Roar

Product Information

Roar PLTP Activity Assay Kit, 100 assays

Phospholipid Transfer Protein Activity Assay Kit
Catalog No. P7700
U.S. Pat. Nos. 7,618,784; 7,851,223

Assay Method:	Fluorometric
Number of Assays:	100 assays in 0.1 ml assay volume
Kit Contents:	Donor particle: 300 µl Acceptor particle: 5 ml Assay buffer: 5 ml
Storage and Handling:	Donor particle: store at room temperature Acceptor particle: store at 4°C If stored properly, components are stable for up to 1 year. DO NOT FREEZE.
Instrumentation:	Fluorescence spectrophotometer: cuvette or microplate reading formats. Excitation: 465 nm / Emission: 535 nm.

Overview

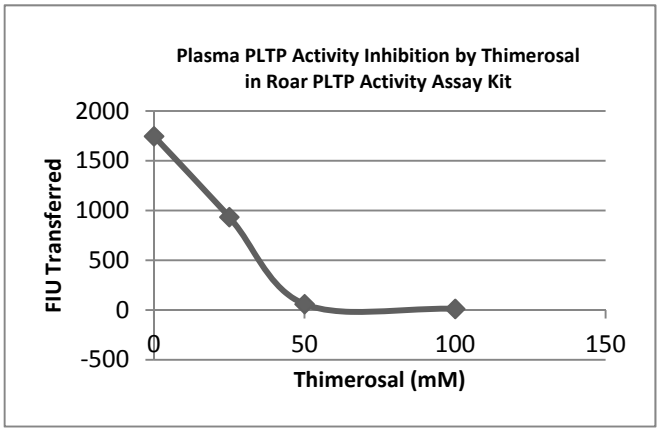
Phospholipid transfer protein (PLTP) is a protein present in normal human plasma. PLTP transfers phospholipids among lipoproteins in plasma. The **Roar PLTP Activity Assay Kit** includes proprietary substrates to detect PLTP mediated transfer of fluorescent substrate. Transfer activity results in increased fluorescent emission intensity from the assay. Interassay coefficient of variation: 3% (Schlitt, 2009; Hudgins, 2003).

Materials Required, But Not Supplied

- Fluorimeter with appropriate wavelength capabilities (Ex: 465 nm; Em: 535 nm)
- Black microtiter plates (recommended: U-bottom, black microplates from Thermo Scientific #7205 or #7005)
- 37°C water bath / incubator
- PLTP source: plasma or serum (fresh or frozen)

Assay Validation

The steps to validate the assay were adapted from a published reference (*J Lipid Res.* 1999;40(4):654-664) using ethylmercurithiosalicylate (thimerosal) to inhibit PLTP activity. Plasma (1:10 dilution) was pre-incubated with different concentrations of thimerosal at room temperature for 30 minutes according to the *Methods* of the reference. Roar PLTP Activity Assays were set up with donor / acceptor / buffer at each respective thimerosal concentration and 15 µl of each plasma dilution was then added. Assays were incubated for 30 minutes at 30° C, and then read in a fluorimeter (Ex 465 nm; Em 535 nm).



Assay Method

1. Add 3 - 5 µl of PLTP source (plasma or serum - fresh or frozen) to the microplate wells. Add a mixture of 3 µl donor and 44 - 42 µl of assay buffer to the wells. Then, add 50 µl acceptor to the wells. Note: the total assay volume should be 0.1 ml.

2. Incubate for 8 – 20 minutes at 37°C

3. Read the plate in a fluorimeter at excitation wavelength of 465 nm and emission wavelength of 535 nm

Standardization

The concentration of fluorescent substrate in the donor particle is listed on the vial label. A standard curve is generated by dispersing a sample of the donor in isopropanol to derive a fluorescence intensity-to-nMoles of substrate relationship. This will allow you to calculate pmoles transferred by your samples in the assay.
NOTE: Do not incubate the standard curve.

1. Spectrally pure (HPLC grade or better) isopropanol is used as the solvent. Please note: there should be no background fluorescence when isopropanol alone is read at EX 465 nm / EM 535 nm.

2. Prepare six test tubes labeled from ‘T0’ to ‘T5’ each containing 1 ml isopropanol; add an additional 1 ml of isopropanol to ‘T5’.

3. Pipette 5 µl of PLTP donor particle to the test tube labeled ‘T5’; thoroughly mix (vortex) to adequately disperse the donor particle in the isopropanol.

4. Transfer 1 ml ‘T5’ to the test tube labeled ‘T4’. Mix and pipette 1 ml from tube ‘T4’ to tube ‘T3’, vortex tube ‘T3’. Pipette 1 ml from tube ‘T3’ to tube ‘T2’, vortex tube ‘T2’. Pipette 1 ml from tube ‘T2’ to tube ‘T1’, vortex. Use tube ‘T0’ as an isopropanol blank in the standard curve.

5. Read the fluorescence intensity (EX 465 / EM 535) of the samples from tubes ‘T0’ to ‘T5’. For example, pipette 100 µl of each tube to a plate and read the plate.

6. The standard curve is created by plotting the fluorescence intensity units of ‘T5’ to ‘T0’ versus the pmole amounts read (80, 40, 20, 10, 5, 0 pmole).

7. Next, from the fluorescence intensity values of the samples that you have measured in the assay (plasma or serum) subtract the buffer blank (assays performed without plasma or serum (i.e. negative control)) to obtain the fluorescence intensity units transferred during the incubation time.

8. The units transferred may then be applied directly to the standard curve to determine pmoles of substrate transferred during the incubation.

Assay Tips

This assay is best set up on ice --- it progresses fairly rapidly even with plasma as the source. A good routine (until you get used to it) is to:

1. Chill your buffer and the acceptor on ice.
2. Place the microplate you will be using in a tray on wet ice, let it chill, then add the components while chilling.
3. The donor can be left at room temperature during the assay set up.
   NOTE: Store the donor at room temperature.

Good results may be obtained by pre-mixing the components, but they must be chilled and kept cold before adding the mixture to the plate. With this assay, temperature and volume are critical factors.

If you try to determine kinetic parameters by varying the components -- it will not work. The components must be present in the specified ratio for the assay to work properly. Any changes will increase the spontaneous transfer in the assay.

For Research Use Only. Not for Diagnostic or Therapeutic Purposes.

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