ab283403 – CETP Inhibitor Screening Kit (Fluorometric)

For the screening of potential CETP inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283403

Storage and Stability

On receipt entire assay kit should be stored at 4°C, protected from light. Upon opening, use kit within 2 months.

Materials Supplied

Item	Quantity	Storage Condition
CETP Assay Buffer	20 mL	4°C
Donor Molecule	0.5 mL	4°C
Acceptor Molecule	0.5 mL	4°C
Enriched Human CETP	1 vial	4°C
Inhibitor (Anacetrapib, 1 mM)	10 µl	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer
- 96-well black/white plate with flat bottom

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

CETP Assay Buffer, Donor Molecule, and Acceptor Molecule: Ready to use. Warm Assay Buffer to room temperature before use. 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 dH_2O, 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 µl of Inhibitor with 248 µl of CETP Assay Buffer to generate an 8 µM stock solution.

Assay Protocol

Screening Compound Preparation:

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

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

Sample Inhibitor and Enzyme/Background Control Reaction Preparation:

1. 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

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

Measurement

1. Pre-incubate at 37°C for 30 minutes protected from light.
2. Measure RFU at Ex/Em = 480/511 nm in kinetic mode for 1-3 hours at 37°C.
3. Choose any two time points (t1 and t2) at least 30 minutes apart in the linear range of the plot and obtain the corresponding values for the RFU (RFU1 and RFU2).

Calculations:

1. Calculate the slope for all samples, including Enzyme Control [EC], dividing the net ΔRFU (RFU2 – RFU1) values by the time ΔT (T2 – T1).
2. 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)

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

$$\text{Slope } S_{Corr} = \frac{\Delta RFU_{S}}{\Delta T_{S}} - \frac{\Delta RFU_{BC}}{\Delta T_{BC}}$$

$$\text{\% Relative inhibition} = \frac{slope\ of\ EC_{Corr} - slope\ of\ S_{Corr}}{slope\ of\ EC_{Corr}} \times 100$$

Where Slope of EC is the slope of Enzyme Control and Slope S is the slope of Sample Screen.

Technical Support

All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)

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