APExBIO
An Apoptosis and Epigenetics Company

Product Information

PLTP Activity Fluorometric Assay Kit

I. Kit Contents:

Components	K2087-100 100 assays	Cap Color	Part Number
PLTP Assay Buffer	20 ml	WM	K2087-C-1
Donor Molecule (30 nmol/ml)	0.2 ml	Green	K2087-C-2
Acceptor Molecule	0.5 ml	Blue	K2087-C-3
Positive Control (rabbit serum)	0.1 ml	Red	K2087-C-4

II. Introduction:

Phospholipid transfer protein (PLTP) is a lipid transfer protein in human plasma and plays an critical role in transferring phospholipids from triglyceride-rich lipoproteins to high density lipoprotein (HDL). PLTP also transfers phospholipids between HDL molecules to modulate HDL composition and size, and control plasma HDL levels. PLTP plays a critical role in reverse cholesterol transport and cholesterol metabolism and may cause atherosclerosis. Therefore, PLTP is a target for pharmacological intervention.

The PLTP Activity Fluorometric Assay Kit provides a simple and convenient way for detection of PLTP activity in various samples based on fluorometric method. The assay utilizes a self-quenched fluorescent phospholipid that can be detected when transferred to an acceptor molecule. The fluorometric intensity is directly proportional to the amount of phospholipid transferred. Rabbit serum is included as a positive control. In addition to detecting PLTP activity in serum, the kit is also suited for detection of recombinant protein activity.

III. Application:

Measurement of PLTP activity in animal serum, plasma and recombinant protein.

IV. Sample Type:

Animal plasma (recommended) or serum, recombinant protein.

V. User Supplied Reagents and Equipment:

100% Isopropanol.

96-well plate with flat bottom, preferably white plate.

Multi-well fluorometer (fluorescence ELISA reader).

VI. Storage Conditions and Reagent Preparation:

Kit is shipped at 4℃. Upon arrival, aliquot and store Positive Control (rabbit serum) at -20℃. Store rest of the kit components at 4℃, 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.

VII. PLTP Activity Assay Protocol:

1. Standard Curve Preparation: Make serial dilutions of the Donor Molecule in 100% isopropanol. Dilute Donor Molecule 100 times by adding 10 µl of Donor Molecule to 990 µl of 100% isopropanol. Dilute further by adding 100 µl of 100 times diluted donor molecule into 900 µl of 100%

isopropanol and label as T5. Label four Eppendorf tubes as T4, T3, T2 and T1 respectively. Aliquot 250 µl of isopropanol into each tube. Add 250 µl from T5 into T4 and mix. Transfer 250 µl from T4 into T3 and mix, and continue for T2 and T1. Add 200 µl from each tube into a series of wells in 96-well plate to generate 0.375, 0.75, 1.5, 3.0 , 6.0 pmol Donor Molecule Standard. Use 200 µl of 100% isopropanol as 0 pmol (blank) Standard. Measure Fluorescence (Ex/Em = 465/535 nm). To save time, Standard Curve can be made during sample incubation.

2. Sample Preparation: Collect plasma or serum by standard methods and keep on ice for immediate use or store at -80℃. To measure sample's PLTP activity, prepare 200 µl mix containing:

Donor Molecule	2 µl
Acceptor Molecule	5 µl
Sample (plasma or serum)	1 - 8 µl
PLTP Assay Buffer To a total of 200 µl	

For positive control, dilute rabbit serum 10 times in Assay Buffer and add 8 µl of diluted Positive Control instead of your sample in desired well(s). For the reagent background control, don't add the PLTP source i.e. plasma, serum, or recombinant protein to the reaction and make up the volume with PLTP Assay Buffer.

Notes:

a. For unknown samples, we suggest doing a pilot experiment by testing several amounts to ensure the readings are within the Standard Curve range.

b. Using higher than recommended amounts of plasma or serum will inhibit the signal (> 2 µl undiluted). Typically diluting human or rabbit plasma 10 times and measuring 2 - 10 µl will give a signal within range of the Standard Curve.

3. Measurement: Pre-incubate at 37℃ for 10 min. protected from light to stabilize the signal. Measure fluorescence (Ex/Em = 465/535 nm) kinetically for 1-3 hr in a microplate reader at 37℃.

Note:

Incubation time depends on sample's PLTP activity. We recommend measuring fluorescence in kinetic mode and choosing two time points (T1 and T2) in the linear range to calculate the PLTP activity of the samples. The Standard Curve can be read in the end point mode.

4. Calculation: Subtract 0 Standard reading from all Standard readings. Plot the Donor Molecule Standard curve. Subtract reagent background control reading from sample reading.

$$RFU_1 = RFU_1S - RFU_1B$$

$$RFU_2 = RFU_2S - RFU_2B$$

Where: RFU_1S & RFU_2S is the sample reading at time T_1 and T_2 respectively

RFU_1B & RFU_2B is the reagent background control reading at time T_1 and T_2 respectively Calculate the PLTP activity of the samples $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the Standard Curve to get B pmol of phospholipids transferred by PLTP during the reaction time ($\Delta T = T_2 - T_1$). Calculate sample's PLTP activity by using the following equation:

$$\text{Sample PLTP Activity (A)} = B/(\Delta T \times V) \times D = \text{pmol/ml/hr} = \text{mU/ml}$$

Where: B is amount of Phospholipid from Standard Curve (pmol)

V is sample volume added into the reaction well (ml)

ΔT is reaction time (hr)

D is sample Dilution factor

Unit Definition: One unit of PLTP is the amount of protein that will transfer 1.0 nmol of donor molecule per hr at 37℃.

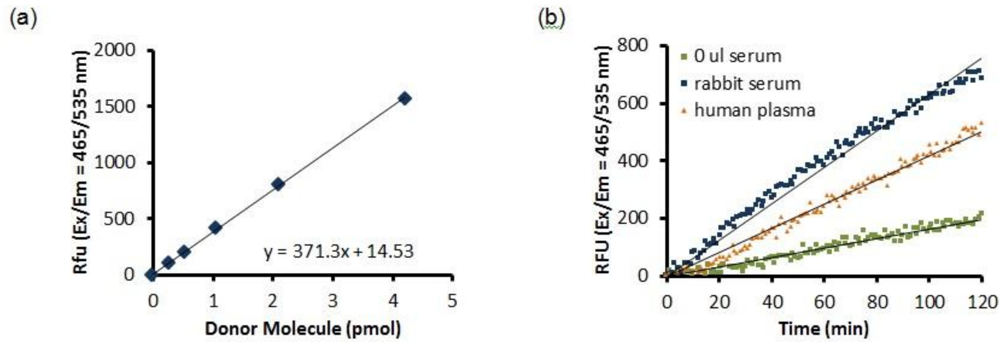

Figure: (a) Donor Molecule Standard Curve (b) Measurement of PLTP activity in rabbit serum (1 µl) or human plasma (1 µl).

Frequently Asked Questions:

1. The enzyme does not fully dissolve in 220 µl of Assay Buffer. Will this affect the results?

The enzyme solution is likely super-saturated and hence some material is insoluble. We have tested the assay with 100% solubilized enzyme and 50% solubilized enzyme and the results were identical. The enzyme solution can be warmed up briefly for 1 - 2 minutes at 37℃ to help solubilize more enzyme.

2. The probe changes to red color after dissolving. Does this affect the assay?

The probe changing to red color after dissolving in DMSO should not affect the quality of the assay. The probe will always be pink to red and the extent of redness depends on the level of oxidation. However, a pinkish/mild red color should not affect the assay significantly.

3. To measure free cholesterol and total cholesterol, are two separate sets of standard curves needed?

No. Only one standard curve after adding the cholesterol esterase should be produced.

4. In serum both esterified and non-esterified cholesterol is detectable. From tissue homogenates only the non-esterified version is detected. Why?

When tissues are homogenized, cellular enzymes including Esterases are released. These enzymes hydrolyze the esterified cholesterol to non-esterified cholesterol.

5. Can EDTA be used for blood collection for this assay?

Yes, EDTA will be fine.

6. Can this kit measure free or protein-bound cholesterol?

This kit can measure both free and apolipoprotein-bound forms of cholesterol.

7. What is the detailed principle of measurement?

Cholesterol esterase hydrolyzes cholesterol ester into free cholesterol. Then cholesterol oxidase in the enzyme mix oxidizes the free cholesterol to generate H_2O_2. H_2O_2 then interacts with the cholesterol probe to generate color/fluorescence.

8. Can this kit be used with tissue samples, e.g. liver?

Yes, tissue samples can be used with this kit. It is critical to homogenize the tissue in chloroform:Isopropanol: NP-40 for effective extraction of lipids from the sample. Please follow the instructions on the Datasheet for further details.

9. Can the Absorbance for this kit be read at 620nm instead of 570nm?

All spectrophotometers have a window within which they are equally accurate. Typically this window is +/-20 to +/-40nm. 620nm seems a bit far-stretched for 570nm recommended wavelength for this assay but I would suggest checking the instrument specifications.

10. Can frozen tissue be used with this kit?

Although not ideal, flash-frozen tissue or cells stored at -80℃ can be used. It is important to remember that results may vary between fresh and frozen samples.

11. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

12. Can alternate buffers for sample preparation (cell lysis, sample dilutions etc) be used?

Our assay buffers are optimized for the reactions in this assay. They not only contain some detergents for efficient lysis of your cells, but also contain some proprietary components required for the detection reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

13. Is it essential to make a standard curve for every expt, or is one curve/kit enough?

Yes, we strongly recommend you measure the standards every time you do an experiment. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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