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Product Manual

# Human ApoCIII ELISA Kit

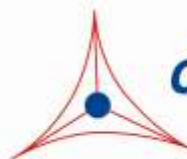
Catalog Number

STA-366

96 assays

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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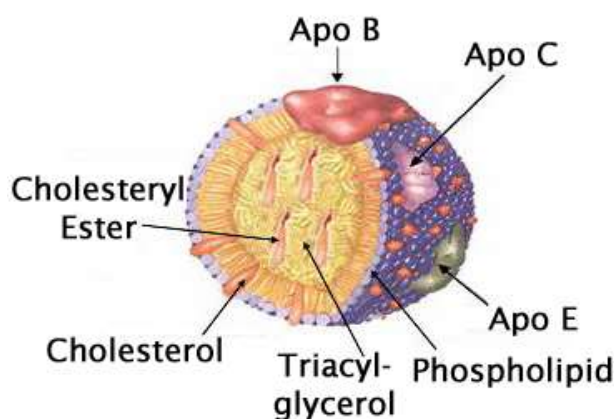


**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **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).

ApoCIII is a 79-amino-acid protein synthesized by liver and intestine, which is an essential constituent of circulating particles rich in triacylglycerol, i.e., chylomicrons and VLDLs. ApoCIII inhibits the hydrolysis of TG-rich particles by the lipoprotein lipase and their hepatic uptake mediated by ApoE.



**Figure 1: Structure of VLDL.**

Cell Biolabs' Human ApoCIII ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human ApoCIII in plasma, serum or other biological fluid samples. The kit has detection sensitivity limit of 50 pg/mL human ApoCIII. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

## **Related Products**

1. STA-214: Copper (Cu<sup>++</sup>) Oxidized Human Low Density Lipoprotein (LDL)
2. STA-362: Human ApoAI ELISA Kit
3. STA-363: Human ApoAII ELISA Kit
4. STA-364: Human ApoCI ELISA Kit
5. STA-365: Human ApoCII ELISA Kit
6. STA-367: Human ApoE ELISA Kit
7. STA-368: Human ApoB-100 ELISA Kit
8. STA-369: Human Oxidized LDL ELISA Kit

## **Kit Components**

### **Box 1 (shipped at room temperature)**

1. Anti-ApoCIII Antibody Coated Plate (Part No. 236601): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-ApoCIII Antibody (1000X) (Part No. 236602): 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 ApoCIII Standard (Part No. 236603): One 50 µL vial of 1 µg/mL Human ApoCIII 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 ApoCIII 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-ApoCIII Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-ApoCIII antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

## **Preparation of Human ApoCIII Standard**

Prepare a dilution series of human ApoCIII standards in the concentration range of 0 to 2,000 pg/mL in Assay Diluent (Table 1).

<b>Standard Tubes</b>	<b>1 µg/mL Human ApoCIII Standard (µL)</b>	<b>Assay Diluent (µL)</b>	<b>Human ApoCIII (pg/mL)</b>
1	4	1996	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 ApoCIII Standards.**

## **Preparation of Samples**

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

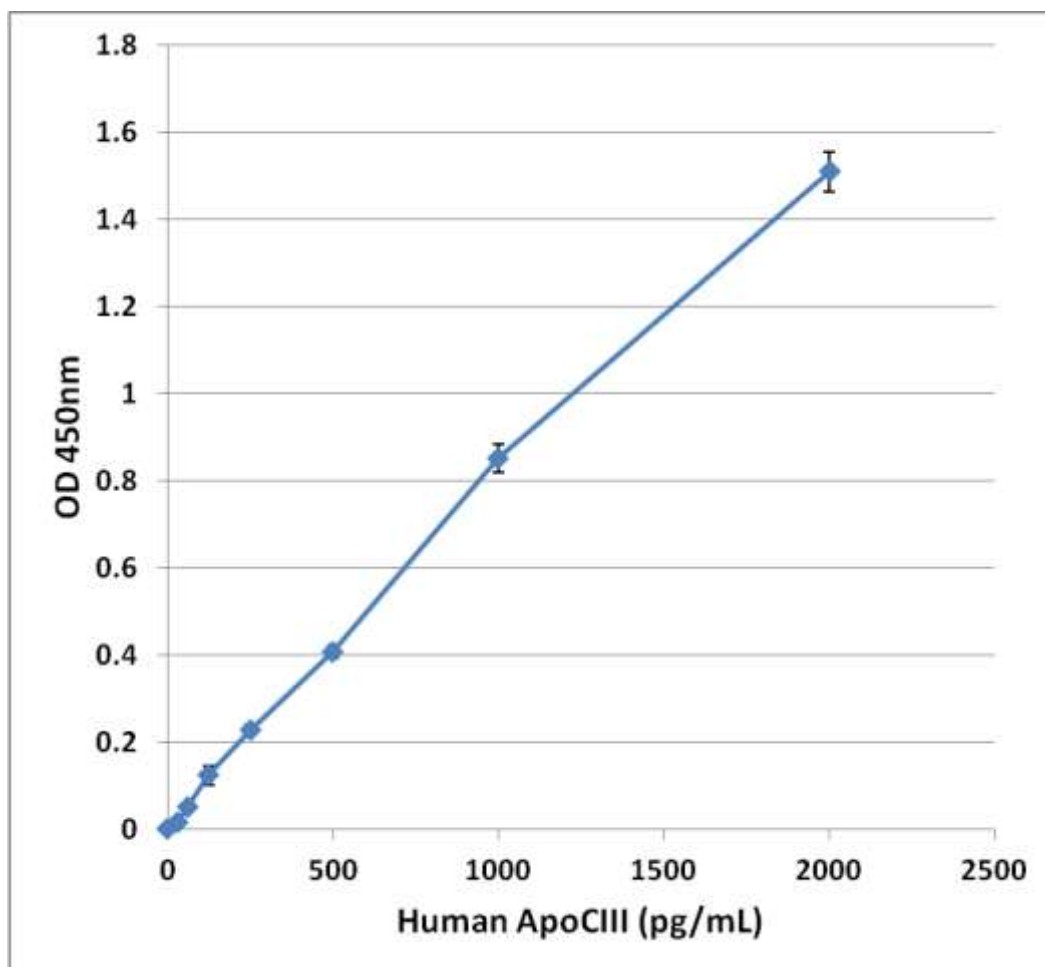
- Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4°C. Remove the plasma and assay immediately or store samples at -80°C up to three months. Normal plasma samples require at least 20,000 fold dilution or more with PBS containing 0.1% BSA immediately before running the ELISA. A sample titration may be performed to ensure sample OD values fall within the middle range of the standard curve.
- 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 at least 20,000 fold dilution or more with PBS containing 0.1% BSA immediately before running the ELISA. A sample titration may be performed to ensure sample OD values fall within the middle range of the standard curve.
- 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  $\mu$ L of ApoCIII unknown sample or standard to the Anti-ApoCIII Antibody Coated Plate. Each ApoCIII 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  $\mu$ 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  $\mu$ L of the diluted Biotinylated Anti-ApoCIII 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  $\mu$ 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  $\mu$ 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  $\mu$ 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 ApoCIII ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 2: Human ApoCIII 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. Mendivil C. O, Zheng C, Furtado J, Lel J, and Sacks F. M. *Arteriosclerosis, Thrombosis and Vascular Biology* **30** (2): 239–45, 2009.

## Recent Product Citation

Wolska, A. et al. (2020). A dual apolipoprotein C-II mimetic-apolipoprotein C-III antagonist peptide lowers plasma triglycerides. *Sci Transl Med.* **12**(528). pii: eaaw7905. doi: 10.1126/scitranslmed.aaw7905.

## **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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