DRG® PLTP Activity Assay Kit (EIA-4415)

Revised 17 Aug. 2007 **RUO**

1 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. For Research Use Only.

2 TEST PRINCIPLE

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).

3 KIT CONTENTS

100 assays; Store at 4°C)

Component	Volume	Cap color
PLTP Donor Molecule	1 ml	Violet
PLTP Acceptor Molecule	1 ml	Blue
PLTP Assay Buffer (10X)	5 ml	Clear
Positive Control (Rabbit Serum)	30 µl	Red

4 PLTP ASSAY PROTOCOL

4.1 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 read.

4.2 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 T0 to T5, each contains 0.2 ml of isopropanol; the tube labeled T5 should contain an additional 0.2 ml of isopropanol.
2. Add 2 µl Donor Molecule to T5, vortex to mix well.
3. Transfer 0.2 ml from T5 to T4. Mix and then transfer 0.2 ml from T4 to T3.
   Mix and then transfer 0.2 ml from T3 to T2. Mix and then transfer 0.2 ml from T2 to T1.
   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 standard samples from T0 to T5.

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).

4.3 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.

2. Incubate for 30-60 minutes at 37°C.
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). 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

   Where: Y = Fluorescence Intensity of Sample – Fluorescence Intensity of Blank
   M = Slope of the Standard Curve
   X = Concentration of Plasma Sample
   B = Y – Intercept

   Example: Y = 16206 – 8956 = 7858
   M = 79.5
   B = 595
   7858 = 79.5 + 595
   X = 91.35 pmol/µl sample/1 hr