CETP Inhibitor Drug Screening Kit

Catalog Number KA0828

100 assays

Version: 01

Intended for research use only

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Introduction

Background

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 Kit 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). Inhibitor of CETP will inhibit the lipid transfer and therefore decrease fluorescence intensity. The kit provides sufficient reagents for 100 CETP inhibitor screening assays.

General Information

Materials Supplied

List of component

Component	Amount
Donor Molecule	1 ml
Acceptor Molecule	1 ml
CETP Assay Buffer (10X)	5 ml
Positive Control (Rabbit Serum)	0.3 ml

Assay Protocol

Reagent Preparation

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 samples should be diluted to 500 μl with 1X CETP Assay Buffer before reading.

Standard Curve

Standard 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, each contains 0.2 ml of isopropanol; the tube labeled T5 should contain an additional 0.2 ml of isopropanol.
2. Add 2 μl 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 standard curve directly to your results. The Donor Molecule solution contains 0.1 mM labeled lipids.

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 no testing inhibitors.
2. Prepare a Master Mix for each assay containing the follows. 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 testing inhibitor is prepared in organic solvent).

5. Comparison of the fluorescence intensity of the testing inhibitor with positive control to determine the inhibition efficiency of the testing inhibitors.