PLTP Activity Assay Kit

(Catalog #K604-100; 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 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 (Ex/Em: 465/535 nm).

II. Kit Contents:

Components	Volume	Cap Code	Part No.
PLTP Donor Molecule	1 ml	Violet	K604-100-1
PLTP Acceptor Molecule	1 ml	Blue	K604-100-2
PLTP Assay Buffer (10X)	5 ml	Clear	K604-100-3
Positive Control (Rabbit Serum)	30 µl	Red	K604-100-4

III. 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 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.

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 Std0 to Std5, each contains 0.2 ml of isopropanol; the tube labeled Std5 should contain an additional 0.2 ml of isopropanol.
2. Add 2 µl Donor Molecule to Std5, vortex to mix well.
3. Transfer 0.2 ml from Std5 to Std4. Mix and then transfer 0.2 ml from Std4 to Std3. Mix and then transfer 0.2 ml from Std3 to Std2. Mix and then transfer 0.2 ml from Std2 to Std1. 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 nm) of the standard samples from Std0 to Std5.
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).

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

C. Assay Procedure:

1. For each reaction, add the following components:

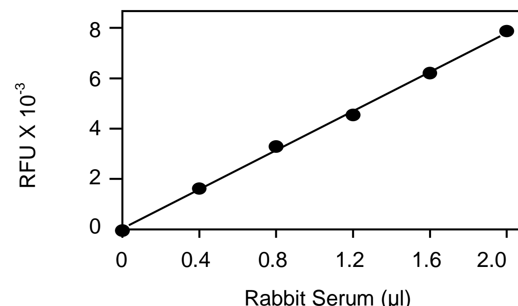
10 µl	Donor Molecule
10 µl	Acceptor Molecule
20 µl	10X PLTP Assay Buffer
1-3 µl	Your Sample (serum or plasma)
ddH_2O	To a total of 200 µl

   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 min to 4 hrs at 37°C, preferably while monitoring fluorescence (i.e. Kinetics for Enzyme Activity).
3. Measure the fluorescence intensity of the blank, samples, and positive control using a fluorescence plate reader or fluorometer (Ex/Em: 465/535 nm) initially after 1-2 min (call this T_1 and absorbance = A_1). Continue to monitor the 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.
4. 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 9 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: Y_1 = 10000-8000 = 2000 (T= 1 min); Y_2 = 17000-8000 = 9000 (T= 2 hrs)
   (Hypothetical) M = 80; B = 600 & assume Volume = 2 ul
   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

PLTP Activity Assay



GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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