



CETP Activity Fluorometric Assay Kit II

rev. 7/19

(Catalog # K595-100; 100 assays; Store at 4°C)

I. Introduction:

Cholesteryl ester transfer protein (CETP) is a plasma protein that transfers a cholesteryl ester from HDL to LDL or VLDL in exchange for a triglyceride. HDL plays an important role in lipid metabolism and cardiovascular health. HDL transports cholesterol to the liver for excretion or to steroidogenic tissues for steroid synthesis. HDL also plays an important role in the reverse cholesterol transport pathway, removing cholesterol from lipid-filled macrophages, protecting against atherosclerosis. Because of this function, CETP is viewed as a target to increase HDL, with CETP inhibition an active area of research and several CETP inhibitors at various stages of drug development. BioVision's CETP Activity Fluorometric Assay Kit uses a lipoprotein donor molecule containing a quenched fluorescent cholesteryl ester analogue that is unquenched upon transfer to an acceptor molecule. The fluorometric intensity is directly proportional to the amount of neutral lipid transfer. Rabbit serum is provided as a positive control and CETP inhibitor torcetrapib is included for assay validation. This Assay Kit, in addition to measuring activity in plasma/serum samples, is also suitable for testing activity of recombinant CETP proteins.

II. Application:

· Measurement of CETP activity in animal serum, plasma and recombinant protein

III. Sample Type:

Animal plasma (recommended) or serum, recombinant CETP protein

IV. Kit Contents:

Components	K595-100	Cap Code	Part Number
CETP Assay Buffer	20 ml	WM	K595-100-1
Donor Molecule (8 nmol/ml)	0.5 ml	Green	K595-100-2
Acceptor Molecule	0.5 ml	Blue	K595-100-3
Positive Control (Rabbit Serum)	0.1 ml	Red	K595-100-4
Inhibitor (Torcetrapib, 1 mM)	10 µl	Yellow	K595-100-5

V. User Supplied Reagents and Equipment:

- 100% Isopropanol
- 96-well plate with flat bottom, preferably white or black plate
- Multiwell fluorescence microplate reader (fluorescence ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Kit is shipped at 4°C. Upon arrival, aliquot and store Positive Control (Rabbit Serum) at -80°C, avoid repeated freeze/thaw cycles. Store the rest of the kit components at 4°C, **protected from light.** Warm CETP Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening and keep Donor Molecule and Acceptor Molecule on ice while in use.

VII. CETP Activity Assay Protocol:

 Standard Curve Preparation: Make serial dilutions of the Donor Molecule in 100% isopropanol. Dilute Donor Molecule 100 times by adding 10 μl of Donor Molecule to 990 μl of 100% isopropanol. Dilute further by adding 250 μl of 100 times diluted donor molecule into 750 μl of 100% isopropanol and label as T5. Label four microcentrifuge tubes as T4, T3, T2 and T1 respectively. Aliquot 250 μl of isopropanol into each tube. Add 250 μl from T5 into T4 and vortex thoroughly. Transfer 250 μl from T4 into T3 and vortex, and then repeat for T2 and T1. Add 200 μl from each tube into a series of wells in 96-well plate to make 0.25, 0.5, 1, 2 and 4 pmole/well solubilized Donor Molecule Standard. Use 200 μl of isopropanol as the 0 pmole/well (Blank) Standard. Measure Fluorescence (Ex/Em = 488/523 nm) of all Standard Curve wells in endpoint mode.

Note: The Standard Curve wells should be read promptly after transferring the serially-diluted Standards from microcentrifuge tubes to the 96-well plate, to minimize the evaporation of the isopropanol.

2. Sample Preparation: Collect plasma (recommended) or serum samples by standard methods (keep on ice for immediate use or aliquot and store at -80°C for future experiments). Prepare assay reaction wells according to the table below. For human samples, we recommend adding 1-10 µl of undiluted serum/plasma per reaction (for Unknown Samples, we suggest doing a pilot experiment by testing several amounts to ensure readings are within the range of the Standard Curve). For Positive Control, dilute rabbit serum 10-fold in CETP Assay Buffer and add 10-20 µl of the diluted Positive Control in desired well(s). For the Reagent Background Control, do not add the CETP source (*i.e.* plasma/serum sample) to the reaction. Adjust the volume of all reaction wells to 200 µl with CETP Assay Buffer.

	Test Sample	Background Control	Positive Control
Donor Molecule	5 µl	5 µl	5 µl
Acceptor Molecule	5 µl	5 µl	5 µl
Sample (Plasma or Serum)	1-10 µl	_	_
Diluted Positive Control	_	_	10-20 µl
CETP Assay Buffer	to a total of 200 µl	to a total of 200 μl	to a total of 200 µl

Optional: For further verification of CETP specific activity, you may prepare CETP Inhibitor Control wells (Sample + 100 nM Torcetrapib). Dilute the included Inhibitor (Torcetrapib, 1 mM) by mixing 2 μ I of 1 mM stock with 98 μ I of DMSO to yield a 20 μ M working solution. Add 1 μ I of the 20 μ M working solution to Test Sample (with Donor Molecule and Acceptor Molecules) and make up the volume to 200 μ I with CETP Assay Buffer. Torecetrapib will inhibit rabbit CETP as well as human CETP.





3. **Measurement:** Preincubate the plate for 10 min at 37°C **protected from light** to allow the reaction temperature to equilibrate. Measure fluorescence (Ex/Em = 488/523 nm) in kinetic mode for 1-3 hr at 37°C. We strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction. Ideal measurement time for the linear range may vary depending upon the sample.

Note: Samples with very high activity (such as rabbit serum), may show a decrease in rate after 1 hr.

4. Calculation: Subtract the zero Standard (0 pmole/well) reading from all of the other solubilized Donor Molecule Standard readings. Plot the Background-subtracted values and calculate the slope of the Standard Curve. For the Test Sample reaction wells, choose two time points (T₁ and T₂) in the linear phase of the reaction progress curves, obtain the corresponding fluorescence values at those points and subtract the Reagent Background Control reading from Sample reading:

$$RFU_1 = RFU_1S - RFU_1BC$$
$$RFU_2 = RFU_2S - RFU_2BC$$

Where: RFU₁S and RFU₂S are the Sample readings at time T₁ and T₂, respectively

RFU₁BC and RFU₂BC are the Reagent Background Control readings at time T₁ and T₂, respectively

Calculate the CETP activity of the Samples Δ RFU = RFU₂ - RFU₁. Apply the Δ RFU to the Standard Curve to get *B* pmole of cholesteryl ester transferred by CETP during the reaction time ($\Delta T = T_2 - T_1$). Calculate Sample's CETP activity by using the following equation:

Sample CETP Activity =
$$\frac{B}{\Delta T \times V} \times D$$
 = pmole/hr/µl

Where: **B** is the amount of solubilized Donor Molecule from the standard curve (in pmole)

V is the Sample volume added into the reaction well (in µl)

 ΔT is the reaction time (in hours)

D is the Sample dilution factor (if applicable)

Unit Definition: One unit of CETP is the amount of protein that facilitates the transfer of 1 µmole of cholesteryl ester from the donor molecule per hour at 37°C.



Figures: (a) Solubilized Donor Molecule Standard Curve. One mole of Donor Molecule Standard corresponds to the unquenched fluorescence of cholesteryl ester analogue molecules released upon solubilization of one mole of Donor Molecule in 100% isopropanol. (b) Reaction kinetics of CETP activity in rabbit serum (1 μ l) and human plasma (4 μ l). (c) Inhibition of CETP activity from rabbit serum (1 μ l/well) by Torcetrapib. The assay was run for 1 hour and the IC₅₀ was determined to be 3.56 nM. (d) Inhibition of CETP activity from human plasma using 80 nM Torcetrapib (assay was run for 2 hours).

VIII. Related Products:

CETP Inhibitor Screening Kit II (Fluorometric) (K594) CETP Antibody (3413) CETP Polyclonal Antibody (6606) EZSolution™ Dalcetrapib (2532) Active Recombinant Human CETP (7606) Lipoproteins, Human Plasma, High Density (4934)

HDL and LDL/VLDL Quantification Colorimetric/Fluorometric Kit (K613) CETP Blocking Peptide (3413BP) Dalcetrapib (2419) Anacetrapib (2418) Torcetrapib (2420) Rabbit Serum (1267)

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FOR RESEARCH USE ONLY! Not to be used on humans.

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