Product Information

CETP Inhibitor Drug Screening Kit (Fluorometric)

I. Kit Contents:

Components	K2088-100 100 assays	Cap Color	Part Number
CETP Assay Buffer	20 ml	WM	K2088-C-1
Donor Molecule	0.5 ml	Green	K2088-C-2
Acceptor Molecule	0.5 ml	Blue	K2088-C-3
Enriched Human CETP	0.1 ml	Red	K2088-C-4
Inhibitor (Anacetrapib, 1 mM)	10 µl	Yellow	K2088-C-5

II. Introduction:

Cholesteryl ester transfer protein (CETP) is a plasma protein that transfers a cholesteryl ester from high-density lipoproteins (HDL) to low-density lipoproteins (LDL) or very-low-density lipoproteins (VLDL) in exchange for a triglyceride, and vice versa. HDL plays important roles in lipid metabolism and cardiovascular health. HDL transports cholesterol to steroidogenic tissues for steroid synthesis or to the liver for excretion. HDL is also involved in the reverse cholesterol transport pathway, protecting against atherosclerosis by removing cholesterol from lipid-filled macrophages. CETP is known as a target to increase HDL.

The CETP Inhibitor Drug Screening Kit (Fluorometric) provides a sensitive, simple and convenient way for screening of CETP inhibitors in various biological fluids based on fluorometric method. The kit contains human CETP enzyme and CETP Inhibitor Anacetrapib, which provide convenience for ultimate user.

III. Application:

Screening/studying/characterizing CETP inhibitors.

IV. Sample Type:

Animal plasma (recommended) or serum, recombinant protein.

V. User Supplied Reagents and Equipment:

96-well plate with flat bottom, preferably white or black plates.

Multi-well fluorometer (fluorescence ELISA plate reader).

VI. Storage Conditions and Reagent Preparation:

Store kit at 4℃ 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℃. Use within two months after opening the kit.

Enriched Human CETP: Reconstitute with 550 µl of dH_2O, make sure the material is completely dissolved. Aliquot and store at -20℃. Avoid repeated freeze/thaw. Keep on ice while in use.

Inhibitor (Anacetrapib, 1 mM): Dilute 2 µl of Inhibitor with 248 µl of CETP Assay Buffer to generate a 8 µM stock solution.

VII. 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	---	---
CETP Assay Buffer	183 µl	185 µl	190 µl

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

3. Measurement: Pre-incubate at 37℃ 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℃. 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)

$$\text{Slope EC}_{Corr} = \Delta \text{RFU}_{EC} / \triangle T_{EC} - \Delta \text{RFU}_{BC} / \triangle T_{BC}$$

$$\text{Slope S}_{Corr} = \Delta \text{RFU}_{S} / \triangle T_{S} - \Delta \text{RFU}_{BC} / \triangle T_{BC}$$

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

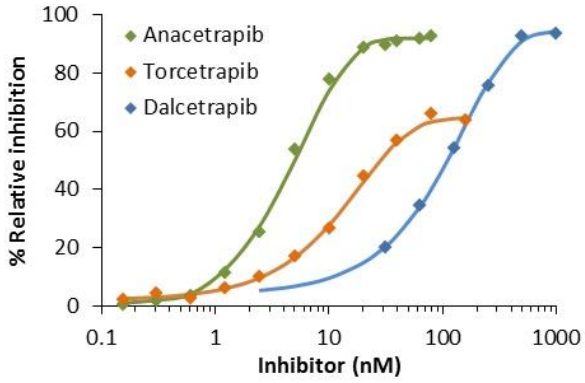

Figure: Semi-log plot using best fit 4-parameter regression to compare inhibition of Enriched Human CETP by Anacetrapib, Torcetrapib, and Dalcetrapib. The IC_{50} of Anacetrapib was determined to be 5 nM. The IC_{50} of Dalcetrapib was determined to be 112 nM.
*Note: Only 64% of Enriched Human CETP activity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

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Tel: +1-832-696-8203; Fax: +1-832-641-3177
http://www.apexbt.com/; Email: sales@apexbt.com.

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Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com