KAMIYA BIOMEDICAL COMPANY

CETP Activity Assay

For the measurement of CETP activity in mammalian serum or plasma including mouse, rat, rabbit, horse, and human.

Cat. No. KT-149

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

PRODUCT INFORMATION

CETP Activity Assay
Cat. No. KT-149
100 tests

PRODUCT
The *-ASSAY®* CETP Activity Assay is a convenient fluorometric assay to measure CETP activity in mammalian serum and plasma including mouse, rat, rabbit, horse and human. For research use only. Not for use in diagnostic procedures.

PRINCIPLE
Cholesteryl ester transfer protein (CETP) is a member of the lipid transfer/lipopolysaccharide binding protein gene family. CETP transfers neutral lipids from high-density lipoprotein (HDL) to very low-density lipoprotein (VLDL) and is present in the plasma and serum of normal humans and animals. The CETP Activity Assay uses a donor molecule containing a fluorescent self-quenched neutral lipid that is transferred to an acceptor molecule in the presence of CETP. CETP-mediated transfer of the fluorescent neutral lipid to the acceptor molecule results in an increase in fluorescence (Excitation: 465 nm; Emission: 535 nm).

COMPONENTS

• Donor Molecule	1 mL	(Green cap)
• Acceptor Molecule	1 mL	(Blue cap)
• CETP Assay Buffer (10X)	5 mL	(Clear cap)
• Positive Control (Rabbit Serum)	30 µL	(Red cap)

PROTOCOLS

A. General Consideration for Using Fluorometer and Plate Reader
We recommend using a microtiter plate for the assay. The microtiter plates should be sealed as tightly as possible with plate sealer and incubated in a sealed, humidified chamber to prevent evaporation.

If using a regular fluorometer for sample reading, the reaction mixture should be diluted to 500 µL with 1X CETP Assay Buffer before read.

B. Preparation of Calibration Curve
Calibration curve is prepared by making serial dilutions of the donor molecule in isopropanol and subsequently recording the fluorescence intensity of each dilution, using isopropanol alone as a blank.

1. Prepare 6 test tubes labeled T0 to T5. Add 0.2 mL of isopropanol to each tube. Add additional 0.2 mL of isopropanol to the tube T5.
2. Add 2 µL Donor Molecule to T5 and vortex to mix well.
3. Transfer 0.2 mL from T5 to T4. Mix and then transfer 0.2 mL from T4 to T3. Mix and then transfer 0.2 mL from T3 to T2. Mix and then transfer 0.2 mL from T2 to T1. The Donor Molecule solution contains 0.1 mM labeled lipids and thus the calibration curve samples contain 0, 6.25, 12.5, 25, 50, 100 pmol donor molecule.
4. Read the fluorescence intensity (Ex. = 465 nm; Em. = 535 nm) of the samples from T0 to T5.
5. Apply the fluorescence intensity values of the calibration curve directly to your results to express specific activity of the serum/plasma sample (pmoles/µL sample/hr), or use Relative Fluorescence Units (RFU)/µL sample/hour.

C. Assay Procedure
1. For each reaction, add the following components:
   10 µL Donor Molecule
   10 µL Acceptor Molecule

20 μL	10X CETP Assay Buffer
1-3 μL	Your Sample (serum or plasma)
ddH_2O	To a total of 200 μL

For positive control, add 1-3 μL of Positive Control (rabbit serum) instead of your sample. Prepare a blank that contains no CETP source (contains all ingredients of the reaction mixture except for serum or plasma).

2. Incubate for 30-60 minutes at 37°C.
3. Measure the fluorescence intensity of the blank, samples, and positive control using a fluorescence plate reader or fluorometer (Ex. = 465 nm; Em. = 535 nm). Due to the nature of the self-quenched probe, background fluorescence can be significant; therefore, fluorescence intensity from each sample can be corrected by subtracting the fluorescent intensity of the blank. The increase in fluorescence intensity is usually 0.2-2 fold over blank.
4. Calculate the activity of the serum (or plasma) sample:

Y = MX + B

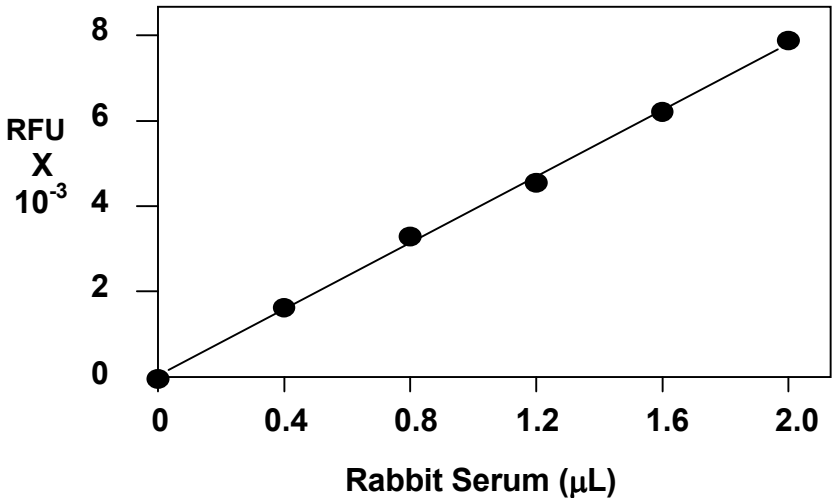
Where: Y = Fluorescence Intensity of Sample – Fluorescence Intensity of Blank
M = Slope of the Calibration Curve
X = Concentration of Serum or Plasma Sample
B = Intercept of the Calibration Curve

Example: Y = 16283-8655 = 7628
M = 74.5
B = 480
7628 = 74.5X + 480
X = 95.9 pmole/μL sample/1 hr

5. Calculate the specific CETP activity of the sample as pmole/μL/hr.

Example: If sample size = 2 μL and incubation time = 1 hr,
the specific CETP activity of the above example = 95.9 pmole/2 μL/1 hr
= 47.95 pmole/μL/hr

CETP Activity Assay with Rabbit Serum



STORAGE

Store at 4°C. The kit is stable until the expiration date shown on the label when stored at 4°C.

FOR RESEARCH USE ONLY

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