Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

PLTP Activity Fluorometric Assay Kit II

rev. 3/16

(Catalog # K593-100; 100 assays; Store at 4°C)

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. Application:

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

III. Sample Type:

Animal plasma (recommended) or serum, recombinant protein

IV. Kit Contents:

Components	K593-100	Cap Code	Part Number
PLTP Assay Buffer	20 ml	WM	K593-100-1
Donor Molecule (30 nmol/ml)	0.2 ml	Green	K593-100-2
Acceptor Molecule	0.5 ml	Blue	K593-100-3
Positive Control (rabbit serum)	0.1ml	Red	K593-100-4

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°C. Upon arrival, aliquot and store Positive Control (rabbit serum) at -80°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.

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°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 = 465/535 nm) 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 (T_1 and T_2) 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.

155 S. Milpitas Blvd., Milpitas, CA 95035 USA | T: (408) 493-1800 F: (408) 493-1801 | www.biovision.com | tech@biovision

$$RFU_1 = RFU_1S - RFU_1B$$
$$RFU_2 = RFU_2S - RFU_2B$$

Where: **RFU_1S** & **RFU_2S** is the sample reading at time T1 and T2 respectively
RFU_1B & **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 transferred by PLTP during the reaction time ($\Delta T = T_2 - T_1$). 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 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°C.

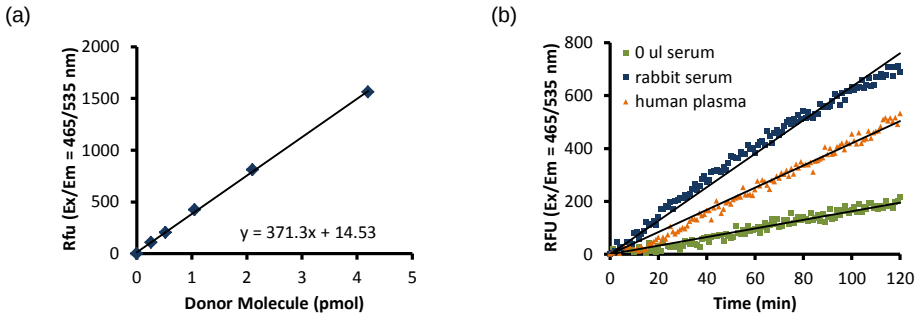

Figure: (a) Donor Molecule Standard Curve (b) Measurement of PLTP activity in rabbit serum (1 µl) or human plasma (1 µl).

VIII. Related Products:

PLTP Antibody (3595)
CETP Activity Fluorometric Assay Kit II (K595)
CETP Inhibitor Screening Kit II (Fluorometric) (K594)
Lipoproteins, Human Plasma, High Density (4934)
CETP Polyclonal Antibody (6606)
Rabbit Serum (1267)
PLTP Blocking Antibody (3595BP)
HDL and LDL/VLDL Quantification Colorimetric/Fluorometric Kit (K613)
CETP Antibody (3413)
CETP Blocking Peptide (3413BP)
Active Recombinant Human CETP (7606)

FOR RESEARCH USE ONLY! Not to be used on humans.