
Product Manual

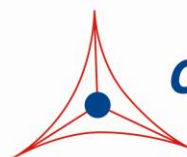
Human Apo(a) ELISA Kit

Catalog Number

STA-359

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

Lipoprotein(a) (Lp(a)) is a low density lipoprotein (LDL)-like particle synthesized by the liver that consists of ApoB-100 molecule covalently linked to a very large glycoprotein known as apolipoprotein(a) (Apo(a)) (see Figure 1). Many epidemiological studies have reported positive associations of baseline Lp(a) concentration with coronary heart disease (CHD) risk. The Apo(a) chain contains five cysteine rich domains known as "kringles". The fourth kringle is homologous with the fibrin-binding domain of plasminogen, a plasma protein that dissolves blood clots when activated. Because of this structural similarity to plasminogen, Lp(a) interferes with fibrinolysis by competing with plasminogen binding to molecules and cells. This impairs plasminogen activation, plasmin generation, and fibrinolysis. Lp(a) also binds to macrophages via a high-affinity receptor that promotes foam cell formation and the deposition of cholesterol in atherosclerotic plaques.

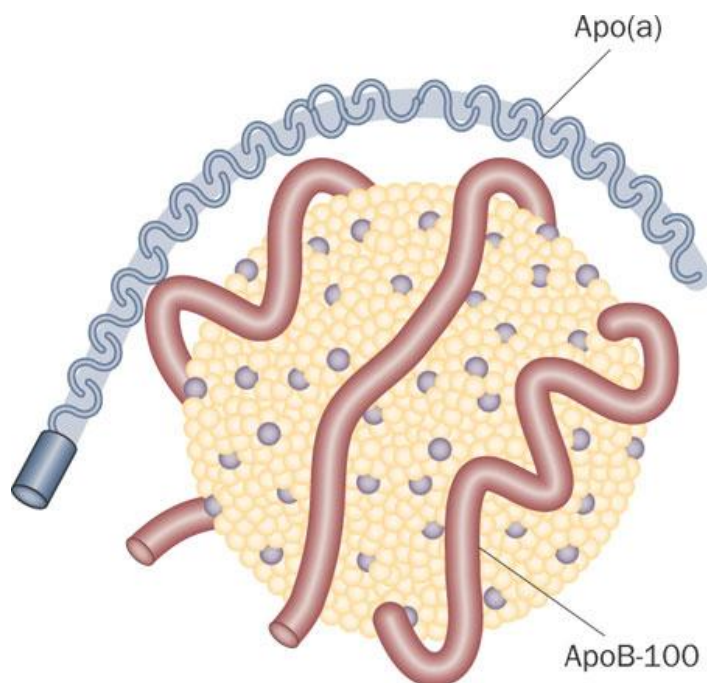


Figure 1: Schematic diagram of lipoprotein(a): Lipoprotein(a) consists of an LDL particle and a glycoprotein molecule, Apo(a), attached to the ApoB-100 moiety of the LDL particle through a disulfide bond.

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

Related Products

1. STA-362: Human ApoAI ELISA Kit
2. STA-363: Human ApoAII ELISA Kit
3. STA-364: Human ApoCI ELISA Kit
4. STA-365: Human ApoCII ELISA Kit
5. STA-366: Human ApoCIII ELISA Kit
6. STA-367: Human ApoE ELISA Kit
7. STA-368: Human ApoB ELISA Kit
8. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
9. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
10. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Apo(a) Antibody Coated Plate (Part No. 235901): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Apo(a) Antibody (1000X) (Part No. 235902): One 20 μ L vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 μ L 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 Apo(a) Standard (Part No. 235903): One 100 μ L vial of 5 μ g/mL Human Apo(a) in PBS plus BSA.

Materials Not Supplied

1. Plasma, Serum or Other Biological Fluids
2. PBS containing 0.1% BSA
3. 10 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
4. 50 μ L to 300 μ L 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 Apo(a) Standard at -20°C to avoid multiple freeze/thaw cycles. Store all other components at 4°C.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Apo(a) Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Apo(a) antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:10,000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human Apo(a) Standard

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

Standard Tubes	5 µg/mL Human Apo(a) Standard (µL)	Assay Diluent (µL)	Human Apo(a) (ng/mL)
1	10	990	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	3.13
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 Apo(a) 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 4°C. Remove the plasma and assay immediately or store samples at -80°C up to three months. Normal plasma samples require about 1,000 to 10,000 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 4°C. Assay immediately or store samples at -80°C up to three months. Normal serum samples require about 1000 to 10,000 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 4°C. Assay immediately or store samples at -80°C 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 μ L of Apo(a) unknown sample or standard to the Anti-Apo(a) Antibody Coated Plate. Each Apo(a) unknown sample, standard and blank should be assayed in duplicate.
3. Incubate at 37°C for at least 2 hours or 4°C overnight.
4. Wash microwell strips 3 times with 250 μ L 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 μ L of the diluted Biotinylated Anti-Apo(a) 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 μ L 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 μ L 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 μ L 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 Apo(a) ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

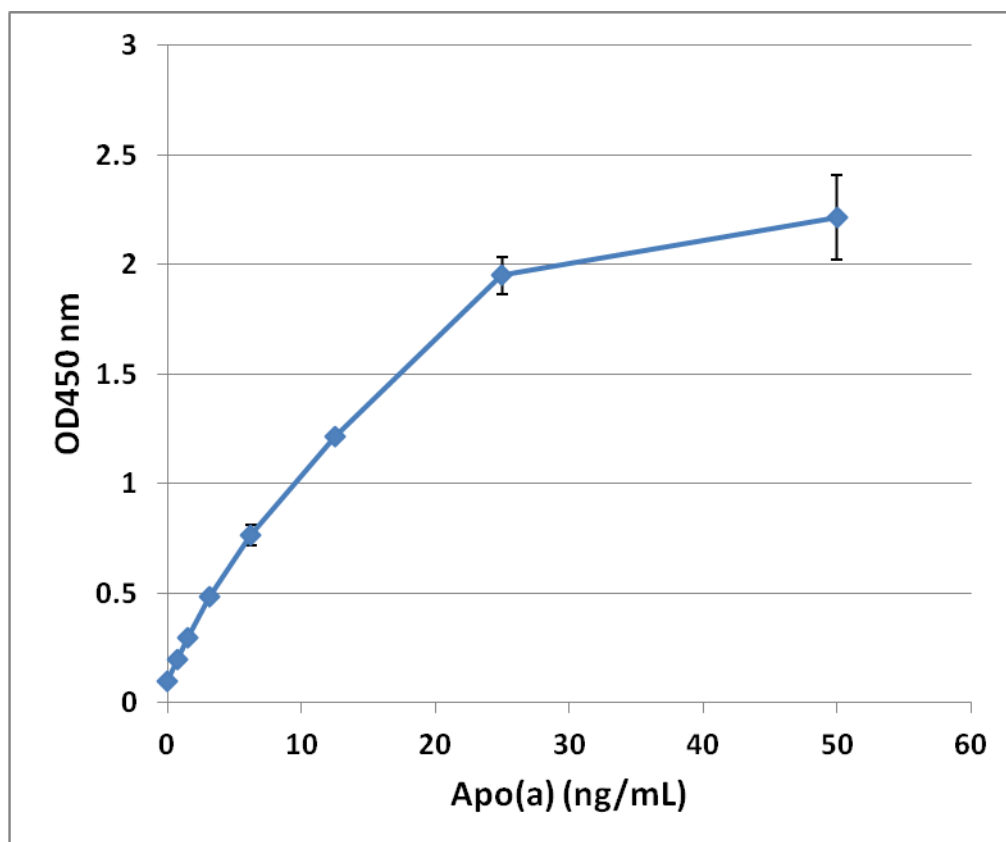


Figure 2: Human Apo(a) ELISA Standard Curve.

References

1. Marcovina SM, Koschinsky ML. (1998) *Am J Cardiol.* **82**:57U–66U.
2. Anuurad E, Boffa MB, Koschinsky ML, Berglund L. (2006) *Clin Lab Med.* **26**:751–772.
3. Hobbs HH, White AL. (1999) *Curr Opin Lipidol.* **10**:225–236.
4. Boffa MB, Marcovina SM, Koschinsky ML. (2004) *Clin Biochem.* **37**(5):333–343.
5. Poon M, Zhang X, Dunsy KG, Taubman MB, Harpel PC. (1997) *Circulation.* **96**:2514–2519.
6. Nielsen LB, Juul K, Nordestgaard BG. (1998) *Arterioscler Thromb Vasc Biol.* **18**:641–649.

Recent Product Citation

Bissig-Choisat, B. et al. (2015). Development and rescue of human familial hypercholesterolaemia in a xenograft mouse model. *Nat Commun.* **6**:7339.

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

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