Human Cholesteryl Ester Transfer Protein (CETP) ELISA Kit

Competitive Enzyme Immunoassay for the quantification of Human Cholesteryl Ester Transfer Protein (CETP) in plasma, serum or other biological fluids samples.

Catalog number: ARG81216

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

CETP (cholesteryl ester transfer protein) is found in plasma, where it is involved in the transfer of cholesteryl ester from high density lipoprotein (HDL) to other lipoproteins. Defects in this gene are a cause of hyperalphalipoproteinemia 1 (HALP1). Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Oct 2013]

Function Involved in the transfer of neutral lipids, including cholesteryl ester and triglyceride, among lipoprotein particles. Allows the net movement of cholesteryl ester from high density lipoproteins/HDL to triglyceride-rich very low density lipoproteins/VLDL, and the equimolar transport of triglyceride from VLDL to HDL. Regulates the reverse cholesterol transport, by which excess cholesterol is removed from peripheral tissues and returned to the liver for elimination. [UniProt]

PRINCIPLE OF THE ASSAY

This is a Competitive Enzyme Immunoassay for the quantification Human Cholesteryl Ester Transfer Protein (CETP) in plasma, serum or other biological fluids samples.

The CETP protein would be coated onto a microtiter plate. CETP standards or samples are then added to the CETP protein coated ELISA plate. After a brief incubation, an anti-CETP antibody is added and competitively binding to the CETP protein on the plate, CETP protein in the samples or recombinant human

CETP protein in the standard. After washing, any antibody unbounded on the plate would be wash way. Then HRP-conjugated secondary antibody is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of CETP-antibody complex present on the wells. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm ±2nm. The concentration of Cholesteryl Ester Transfer Protein (CETP) in the sample is then determined by comparing the O.D of samples to the standard curve.

CETP protein in the samples or recombinant human CETP protein in the standards compete with the CETP-coated plate for antibody binding. High CETP protein content in a sample or high concentration CETP protein standard results in less CETP-antibody binding complex on the plate, resulting in a low signal. Conversely, low CETP protein content in a sample or low concentration CETP protein standard result in most antibody binding to the CETP protein on the plate, producing a higher signal.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store all kit components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
Microplate	1 strips X 96 wells	4°C
10X Wash Buffer	100 ml	4°C
500X Anti-CETP Antibody	15 µl	4°C
1000X HRP-Conjugated Secondary Antibody	20 µl	4°C
Recombinant CETP Protein Standard (400 µg/ml)	20 µl	-20°C
1000X CETP Conjugate (1 mg/ml)	20 µl	4°C
Assay Diluent	50 ml (Ready-to-use)	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: 620 nm as optional reference wave length)
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- 1X PBS.
- 1X PBS containing 0.1% BSA.

- Microplate shaker.
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, the standard can be stored at -20°C to avoid repeated freeze-thaw cycles. Store all other kit components at 4°C. Use the kit before expiration date.
- Briefly spin down the CETP-BSA Standard, Anti-CETP Antibody and HRP-Conjugated Secondary Antibody before use.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for few minutes before centrifugation for 10 minutes at 1000 x g at 4°C. Collect serum and assay immediately or aliquot and store samples at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles. Normal human serum samples require to dilute about 2 to 10 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant at 4°C. Centrifuge for 10 minutes at 1000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at-80°C for up to 3 months. Avoid repeated freeze-thaw cycles. Normal human plasma samples require to dilute about 2 to 10 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. Collect samples and assay immediately or aliquot and store samples at-80°C for up to 3 months. Avoid repeated freeze-thaw cycles. Dilute samples with 0.1% BSA contained 1X PBS if needed.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. Storage at 2-8°C.
- Anti-CETP Antibody and Secondary Antibody: Dilute the antibodies immediately before use, dilute the 500X Anti-CETP antibody and 1000X HRP-Conjugated Secondary Antibody into Assay Diluent to yield 1X antibody working solutions. Do not store diluted solutions.
- 1X CETP Conjugate working solution: Dilute reagent immediately before use, diluting the 1000X CETP Conjugate concentrate into 1X PBS to yield 1X CETP Conjugate working solution. (e.g. Example: Add 10 µl of 1000X CETP Conjugate concentrate into 9.990 mL of 1X PBS, mix well)

  Note: for plate coating, add 100 µl of 1X CETP Conjugate working solution for each well and incubate for overnight at 2-8°C. Aspirate each well and wash with 1× PBS, repeating the process 2 times for a total 3 washes. Add 200 µl Assay Diluent to each wells for block for 1 hr at RT.
- Sample: Normal plasma or serum samples should be diluted at 2 to 10 fold dilution with 0.1% BSA contained 1X PBS immediately before assay. If the assay found samples (diluted serum, plasma or other samples) contain CETP still higher than the highest standard, the samples can be further diluted with 0.1% BSA contained 1X PBS and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

a) Dilution 1:10: 10 µl of sample + 90 µl of 0.1% BSA contained 1X PBS (mix thoroughly).

b) Dilution 1:100: 10 µl of 1:10 diluted a) + 90 µl of 0.1% BSA contained 1X PBS (mix thoroughly).

(It is recommended to do pre-test to determine the suitable dilution factor).

- **Recombinant CETP Protein standard:** Prepare a series dilution of Recombinant CETP Protein Standard with Assay Diluent. The Assay Diluent serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

Standard No	CETP concentration (ng/ml)	Assay Diluent (µl)	Standards (µl)
S1	4000	495	5 (400 µg/ml stock)
S2	2000	250	250 µl (S1)
S3	1000	250	250 µl (S2)
S4	500	250	250 µl (S3)
S5	250	250	250 µl (S4)
S6	125	250	250 µl (S5)
S7	62.5	250	250 µl (S6)
S8	0	250	0

Note: Dilutions for the standard curve and zero standard must be made and applied to the plate immediately. S0 serves as background.

ASSAY PROCEDURE

Warm TMB Substrate Solution to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Standards and samples should be assayed in duplicates.
2. Add **50 µl of the Standards and diluted samples** into the appropriate wells on the CETP Coated Plate. Incubate for **5 minutes at RT** on a microplate shaker.
3. Add **50 µl** of the **diluted anti-CETP antibody working solution** to each well, incubate for **1 hours at RT** on a microplate shaker.
4. Aspirate each well and wash, repeating the process 2 times for a total **3 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting
5. Add **100 µl** of the **diluted HRP-conjugated secondary antibody working solution** to all wells and incubate for **1 hour at RT** on a microplate shaker.
6. **Warm TMB substrate solution to RT** before next wash step. Aspirate each well and wash as step 4. Proceed immediately to the next step.
7. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **2-30 mins at RT** on microplate shaker. Avoid exposure to light.
   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

8. Add 100 µl of Stop Solution to each well. Gently tap the plate to ensure thorough mixing. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately (optional: read at 620 nm as reference wave length).

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

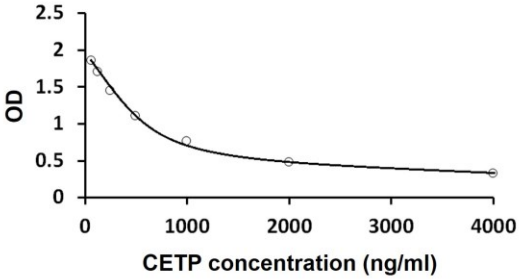
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

60 ng/ml

Assay Range

62.5 - 4000 ng/ml