



CETP Activity Assay Kit

Catalog Number KA0790

100 assays

Version: 05

Intended for research use only

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Introduction

Background

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. Abnova's CETP Activity 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.

- ✓ Application:
Measurement of CETP activity in animal serum, plasma and recombinant protein
- ✓ Sample Type:
Animal plasma (recommended) or serum, recombinant protein

General Information

Materials Supplied

List of component

Component	Amount
CETP Assay Buffer	20 mL
Donor Molecule (2.4 nmol/mL)	0.5 mL
Acceptor Molecule	0.5 mL
Positive Control (Rabbit Serum)	0.1 mL
Inhibitor (Torcetrapib, 1 mM)	10 μ L

Storage Instruction

Kit is shipped at 4°C. Upon arrival, aliquot and store Positive Control (rabbit serum) at -20°C. Store rest of the kit components at 4°C, 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.

Materials Required but Not Supplied

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

Precautions for Use

- ✓ For research use only! Not to be used on humans.

Assay Protocol

Assay Procedure

1. 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 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 Standard. Use 200 μL of isopropanol as 0 (blank) pmol Standard. Measure Fluorescence (Ex/Em = 480/511 nm). To save time, Standard 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:

- ✓ *For unknown samples, we suggest doing a pilot experiment by testing several amounts to ensure the readings are within the Standard Curve range.*
- ✓ *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 Standard Curve.*
- ✓ *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 Standard 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.

Data Analysis

Calculation of Results

Calculation: Subtract 0 Standard reading from all Standard readings. Plot the Donor Molecule Standard curve. Subtract reagent background control reading from sample reading.

$$RFU_1 = RFU_1S - RFU_1B$$

$$RFU_2 = RFU_2S - RFU_2B$$

Where: RFU1S & RFU2S is the sample reading at time T1 and T2 respectively

RFU1B & RFU2B 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 Standard 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:

$$\text{Sample CETP Activity (A)} = B / (\Delta T \times V) \times D = \text{pmol} / \mu\text{L} / \text{hr}$$

Where: B is amount of Cholesteryl ester from Standard 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.

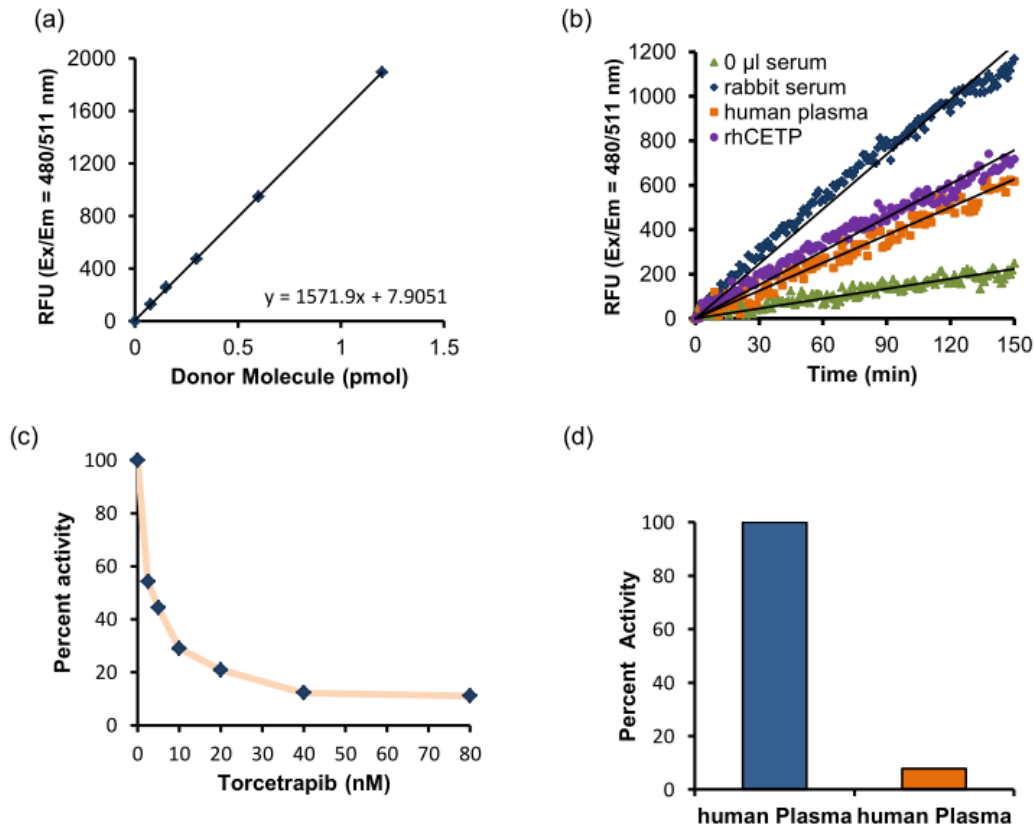


Figure: (a) Donor Molecule Standard Curve, (b) Measurement of CESTP activity of rabbit serum (1 μ L), human plasma (1 μ L) or recombinant human CESTP (800 ng), (c) Inhibition of CESTP activity from rabbit serum by Torcetrapib. The assay was run for 1 hr and the IC₅₀ was determined to be 3.56 nM and (d)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