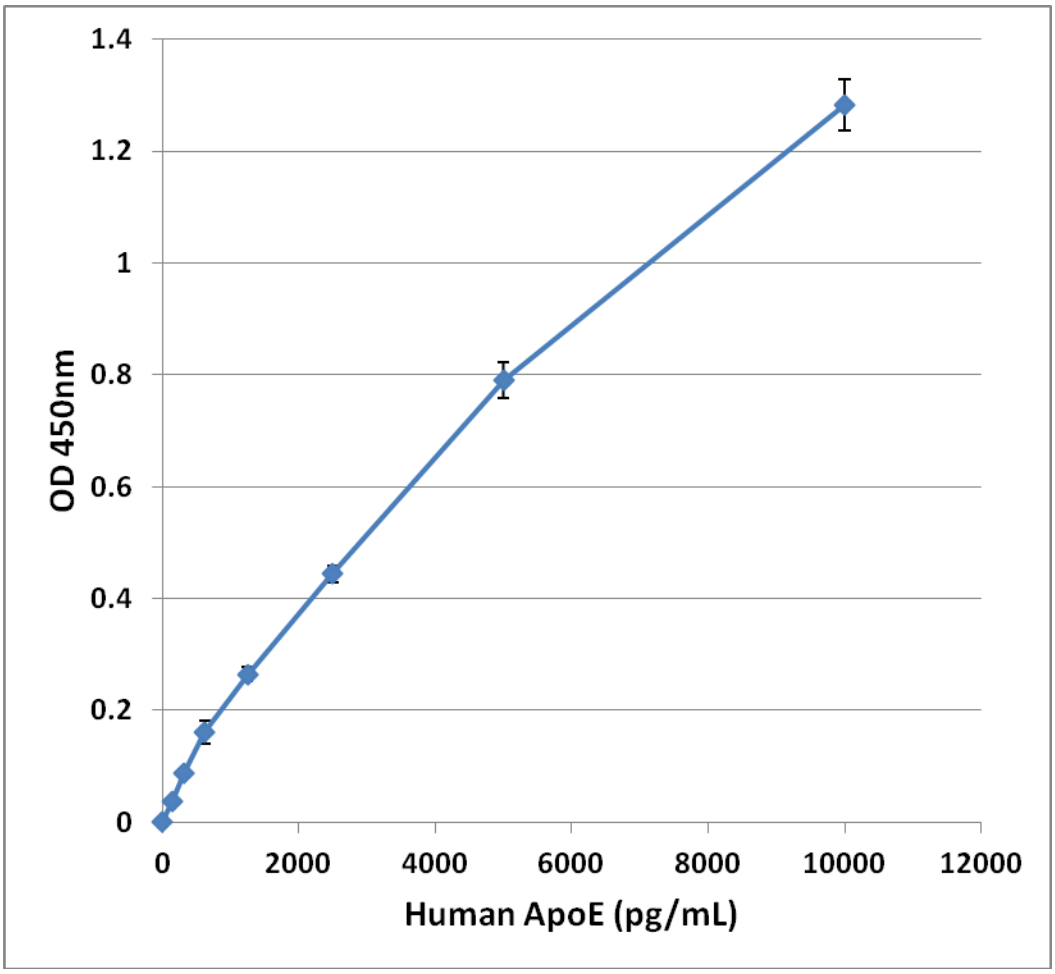

Figure 2: Human ApoE ELISA Standard Curve.

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. Weisgraber K. H. *Adv. Protein Chem.* **45**:249–302, 1994.
4. Brown M. S., Herz J., and Goldstein J. L. *Nature* **388**:629–630, 1997.

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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