



CETP Inhibitor Screening Kit (Fluorometric) II

9/14

(Catalog # K594-100; 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 being an active area of research and several CETP inhibitors at various stages of drug development. BioVision's CETP Inhibitor Screening Kit uses a self-quenched fluorescent neutral lipid that can be measured when transferred to an acceptor molecule. The fluorometric intensity is directly proportional to the amount of neutral lipid transfer. Enriched Human CETP from plasma is included for screening. A CETP inhibitor (Anacetrapib) is included as a positive control.

II. Application:

· Screening/studying/characterizing CETP inhibitors

III. Kit Contents:

Components	K594-100	Cap Code	Part Number
CETP Assay Buffer	20 ml	WM	K594-100-1
Donor Molecule	0.5 ml	Green	K594-100-2
Acceptor Molecule	0.5 ml	Blue	K594-100-3
Enriched Human CETP	1 vial	Red	K594-100-4
Inhibitor (Anacetrapib, 1 mM)	10 µl	Yellow	K594-100-5

IV. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom, preferably white or black plates
- Multi-well fluorometer (fluorescence ELISA plate reader)

V. Storage Conditions and Reagent Preparation:

Store kit at 4°C protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

- CETP Assay Buffer, Donor Molecule, and Acceptor Molecule are ready to use as supplied. Keep on ice while in use. Store at 4°C. Use within two months after opening the kit.
- Enriched Human CETP: Reconstitute with 550 µl of dH₂O, make sure the material is completely dissolved. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use.
- Inhibitor (Anacetrapib, 1 mM): Dilute 2 µI of Inhibitor with 248 µI of CETP Assay Buffer to generate a 8 µM stock solution.

VI. CETP Activity Assay Protocol:

1. **Screening Compound Preparation:** Dissolve test inhibitors in appropriate solvents to generate 100X stock solutions of the highest desired test concentration. For the Inhibitor provided, use 2 μl of the 8 μM diluted Inhibitor solution (final Anacetrapib working concentration is 80 nM) per well.

Note: Final solvent concentration should not exceed 2% of total volume. If solvent exceeds 2%, include a Solvent Control.

2. Sample Inhibitor and Enzyme/Background Control Reaction Preparation: For each well, prepare 200 µl mix containing:

	+ Inhibitor	Enzyme Control (EC)	Background Control (BC)
Donor Molecule	5 µl	5 µl	5 μl
Acceptor Molecule	5 µl	5 µl	5 μl
Enriched Human CETP	5 µl	5 µl	- ·
Inhibitor	2 µl	<u>-</u> `	-
CFTP Assay Buffer	183 ul	185 ul	190 ul

Mix well and add 200 µl mix to the appropriately assigned wells.

- Measurement: Pre-incubate at 37°C for 30 min. protected from light. Measure fluorescence (Ex/Em = 480/511 nm) in kinetic mode for 1-3 hr in a microplate reader at 37°C. Choose two points (T₁ and T₂) at least 30 min apart in the linear range of the plot and obtain the corresponding values (RFU₁ and RFU₂).
- 4. Calculation: Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (RFU₂ RFU₁) values by the time ΔT (T₂ T₁). Subtract the slope of the Background Control (BC) from the slope of the Enzyme Control (EC) and Inhibitor (S). (Optional: slope can be obtained by plotting a graph {using a program such as Excel} and taking the m value from the y = mx + b equation. Use only linear portion of graph when obtaining the m value)

$$Slope \; EC_{Corr} = \frac{\Delta RFU_{EC}}{\Delta T_{EC}} - \frac{\Delta RFU_{BC}}{\Delta T_{BC}}$$

$$Slope \ S_{Corr} = \frac{\Delta RFU_S}{\Delta T_S} - \frac{\Delta RFU_{BC}}{\Delta T_{BC}}$$





% Relative Inhibition =
$$\frac{\text{Slope EC}_{\text{Corr}} - \text{Slope S}_{\text{Corr}}}{\text{Slope EC}_{\text{Corr}}} \times 100$$

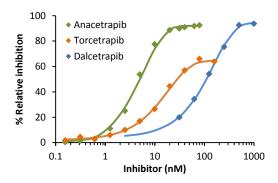


Figure: Semi-log plot using best fit 4-parameter regression to compare inhibition of Enriched Human CETP by Anacetrapib, Torcetrapib, and Dalcetrapib. The IC_{50} of Anacetrapib was determined to be 5 nM. The IC_{50} of Dalcetrapib was determined to be 112 nM. *Note: Only 64% of Enriched Human CETP activity was inhibited by 160 nM Torcetrapib.

VII. RELATED PRODUCTS:

Cholesterol/Cholesteryl Ester Quantitation Colorimetric Kit II (K623)
Cholesterol/Cholesteryl Ester Quantitation Colorimetric/Fluorometric Kit (K603)
HDL and LDL/VLDL Quantification Colorimetric/Fluorometric Kit (K613)
CETP Activity Fluorometric Assay Kit II (K595)
Rabbit Serum (1267)
CETP Antibody (3413)
Active Recombinant Human CETP (7606)
Lipoproteins, Human Plasma, High Density (4934)
Anacetrapib (2418)
Dalcetrapib (2419)
Torcetrapib (2420)

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