RayBio® PLTP Activity Assay Kit

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RayBio® PLTP Activity Assay Kit Protocol

(Cat#: 68AT-PLTP-S100)

RayBiotech, Inc.

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TABLE OF CONTENTS

I. INTRODUCTION

Phospholipid transfer protein (PLTP) plays an important role in transferring phospholipids between HDL molecules to modulate HDL size and composition and controlling plasma HDL levels. PLTP also transfers phospholipids from lipoproteins to HDL. PLTP plays a key role in reverse cholesterol transport and may promote atherosclerosis. PLTP is, therefore, considered a promising target for pharmacological intervention. BioVision's PLTP Activity Fluorometric Assay Kit II uses a self-quenched fluorescent phospholipid that can be measured when transferred to an acceptor molecule. The fluorometric intensity is directly proportional to the amount of phospholipid transferred.
Rabbit serum is provided as a positive control. This Assay Kit, in addition to measuring activity in serum, is also suitable for testing activity of recombinant protein.

II. REAGENTS

Components	PLTP-S100	Cap Code	Part Number
PLTP Assay Buffer	20 mL	WM	Item A
Donor Molecule (30 nmol/ml)	0.2 mL	Green	Item B
Acceptor Molecule	0.5 mL	Blue	Item C
Positive Control (Rabbit Serum)	0.1 mL	Red	Item D

User Supplies Reagents and Equipment:

- 100% Isopropanol
- 96-well plate with flat bottom, preferable white plate.
- Multi-well fluorometer (fluorescence ELISA reader)

III. REAGENT STORAGE & PREPARATION

Kit is shipped at 4°C. Upon arrival, aliquot and store Positive Control (rabbit serum) 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 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 (b lank) 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°C. 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°C for 10 min. protected from light to stabilize the signal. Measure fluorescence (Ex/Em = 465/535nm) kinetically for 1-3 hr in a microplate reader at 37°C.

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.

$$\mathbf{RFU_1 = RFU_1S - RFU_1B}$$
$$\mathbf{RFU_2 = RFU_2S - RFU_2B}$$

Where: $\mathbf{RFU_1S}$ & $\mathbf{RFU_2S}$ is the sample reading at time T1 and T2 respectively.
$\mathbf{RFU_1B}$ & $\mathbf{RFU_2B}$ is the reagent background control reading at time T1 and T2 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 transfered by PLTP during the

reaction time (ΔT = T2 - T1). Calculate sample's PLTP activity by using the following equation:

Sample PLTP Activity (A) = B/(ΔT x V) X D = pmol/ml/hr = mU/ml

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

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

ΔT us 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°C.

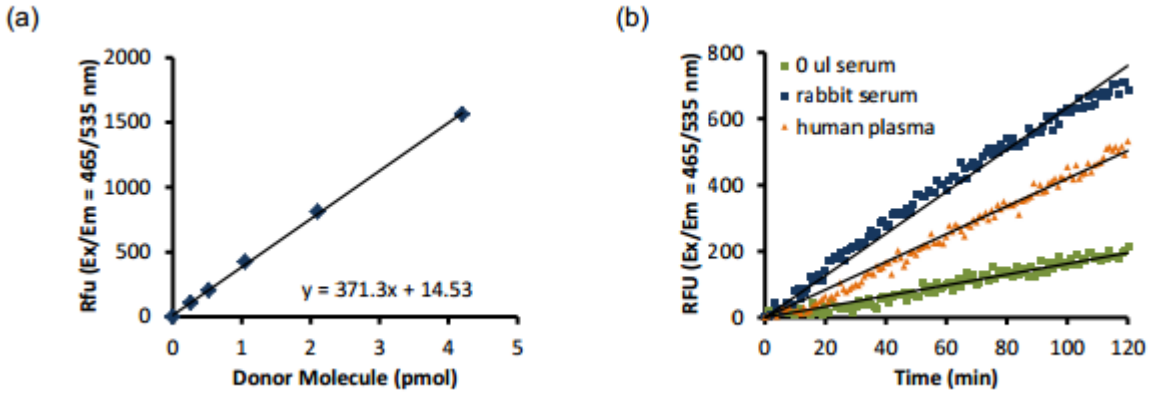

Figure: (a) Donor Molecule Standard Curve (b) Measurement of PLTP activity in rabbit serum (1 µl) or human plasma (1 µl).

This product is for research use only.