PLTP Activity Assay Kit

Catalog Number KA0791

100 assays

Version: 02

Intended for research use only

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Introduction

Background

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 Activity Assay Kit 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 (Excitation: 465 nm; Emission: 535 nm)

General Information

Materials Supplied

List of component

Component	Amount
PLTP Donor Molecule	1 ml
PLTP Acceptor Molecule	1 ml
PLTP Assay Buffer (10X)	5 ml
Positive Control (Rabbit Serum)	30 µl

Storage Instruction

Store at 4℃.

Precautions for Use

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 PLTP Assay Buffer before reading.

Assay Protocol

Reagent Preparation

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 Std 0 to Std 5, each contains 0.2 ml of isopropanol; the tube labeled Std 5 should contain an additional 0.2 ml of isopropanol.
2. Add 2 µl Donor Molecule to Std 5, vortex to mix well.
3. Transfer 0.2 ml from Std 5 to Std 4. Mix and then transfer 0.2 ml from Std 4 to Std 3. Mix and then transfer 0.2 ml from Std 3 to Std 2. Mix and then transfer 0.2 ml from Std 2 to Std 1. 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. = 465 nm; Em. = 535 nm) of the samples from Std 0 to Std 5.
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).

Assay Procedure

1. For each reaction, add the following components:
   10 µl Donor Molecule
   10 µl Acceptor Molecule
   20 µl 10X PLTP 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 Rabbit Serum instead of your sample. Prepare a blank that contains no PLTP Source as background.
2. Incubate for 30 minutes to 4 hours at 37℃, preferably while monitoring fluorescence. (i.e. Kinetic for enzyme activity)
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) initially after 1-2 min (call this T_1 and absorbance = A_1). Continue to kinetics to measure a few time points throughout the incubation (T_2, T_3, ..; A_2, $A_{3,..}$). Due to the nature of the self-quenched probe, background fluorescence can be significant; therefore, fluorescence intensity from each sample should be corrected by subtracting the blank fluorescence intensity. The increase in fluorescence intensity is usually 0.2-2 fold over blank.

Data Analysis

Calculation of Results

Calculate the activity of the plasma sample:

$Y = MX + B$

Do this for initial and final readings that fit within the linear range of the standard curve (e.g. Y_1 at T_1 & A_1; Y_2 at T_2 & A_2)

Where:

Y = Fluorescence Intensity of Sample – Fluorescence Intensity of Blank

M = Slope of the Standard Curve

X = Concentration of Plasma Sample

B = Intercept

Example (Hypothetical)

Y_1 = 10000-8000 = 2000 (T = 1 min); Y_2 = 17000-8000 = 9000 (T = 2 hours)

M = 80

B = 600 & assume volume = 2 µl

Then solving; X1 = 17.5; X2 = 105; ΔX =97.5 pmol & ΔT = 119 min

So activity = (97.5)/(2*119) = 0.41 pmol/µl/min or 0.41 nmol/ml/min