Product Manual

Human Apolipoprotein D (ApoD) ELISA Kit

Catalog Numbers

MET- 5074 **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 (ApoA-I, ApoA-II, ApoA-IV, ApoC-I, ApoC-II, ApoC-III, ApoD and ApoE).

ApoD is a component of HDL (Figure 1) and is mainly produced in the brain and testes. It has an approximate molecular weight of 33 kDa but little sequence homology to other apolipoproteins. In fact, ApoD is closely associated to carrier proteins of the lipocalin family, involved in lipoprotein metabolism and the interaction between HDL particles and cells.

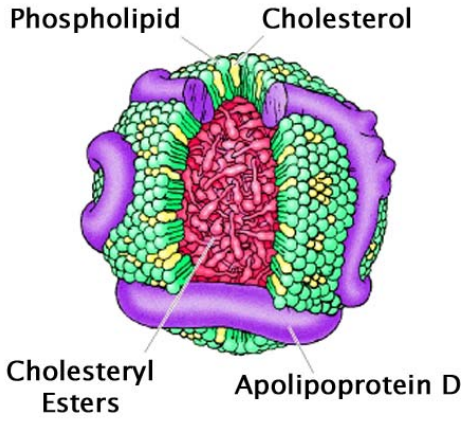

Figure 1: Structure of HDL.

Cell Biolabs' Human Apolipoprotein D ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the ApoD protein. The kit has detection sensitivity limit of ~ 3 ng/mL ApoD. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and ApoD samples.

Assay Principle

An anti-ApoD coating antibody is adsorbed onto a microtiter plate. ApoD protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-ApoD antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin-enzyme conjugate is added and binds to the biotinylated anti-ApoD antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of ApoD present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified ApoD and sample concentration is then determined.

Related Products

1. STA-134: Goat Anti-Human Apolipoprotein B-100/48 Polyclonal Antibody
2. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
3. STA-234: Human Apolipoprotein B-100
4. STA-241: Human Low Density Lipoprotein (LDL)
5. STA-362: Human Apo AI ELISA Kit
6. STA-363: Human Apo AII ELISA Kit
7. STA-365: Human Apo CII ELISA Kit
8. STA-366: Human Apo CIII ELISA Kit
9. STA-368: Human Apo B ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-ApoD Antibody Coated Plate (Part No. 50741B): One strip well 96-well plate.
2. Biotinylated Anti-ApoD Antibody (500X) (Part No. 50742D): One 25 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. Recombinant Human ApoD Standard (Part No. 50743D): One 100 µL vial of 20 µg/mL ApoD.

Materials Not Supplied

1. ApoD sample: serum, plasma, 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 ApoD Standard at -20ºC and avoid freeze/thaw. 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-ApoD Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-ApoD Antibody 1:500 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of ApoD Standard in the concentration range of 200 ng/mL – 3.13 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	20 µg/mL Human ApoD Standard (µL)	Assay Diluent (µL)	ApoD (ng/mL)
1	10	990	200
2	500 of Tube #1	500	100
3	500 of Tube #2	500	50
4	500 of Tube #3	500	25
5	500 of Tube #4	500	12.5
6	500 of Tube #5	500	6.25
7	500 of Tube #6	500	3.13
8	0	500	0

Table 1. Preparation of ApoD Standard

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 and mix all reagents thoroughly before use.
2. Add 100 µL of ApoD sample or standard to the Anti-ApoD Antibody Coated Plate. Each ApoD sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
4. Remove plate cover and empty wells. 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-ApoD Antibody to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
9. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.
11. 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 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 figure demonstrates typical Human ApoD ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

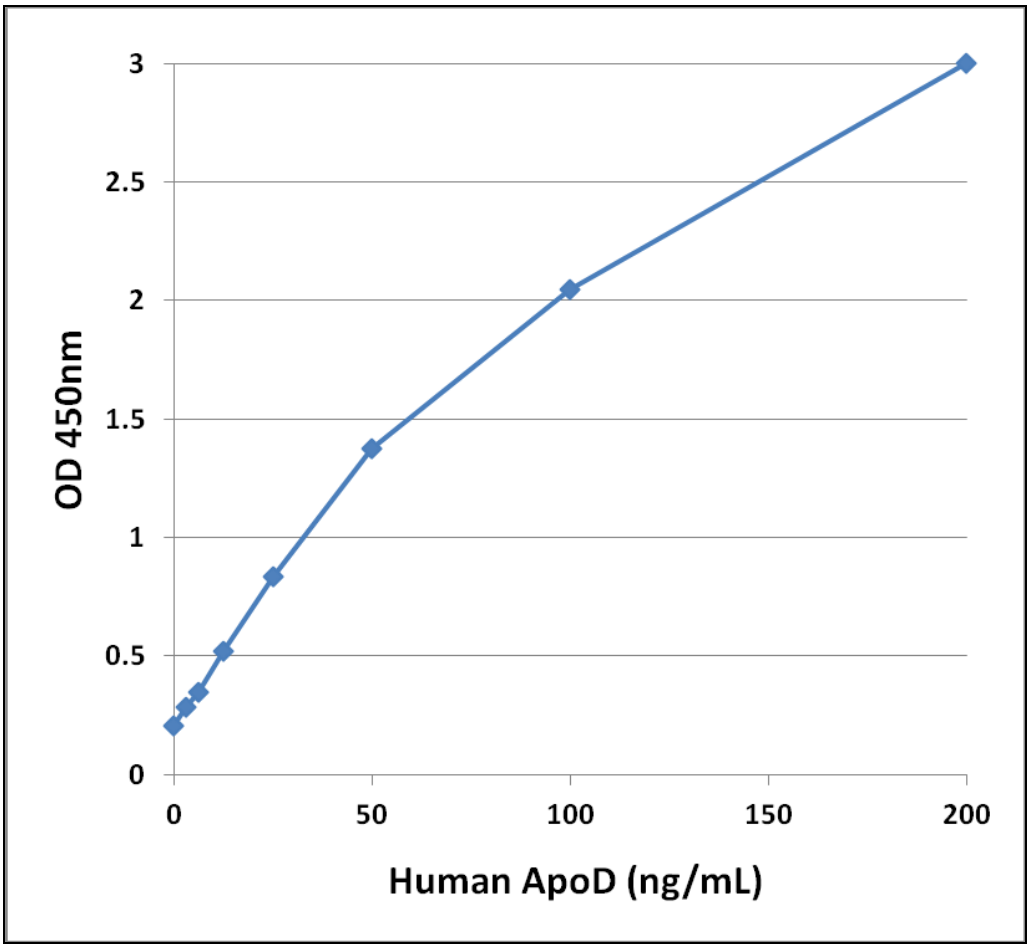

Figure 2: Human ApoD ELISA Standard Curve

References

1. Ganfornina, M., Do Carmo, S., Lora, J., Torres-Schumann, S., Vogel, M., et al. (2008) *Aging Cell* **7**:506-515.
2. Muffat, J., Walker, D. (2010) *Cell Cycle* **9**:269-273.
3. Perdomo, G., Henry Dong, H. (2009) *Aging (Albany NY)* **1**:17-27.

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's 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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