PLTP Inhibitor Drug Screening Kit (Fluorometric)

(Catalog #K605-100; 100 assays; Store at 4˚C)

I. Introduction:

Plasma phospholipid transfer protein (PLTP) is thought to play a major role in the facilitated transfer of phospholipids between lipoproteins and in the modulation of high-density lipoprotein (HDL) particle size and composition. PLTP-facilitated lipid transfer activity is related to HDL and LDL metabolism, as well as lipoprotein lipase activity, adiposity, and insulin resistance. The PLTP Drug Screening Kit utilizes rabbit serum as PLTP activity source to screen PLTP inhibitors. The assay uses a donor molecule containing a fluorescent self-quenched phospholipid that is transferred to an acceptor molecule in the presence of PLTP. PLTP-mediated transfer of the fluorescent phospholipid to the acceptor molecule results in an increase in fluorescence (Ex/Em: 465/535). Inhibitor of PLTP will inhibit the lipid transfer and therefore decrease fluorescence intensity. The kit provides sufficient reagents for 100 PLTP inhibitor screening assays.

II. Kit Contents:

Component	Volume	Cap color
PLTP Donor Molecule	1 ml	Violet
PLTP Acceptor Molecule	1 ml	Blue
PLTP Assay Buffer (10X)	5 ml	Clear
Positive Control (Rabbit Serum)	0.3 ml	Green

III. Storage Conditions and Reagent Preparation:

Kit is shipped at 4°C. Upon arrival, aliquot and store Positive Control 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.

IV. PLTP Assay Protocol:

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 and incubate 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 PLTP Assay Buffer before read.

B. Preparation of 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. The Donor Molecule solution contains 0.1 mM labeled lipids and thus the standard curve samples contain 0, 6.25, 12.5, 25, 50, 100 pmol donor molecule.
4. Read the fluorescence intensity (Ex/Em: 465/535) of the standard samples from T0 to T5.
5. Apply the fluorescence intensity values of the standard curve directly to your results to express specific activity of the plasma sample (moles/µl plasma/hr).

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 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 PLTP Assay Buffer

3. Add 40 µl of the Master Mix into each sample including the blank and positive control also as prepared in Step 1. Mix well and incubate at 37˚C for 30-60 min.
4. Measure the fluorescence intensity of the blank, test samples, and positive control using a fluorescence plate reader or fluorometer (Ex/Em: 465/535).

   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 (without rabbit serum). The increase in fluorescence intensity with PLTP (Rabbit Serum) is usually 1.5-2.0 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.

RELATED PRODUCTS:

- CETP Activity Assay and Inhibitor Drug Screening Kit
- Anti-CETP Antibody
- PLTP Activity Assay Kit
- Anti-PLTP Antibody
- Rabbit Serum
- Cholesterol Quantitation Kit
- Adipocyte Related Proteins, Antibodies, and Elisa Kits

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