



KAMIYA BIOMEDICAL COMPANY

CETP Inhibitor Drug Screening Assay

For the screening of CETP inhibitors using a fluorometric method

Cat. No. KT-150

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

PRODUCT INFORMATION

CETP Inhibitor Drug Screening Assay

Cat. No. KT-150

100 tests

PRODUCT

The **K-ASSAY®** CETP Inhibitor Drug Screening Assay is a convenient fluorometric assay to screen CETP inhibitor samples. 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 normal human plasma and serum. The CETP Inhibitor Drug Screening Assay uses a donor molecule containing a fluorescent self-quenched neutral lipid that is transferred to an acceptor molecule in the presence of CETP (rabbit serum). CETP-mediated transfer of the fluorescent neutral lipid to the acceptor molecule results in an increase in fluorescence (Excitation: 465 nm; Emission: 535 nm). Inhibitors of CETP will prevent the lipid transfer and therefore decrease fluorescence intensity. The kit provides sufficient reagents for 100 CETP inhibitor screening assays.

COMPONENTS

- Donor Molecule 1 mL (Green cap)
- Acceptor Molecule 1 mL (Blue cap)
- CETP Assay Buffer, 10X 5 mL (Clear cap)
- Rabbit Serum (CETP source) 0.3 mL (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 a plate sealer and incubated in a sealed, humidified chamber to prevent evaporation.

If using a regular fluorometer for sample reading, the samples should be diluted to 500 μ L with 1X CETP Assay Buffer before reading.

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 μ L Donor Molecule to T5, 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.
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. The Donor Molecule solution contains 0.1 mM labeled lipids.

C. Assay Procedure

1. Prepare each testing sample in 160 μL of dH_2O . Add 3 μL of Rabbit Serum. Prepare a blank that contains no rabbit serum as background. Prepare a positive control assay containing 3 μL of Rabbit Serum, but with no inhibitors.
2. Prepare a Master Mix for each assay containing the following. Mix well.
 - 10 μL Donor Molecule
 - 10 μL Acceptor Molecule
 - 20 μL 10X CETP Assay Buffer
3. Add 40 μL of the Master Mix into each sample prepared in Step 1. Mix well and incubate at 37°C for 30-60 minutes.
4. Measure the fluorescence intensity of the blank, test samples, and positive control using a fluorescence plate reader or fluorometer (Ex. = 465 nm; Em. = 535 nm).

Note:

Due to the nature of the self-quenched probe, background fluorescence is usually high and therefore fluorescence intensity from each sample should be corrected by subtracting the background fluorescence intensity. The increase in fluorescence intensity with CETP (Rabbit Serum) is usually 1.5-2 fold over blank (Organic solvent may increase background readings and therefore proper control may be needed if the inhibitor is prepared in organic solvent).

5. Compare the fluorescence intensity of the inhibitor with positive control to determine the inhibition efficiency of the inhibitors being tested.

STORAGE

Store at 4°C. Stable until expiration date on label when stored at 4°C.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY

12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: techsupport@kamiyabiomedical.com
www.kamiyabiomedical.com