KAMIYA BIOMEDICAL COMPANY

CETP Activity Assay

For the measurement of CETP activity in animal or human serum, plasma and recombinant protein

Cat. No. KT-782

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** CETP Activity Assay is for the measurement of CETP activity in animal or human serum, plasma and recombinant protein. For research use only. Not for use in diagnostic procedures.

PRINCIPLE
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. The **K-*ASSAY*®** CETP Activity Fluorometric Assay 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. 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 serum, is also suitable for testing activity of recombinant protein.

COMPONENTS

• Donor Molecule (2.4 nmol/mL)	0.5 mL	(Green cap)
• Acceptor Molecule	0.5 mL	(Blue cap)
• CETP Assay Buffer	20 mL	(Clear cap)
• Positive Control (Rabbit Serum)	0.1 mL	(Red cap)
• Inhibitor (Torcetrapib, 1 mM)	10 µL	(Yellow cap)

USER SUPPLIED REAGENTS AND EQUIPMENT
- 100% Isopropanol
- 96-well plate with flat bottom, preferably white or black plate
- Multi-well fluorometer (fluorescence ELISA reader)

STORAGE CONDITIONS AND REAGENT PREPARATION
Kit is shipped at 4℃. Upon arrival, aliquot and store Positive Control (rabbit serum) at -20℃. Store rest of the kit components at 4℃, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. All kit components are supplied as ready to be used. Keep on ice while in use.

CETP ACTIVITY ASSAY PROTOCOL
1. **Calibration 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 eppendorf tubes as T4, T3, T2 and T1 respectively. Aliquot 250 µL of isopropanol into each tube. Add 250 µL from T5 into T4 and mix. Transfer 250 µL from T4 into T3 and mix, repeat for T2 & T1. Add 200 µL from each tube into a series of wells in 96-well plate to make 0.075, 0.15, 0.3, 0.6 and 1.2 pmol Donor Molecule Calibrator. Use 200 µL of isopropanol as 0 (blank) pmol Calibrator. Measure Fluorescence (Ex/Em = 480/511 nm). To save time, Calibration Curve can be made during sample incubation.

2. **Sample Preparation:** Collect plasma (recommended) or serum by standard methods and keep on ice for immediate use or store at -80°C. To measure sample's CETP activity, prepare 200 µL mix containing:

Donor Molecule 5 µL
Acceptor Molecule 5 µL
Sample (plasma or serum) 1-10 µL
CETP Assay Buffer To a total of 200 µL

For positive control, dilute rabbit serum 10 times and add 10 µL of diluted Positive Control instead of your sample in desired well(s). For the reagent background control, don't add the CETP source i.e. plasma, serum, or recombinant protein to the reaction and make up the volume with CETP Assay Buffer.

Notes:
a. For unknown samples, we suggest doing a pilot experiment by testing several amounts to ensure the readings are within the Calibration Curve range.
b. Using higher than recommended amounts of plasma or serum will inhibit the signal (>2 µL undiluted). Typically diluting human or rabbit plasma 10 times and measuring 2-10 µL will give a signal within range of the Calibration Curve.
c. Optional: To validate the CETP specific activity, dilute Inhibitor by adding 4 µL of Inhibitor to 496 µL of DMSO. Add 2 µL of diluted Inhibitor to the Donor Molecule, Acceptor Molecule and sample and make up the volume to 200 µL with CETP Assay Buffer. Torecetrapib will inhibit rabbit CETP as well as human CETP.

3. **Measurement:** Pre-incubate at 37°C for 30 min. protected from light to stabilize the signal. Measure fluorescence (Ex/Em = 480/511 nm) kinetically for 1-3 hr in a microplate reader at 37°C.

Note:
Incubation time depends on sample's CETP activity. We recommend measuring fluorescence in kinetic mode and choosing two time points (T1 and T2) in the linear range to calculate the CETP activity of the samples. The Calibration Curve can be read in the end point mode. High activity samples, such as rabbit serum, may have decreased activity rate after 1 hr. If you want to run the assay for longer period, use less sample.

4. **Calculation:** Subtract 0 Calibrator reading from all Calibrator readings. Plot the Donor Molecule Calibration curve. Subtract reagent background control reading from sample reading.

$RFU_1 = RFU_1S - RFU_1B$
$RFU_2 = RFU_2S - RFU_2B$
Where: **RFU_1S** & **RFU_2S** is the sample reading at time T1 and T2 respectively
RFU_1B & **RFU_2B** is the reagent background control reading at time T1 and T2 respectively

Calculate the CETP activity of the samples $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the Calibration Curve to get B pmol 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 (A) = $B/(\Delta T \times V) \times D$ = pmol/µL/hr

Where: **B** is amount of Cholesteryl ester from Calibration Curve (pmol)
V is sample volume added into the reaction well (µL)
ΔT is reaction time (hr)
D is sample Dilution factor
Unit Definition: One unit of CETP is the amount of protein that will transfer 1.0 µmoL of donor molecule per hr at 37°C.

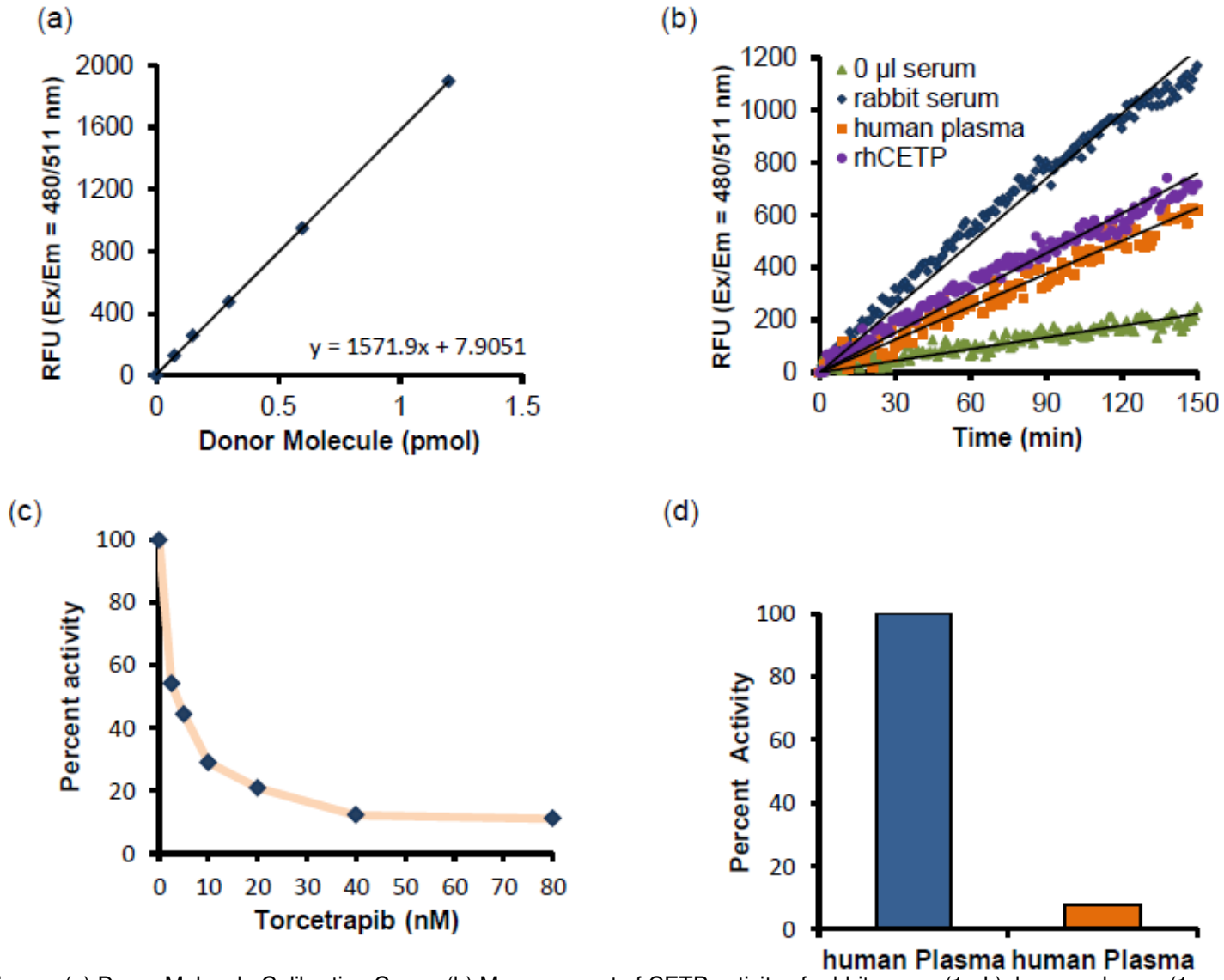

Figure: (a) Donor Molecule Calibration Curve, (b) Measurement of CETP activity of rabbit serum (1 µL), human plasma (1 µL) or recombinant human CETP (800 ng), (c) Inhibition of CETP activity from rabbit serum by Torcetrapib. The assay was run for 1 hr and the IC50 was determined to be 3.56 nM and (d) Inhibition of CETP activity from human plasma using 80 nM Torcetrapib, assay was run for 2 hrs.

FOR RESEARCH USE ONLY

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