Product Manual

Human Cholesteryl Ester Transfer Protein (CETP) ELISA Kit

Catalog Number

STA-614 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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.

Cholesteryl Ester Transfer Protein (CETP) promotes the bidirectional transfer of cholesteryl esters (CEs) as well as triglycerides (TGs) between plasma lipoproteins (Figure 1). While most of the CEs are created on HDLs by Lecithin Cholesterol Acyltransferase (LCAT), CETP serves to equilibrate CEs between HDL and other lipoproteins such as chylomicrons, VLDLs, IDLs, and LDLs.

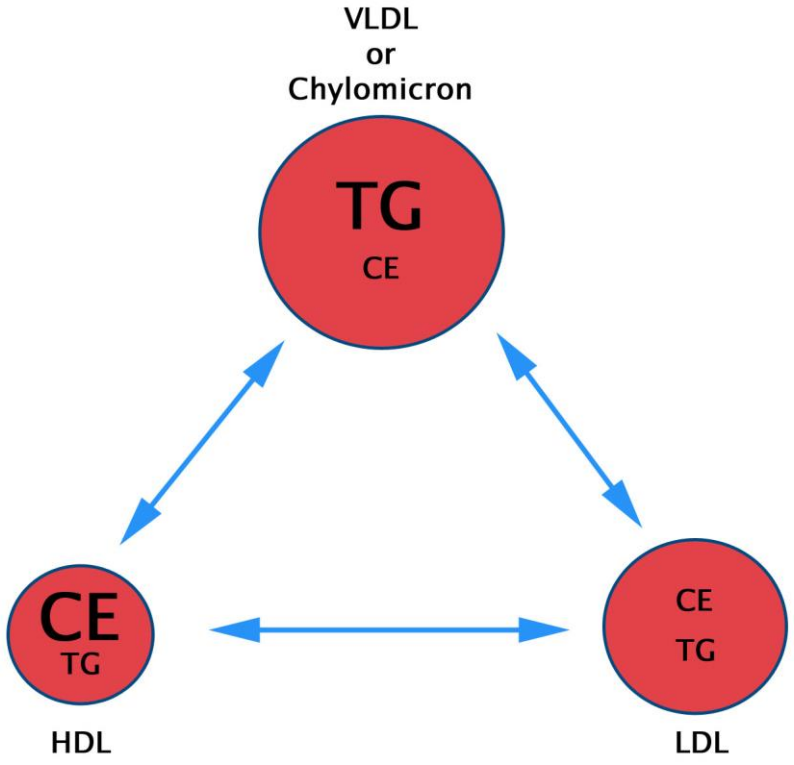

Figure 1. CETP promotes bidirectional transfer of cholesteryl esters (CE) and triglycerides (TG) between lipoproteins.

Cell Biolabs' CETP ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of Cholesteryl Ester Transfer Protein in human plasma, serum or other biological fluid samples. The kit detects CETP from human samples and has a detection sensitivity limit of 60 ng/mL CETP. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
2. STA-390: Total Cholesterol Assay Kit
3. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
4. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
5. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
6. STA-398: Free Glycerol Assay Kit (Colorimetric)
7. STA-399: Free Glycerol Assay Kit (Fluorometric)
8. STA-610: Lipoprotein Lipase (LPL) Activity Assay Kit (Fluorometric)

Kit Components

1. 96 Well Protein Binding Plate (Part No. 231001): One strip well 96 well plate.
2. CETP Conjugate (1000X) (Part No. 261401): One 20 µL vial.
3. Anti-CETP Antibody (500X) (Part No. 261402): One 15 µL vial.
4. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231009): 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. Recombinant CETP Protein Standard (Part No. 261403): One 20 µL vial of 0.4 mg/mL recombinant human CETP protein (55 kDa) in 6 M Guanidine HCl, PBS pH 8.0.

Materials Not Supplied

1. Human Plasma, Serum, or other Biological Fluids
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 kit at 4ºC.

Preparation of Reagents

- CETP Coated Plate: Determine the number of wells to be used, and dilute the CETP Conjugate 1:1000 into PBS. Add 100 uL of diluted CETP conjugate to each well of the 96-well Protein Binding Plate. Incubate for 2 hrs at 37ºC or overnight at 4ºC. Remove the diluted CETP conjugate, blotting plate on paper towels to remove excess fluid. Wash wells 3 times with PBS and blot on paper towels to remove excess fluid. Add 200 uL of Assay Diluent to each well and block for 1 hour at room temperature. Transfer the plate to 4ºC and remove the Assay Diluent immediately before use.

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

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-CETP Antibody and Secondary Antibody HRP Conjugate: Immediately before use dilute the anti-CETP antibody 1:500 and the Secondary Antibody HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of CETP standards in the concentration range of 0 to 4000 ng/mL in assay diluent (Table 1).

Standard Tubes	0.4 mg/mL CETP Standard (µL)	Assay Diluent (µL)	CETP (ng/mL)
1	4	396	4000
2	200 of Tube #1	200	2000
3	200 of Tube #2	200	1000
4	200 of Tube #3	200	500
5	200 of Tube #4	200	250
6	200 of Tube #5	200	125
7	200 of Tube #6	200	62.5
8	0	200	0

Table 1. Preparation of CETP 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 for up to three months. Normal plasma samples should be diluted 2- to 10-fold 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 for up to three months. Normal serum samples should be diluted 2- to 10-fold 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 for up to three months.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each unknown sample (see Preparation of Samples section), CETP standard, and blank should be assayed in duplicate.
3. Add 50 µL of human unknown sample or standard to the CETP Coated Plate. Incubate at room temperature for 5 minutes on an orbital shaker.
4. Add 50 µL of diluted Anti-CETP Antibody (see Preparation of Reagents section) to each tested well. Incubate at room temperature 1 hour on an orbital shaker.
5. Wash microwell strips 3 times with 250 µL 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 µL of the diluted Secondary Antibody HRP Conjugate 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 Cholesteryl Ester Transfer Protein (CETP) ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

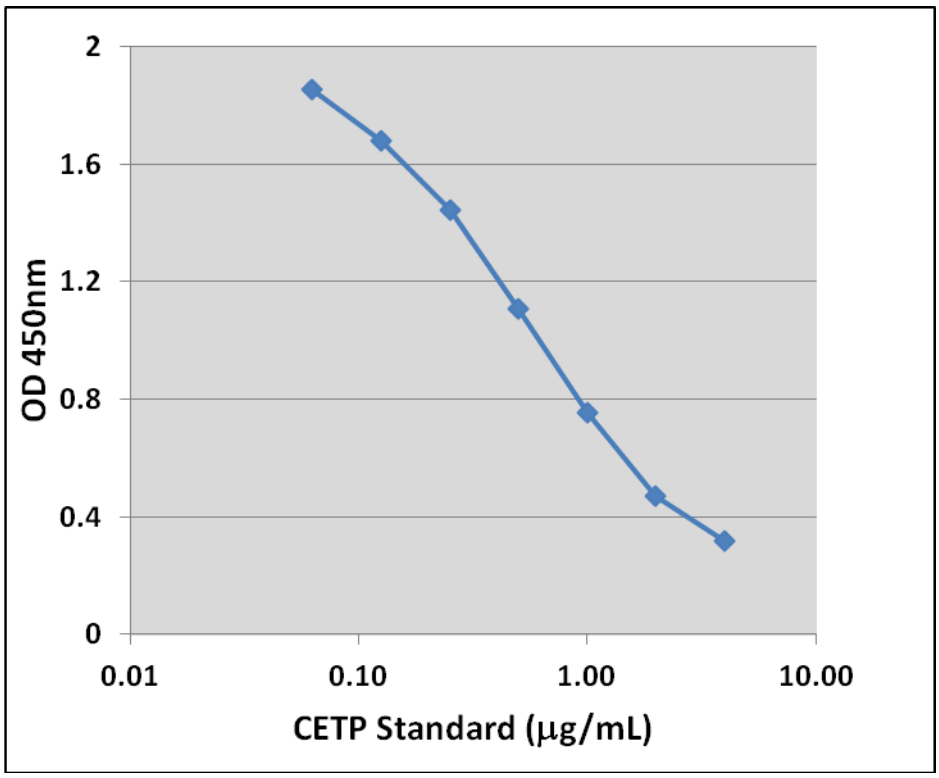

Figure 2: Cholesteryl Ester Transfer Protein (CETP) Standard Curve.

References

1. Barter PJ, Brewer HB,Chapman MJ, Hennekens CH, Rader DJ, and AR Tall (2003) *Arterioscler Thromb Vasc Biol*. **23**:160-167
2. Weber O, Bischoff H, Schmeck C, and M-F Bottcher (2010) *Cell. Mol. Life Sci.* **67**:3139–3149
3. Klerkx AH, El Harchaoui K, van der Steeg WA, Boekholdt SM, Stroes ES, Kastelein JJ, and JA Kuivenhoven (2006) *Arterioscler Thromb Vasc Biol*. **26**:706-15

Recent Product Citations

1. Otsuji, S. et al. (2022). et al. (2022). Clinical diversity and molecular mechanism of VPS35L-associated Ritscher-Schinzel syndrome. *J Med Genet*. doi: 10.1136/jmg-2022-108602.
2. Ying, Q. et al. (2022). Effect of a PCSK9 inhibitor and a statin on cholesterol efflux capacity: A limitation of current cholesterol-lowering treatments? *Eur J Clin Invest*. doi: 10.1111/eci.13766.
3. Low, H. et al. (2019). HIV disease, metabolic dysfunction and atherosclerosis: A three year prospective study. *PLoS One*. **14**(4):e0215620. doi: 10.1371/journal.pone.0215620.
4. Mihajlovic, M. et al. (2019). Changes in lecithin: cholesterol acyltransferase, cholesteryl ester transfer protein and paraoxonase-1 activities in patients with colorectal cancer. *Clin Biochem*. **63**:32-38. doi: 10.1016/j.clinbiochem.2018.11.010.

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

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