
Product Manual

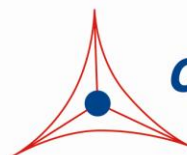
Lecithin Cholesterol Acyltransferase (LCAT) ELISA Kit

Catalog Number

STA-616

96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Cholesterol is a lipid sterol that is produced in and transported throughout the bloodstream in eukaryotes. Cholesterol is a critical compound used in the structure of cell membranes, hormones, and cell signaling. Cholesterol is transported throughout the body within lipoproteins, which have cell-specific signals that direct the lipids they transport to certain tissues. For this reason, lipoproteins exist in different forms within the blood based on their density. These include chylomicrons, very-low density lipoproteins (VLDLs), low-density lipoproteins (LDLs), intermediate-density lipoproteins (IDLs), and high-density lipoproteins (HDLs). The higher the lipid content within a lipoprotein, the lower its density. Cholesterol exists within a lipoprotein as a free alcohol and as a fatty cholesteryl ester, which is the predominant form of cholesterol transport and storage.

Lecithin Cholesterol Acyltransferase (LCAT) catalyzes the transfer of an sn-2 acyl group from phosphatidylcholine to cholesterol to form a cholesteryl ester (Figure 1). LCAT is associated with lipoproteins and plays a key role in promoting the transfer of excess cell-associated cholesterol from peripheral tissues to the liver to be excreted.

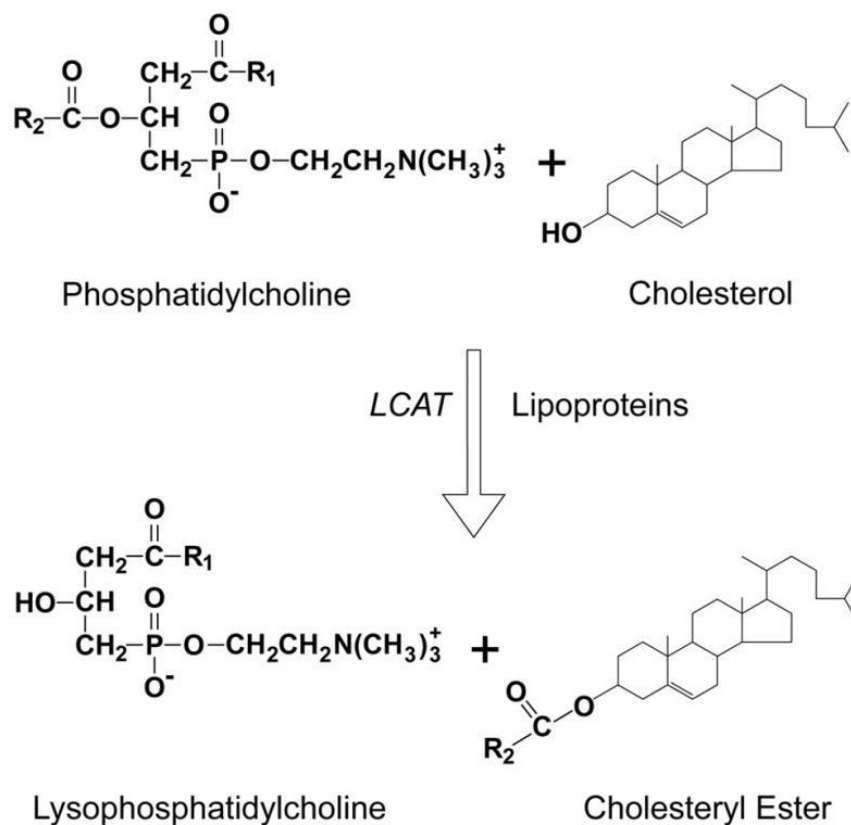


Figure 1. Conversion of free cholesterol to esterified cholesterol by LCAT

Cell Biolabs' LCAT ELISA Kit is a competitive ELISA developed for the detection and quantitation of LCAT protein in plasma, serum or other biological fluid samples of human, mouse, rat and rabbit. The kit has a detection sensitivity limit of 30 ng/mL LCAT. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-361: Human ApoAI and ApoB Duplex ELISA Kit
2. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
3. STA-390: Total Cholesterol Assay Kit
4. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
5. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
6. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
7. STA-398: Free Glycerol Assay Kit (Colorimetric)
8. STA-399: Free Glycerol Assay Kit (Fluorometric)
9. STA-610: Lipoprotein Lipase (LPL) Activity Assay Kit (Fluorometric)
10. STA-611: Lipoprotein Lipase (LPL) ELISA Assay Kit
11. STA-614: Human Cholesteryl Ester Transfer Protein (CETP) ELISA Kit
12. STA-615: Lecithin Cholesterol Acyltransferase (LCAT) Activity Kit (Fluorometric)

Kit Components

1. 96 Well Goat Anti-Mouse Antibody Coated Plate (Part No. 261601): One strip well 96 well plate.
2. Biotin-LCAT Conjugate (1000X) (Part No. 261602): One 10 µL vial.
3. Anti-LCAT Antibody (1000X) (Part No. 261603): One 15 µL vial.
4. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
5. Assay Diluent (Part No. 310804): One 50 mL bottle.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.
9. Human LCAT Protein Standard (Part No. 261604): One 300 µL vial of 8 µg/mL Human LCAT Standard in 150 mM NaCl, 10 mM Tris, 1 mM EDTA pH 7.4.

Materials Not Supplied

1. Plasma, Serum, or other Biological Fluids from Human, Mouse, Rat or Rabbit
2. Phosphate Buffered Saline (PBS)
3. PBS containing 0.1% Bovine Serum Albumin (BSA)
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir

7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, store the Human LCAT Protein Standard at -20°C and avoid multiple freeze/thaws by aliquoting. Store the remainder of the kit at 4°C.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- LCAT antibody-coated Plate: Determine the number of wells to be used, and dilute the LCAT antibody 1:1000 into assay diluent. Add 100 uL of diluted LCAT antibody to each well of the 96 Well Goat Anti-Mouse Antibody Coated Plate. Incubate for at least 2 hr at 37°C or overnight at 4°C. Remove the diluted antibody solution, blotting plate on paper towels to remove excess fluid. Wash wells 3 times with 200 uL 1X wash buffer and blot on paper towels to remove excess fluid. Add 200 uL of Assay Diluent to each well and transfer the plate to 4°C. Remove the Assay Diluent immediately before use.

Note: The LCAT antibody-coated Plate is not stable long-term. We recommend using it within 24 hours after coating.

- Biotin-LCAT conjugate: Immediately before use dilute the Biotin-LCAT conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.
- Streptavidin Enzyme Conjugate: Immediately before use dilute the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

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

Standard Tubes	Human LCAT Protein Standard (µL)	Assay Diluent (µL)	Human LCAT (ng/mL)
1	60	180	2000
2	120 of Tube #1	120	1000
3	120 of Tube #2	120	500
4	120 of Tube #3	120	250
5	120 of Tube #4	120	125
6	120 of Tube #5	120	62.5
7	120 of Tube #6	120	31
8	0	120	0

Table 1. Preparation of LCAT Standards.

Preparation of Samples

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for up to 3 months. Normal plasma samples should be diluted 4- to 10-fold with PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for up to 3 months. Normal serum samples should be diluted 4- to 10-fold with PBS containing 0.1% BSA immediately before running the ELISA.
- Cell Lysates: Collect 10^6 to 10^7 cells by centrifugation at 1000 x g for 10 minutes. Discard the supernatant and resuspend in 1 mL of cold 20 mM Tris, pH 7.5, 150 mM NaCl. Homogenize or sonicate the cell suspension. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the lysate at -80°C for up to 3 months.
- Tissue Samples: Weigh out 200 mg of tissue and mince into small pieces. Rinse the tissue with cold PBS to remove red blood cells and clots. Homogenize the minced tissue in 1 mL of cold 20 mM Tris, pH 7.5, 150 mM NaCl. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the homogenate at -80°C for up to 3 months.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each unknown sample (see Preparation of Samples section), LCAT standard (see Preparation of Standard Curve section), and blank should be assayed in duplicate.
3. Combine 55 μ L of diluted biotin-LCAT conjugate (see Preparation of Reagents section) to 55 μ L of human unknown sample or standard in a microtube and mix thoroughly.
4. Transfer 100 μ L of mixture from step 3 to each tested well of LCAT antibody-coated plate (see Preparation of Reagents section). Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash microwell strips 3 times with 250 μ L 1X Wash Buffer per well with thorough aspiration between each wash. After each wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 μ L of the diluted Streptavidin Enzyme Conjugate (See Preparation of Reagents section) to each well. Incubate at room temperature for 1 hour on an orbital shaker. During this incubation, warm Substrate Solution to room temperature.

7. Wash the strip wells 3 times according to step 5 above. Proceed immediately to the next step.
8. 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.

9. 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).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Lecithin Cholesterol Acyltransferase (LCAT) ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

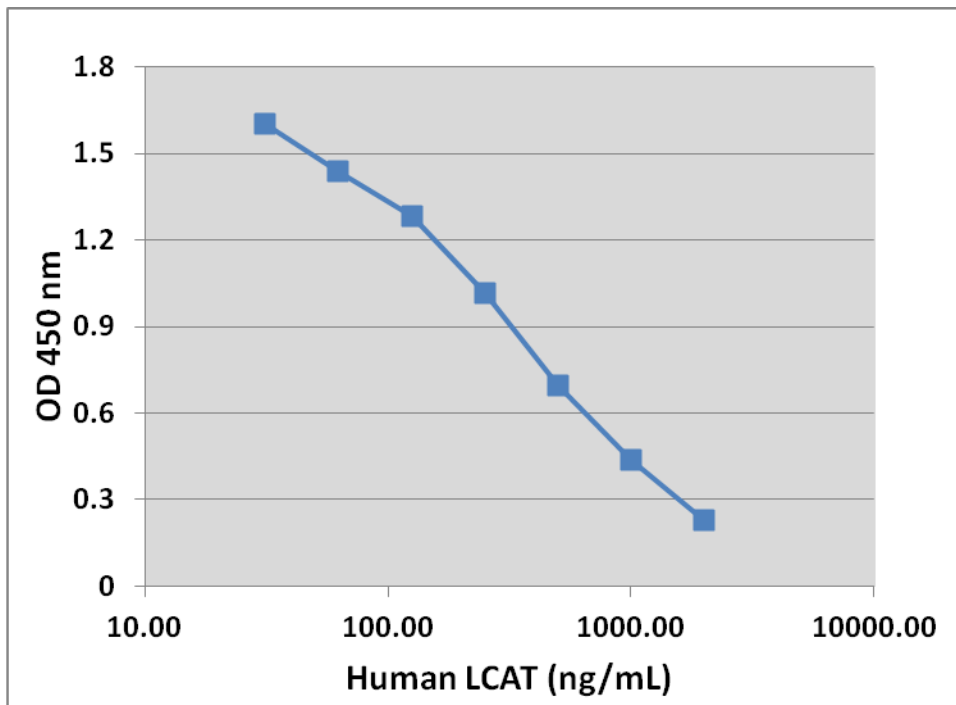


Figure 2: Lecithin Cholesterol Acyltransferase (LCAT) Standard Curve.

References

1. Rousset X, Vaisman B, Amar M, Sethi AA, Remaley AT (2009), *Curr Opin Endocrinol Diabetes Obes.* **16**: 183-71.
2. Rousset X, Shamburek R, Vaisman B, Amar M, Remaley AT (2011), *Curr Atherosler Rep* **13**:249-256
3. Dobiasova M, Frolich JJ (1999), *Clinica Chimica Acta*, **286**: 257-271

Recent Product Citation

Soupeno, E. et al. (2016). Alterations of lecithin cholesterol acyltransferase activity and apolipoprotein AI functionality in human sickle blood. *Exp Biol Med.* doi:10.1177/1535370216657447.

Warranty

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