Human CETP ELISA Kit

Catalog #: BG-HUM10544

User Manual

This kit is designed to quantitatively detect the levels of human CETP in serum/plasma and other suitable sample solution.
I. Introduction

Cholesteryl ester transfer protein (CETP), also called plasma lipid transfer protein, is a plasma protein that facilitates the transport of cholesteryl esters and triglycerides between the lipoproteins. It collects triglycerides from very-low-density (VLDL) or low-density lipoproteins (LDL) and exchanges them for cholesteryl esters from high-density lipoproteins (HDL), and vice versa. Most of the time, however, CETP does a heteroexchange, trading a triglyceride for a cholesteryl ester or a cholesteryl ester for a triglyceride.

Rare mutations leading to reduced function of CETP have been linked to accelerated atherosclerosis. In contrast, a polymorphism (I405V) of the CETP gene leading to lower serum levels has also been linked to exceptional longevity and to metabolic response to nutritional intervention. However, this mutation also increases the prevalence of coronary heart disease in patients with hypertriglyceridemia. The D442G mutation, which lowers CETP levels and increases HDL levels also increases coronary heart disease.

As HDL can alleviate atherosclerosis and other cardiovascular diseases, and certain disease states such as the metabolic syndrome feature low HDL, pharmacological inhibition of CETP is being studied as a method of improving HDL levels. To be specific, in a 2004 study, the small molecular agent torcetrapib was shown to increase HDL levels, alone and with a statin, and lower LDL when co-administered with a statin. Studies into cardiovascular endpoints, however, were largely disappointing. While they confirmed the change in lipid levels, most reported an increase in blood pressure, no change in atherosclerosis, and, in a trial of a combination of torcetrapib and atorvastatin, an increase in cardiovascular events and mortality.

II. Assay Principle

The Novateinbio CETP ELISA Kit is an in vitro quantitative assay for detecting CETP based on the competitive enzyme immunoassay principle.

In this assay, a biotinylated CETP is spiked into the samples and standards. The samples and standards are then added to the plate, where the biotinylated CETP competes with endogenous (unlabeled) CETP for binding to the anti-CETP antibody. After a wash step, any bound biotinylated GLP-1 then interacts with horseradish peroxidase (HRP)-streptavidin, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of captured biotinylated CETP and inversely proportional to the amount of endogenous CETP in the standard or samples. A standard curve of known concentration of CETP can be established and the concentration of CETP in the samples can be calculated accordingly.
### III. Storage

The kit except Streptavidin-HRP may be stored at -20°C to -80°C for up to 6 months from the date of shipment. For extended storage, it is recommended to store at -80°C. **Avoid repeated freeze-thaw cycles.** For prepared reagent storage, see table below.

### IV. Reagents

<table>
<thead>
<tr>
<th>Component</th>
<th>Size, Quantity</th>
<th>Storage / Stability After Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary antibody coated Microplate</td>
<td>96 wells (12 strips x 8 wells) coated with secondary antibody.</td>
<td>6 month at 4°C</td>
</tr>
<tr>
<td>Standard CETP</td>
<td>2 vials of lyophilized CETP (1 ug). 1 vial is enough to run each standard in duplicate.</td>
<td>1 week at 4°C after resuspension, 6 months at 4°C lyophilized. 12 months at -20 °C</td>
</tr>
<tr>
<td>Anti-CETP antibody</td>
<td>180 ul at 60x concentrate</td>
<td>6 month at 4°C</td>
</tr>
<tr>
<td>Biotinylated CETP</td>
<td>1 vials of Biotinylated CETP, 1 vial is enough to assay the whole plate.</td>
<td>Lyophilized, 12 months at -20 °C</td>
</tr>
<tr>
<td>HRP-Streptavidin Concentrate</td>
<td>60 µl 200X concentrated HRP-conjugated streptavidin.</td>
<td><strong>12 months at 4°C, do not freeze</strong></td>
</tr>
<tr>
<td>Assay Diluent</td>
<td>12 ml x 4</td>
<td>6 month at 4°C</td>
</tr>
<tr>
<td>Wash Buffer Concentrate (20X)</td>
<td>25 ml of 20X concentrated solution</td>
<td>24 month at RT</td>
</tr>
<tr>
<td>Chromogen A (H2O2)</td>
<td>6 ml</td>
<td>12 months at 4°C</td>
</tr>
<tr>
<td>Chromogen B (TMB)</td>
<td>6 ml</td>
<td>12 months at 4°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>6 ml 1 M sulfuric acid.</td>
<td>RT</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td></td>
<td>RT</td>
</tr>
</tbody>
</table>

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

### V. Additional Materials Required

a. Microplate reader capable of measuring absorbance at 450 nm
b. Precision pipettes to deliver 2 µl to 1 ml volumes
c. Adjustable 1-25 ml pipettes for reagent Preparation
d. 100 ml and 1 liter graduated cylinders
e. Absorbent paper
f. Distilled or deionized water
g. SigmaPlot software (or other software which can perform four-parameter logistic regression models)

h. Tubes to prepare standard or sample dilutions

i. Orbital shaker

j. Aluminum foil

k. Plastic wrap

VI. Reagent Preparation

Keep kit reagents on ice during reagent Preparation steps.

A. Preparation of Plate and Anti- CETP Antibody

1. Equilibrate plate to room temperature before opening.

2. Label removable 8-well strips as appropriate for your experiment.

3. Briefly centrifuge the anti- CETP antibody vial.

4. The antibody concentrate should then be diluted 60-fold with 1X Assay Diluent. This is your anti- CETP antibody working solution, which will be used in step 2 of Assay Procedure.

Note: The following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure)
**B. Preparation of Biotinylated CETP**

5. Briefly centrifuge the vial of Biotinylated CETP before use.

6. Dissolve in 1 ml for 10 minutes with interval vortexing. Transfer the entire contents of the vial into a tube containing 11 ml of 1X Assay Diluent. This is your Working Stock of Biotinylated CETP. Pipette up and down to mix gently.

**C. Preparation of Standards**

7. Dissolve one vial of standard (1 ug) in 1 ml Assay Diluent for at least 10 min on ice, this is 1000,000 pg/ml. Make 1 ml of 90,000 pg/ml standard by mixing 90 ul of 1000,000 pg/ml standard and 910 ul of Assay Diluent. This is Standard #1.

8. Label 7 microtubes with the following concentrations: 90,000 pg/ml, 30,000 pg/ml, 10,000 pg/ml, 3,333 pg/ml, 1111 pg/ml, 370 pg/ml, and 0 pg/ml.

Follow the table to make the standards by mixing standard with higher concentration with Assay Diluent.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration (pg/ml)</th>
<th>Volume of defined standard</th>
<th>Assay Diluent(ul)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard#1</td>
<td>90,000</td>
<td>Standard#1, 600 ul</td>
<td>0</td>
</tr>
<tr>
<td>Standard#2</td>
<td>30000</td>
<td>Standard#1, 200 ul</td>
<td>400</td>
</tr>
<tr>
<td>Standard#3</td>
<td>10000</td>
<td>Standard#2, 200 ul</td>
<td>400</td>
</tr>
<tr>
<td>Standard#4</td>
<td>3333.333333</td>
<td>Standard#3, 200 ul</td>
<td>400</td>
</tr>
<tr>
<td>Standard#5</td>
<td>1111.111111</td>
<td>Standard#4, 200 ul</td>
<td>400</td>
</tr>
<tr>
<td>Standard#6</td>
<td>370.3703704</td>
<td>Standard#4, 200 ul</td>
<td>400</td>
</tr>
<tr>
<td>Blank</td>
<td>0</td>
<td>0</td>
<td>600</td>
</tr>
</tbody>
</table>

**D. Sample Preparation**

Dilute sample 30-fold (4 μl of sample + 116 μl of 1X Assay Diluent).

Note: Optimal sample dilution factors should be determined empirically, however you may reference below for recommended dilution factors for serum: plasma/serum 1: 30 to Assay Diluent.

If you have any questions regarding the recommended dilutions you may contact technical support at techsupport@Novateinbio.com.
E. Preparation of Wash Buffer and HRP

9. If 20X Wash Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved.

10. Dilute 25 ml of Wash Buffer Concentrate into deionized or distilled water to yield 500 ml of 1X Wash Buffer.

11. Briefly centrifuge the HRP-Streptavidin vial before use.

12. Dilute the HRP-Streptavidin concentrate 200-fold with 1X Assay Diluent

VIII. Assay Procedure

1. Keep kit reagents on ice during reagent Preparation steps. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 µl of Anti-CETP Antibody to each well. Incubate for 1.5 hours at room temperature with gentle shaking (400 rpm). You may also incubate overnight at 4ºC.

3. Discard the solution and wash wells 4 times with 1X Wash Solution Buffer (200-300 µl each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 75 µl of each standard and sample in appropriate wells, followed by 75 µl of biotinylated CETP. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4ºC.

5. Discard the solution and wash 4 times as directed in Step 3.

6. Add 100 µl of prepared HRP-Streptavidin solution to each well. Incubate for 30 minutes at room temperature with gentle shaking.

7. Discard the solution and wash 4 times as directed in Step 3.

8. Mix Chromogen A and B to make 1X TMB reagent. Add 100 µl of TMB to each well. Incubate for 5 to 30 minutes or stop when there is blue color gradient formation AND visible blue color appears for 90,000 pg/mL standard (keep watching!) at room temperature in the dim light with
gentle shaking.

9. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.

IX. Assay Procedure Summary

a. Prepare all reagents, samples and standards as instructed.

b. Add 100 µl anti-CETP to each well. Incubate 1.5 hours at room temperature or overnight at 4ºC.

c. Add 75 µl standard or sample, and 75 µl biotinylated CETP to each well. Incubate 2.5 hours at room temperature or overnight at 4ºC.

d. Add 100 µl prepared Streptavidin solution. Incubate 30 minutes at room temperature.

e. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

f. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

X. Calculation of Results

Calculate the mean absorbance for each set of duplicate stands, controls, and samples and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

A. Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.
**B. Sensitivity**
The minimum detectable concentrations of CETP is 100 pg/ml.

**C. Detection Range**

370 - 90,000 pg/ml

**D. Reproducibility**

Intra-Assay: CV<8%
Inter-Assay: CV<12%

**Sample Reference value CETP** *(Shimada et al. Lipids in Health and Disease (2016) 15:57 DOI 10.1186/s12944-016-0223-6): 2.54 +/- 0.60 ug/ml*

**Reference:**


2. Chapman MJ, Le Goff W, Guerin M, Kontush A. Cholesteryl ester transfer protein: at the heart of the action of lipid-modulating therapy with statins,


