Product Information

Roar PLTP Activity Assay Kit, 250 assays
Phospholipid Transfer Protein Activity Assay Kit
Catalog No. P7700
U.S. Pat. No. 7,618,784 and patents pending

Assay Method: Fluorometric

Number of Assays: 250 assays in 0.1 ml assay volume

Kit Contents: Donor particle: 0.75 ml
Acceptor particle: 12.5 ml
Assay buffer: 5 ml 10X

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.

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%21

Materials Required, But Not Supplied
Fluorimeter with appropriate wavelength capabilities (Ex: 465 nm; Em: 535 nm)
Black microtiter plates (recommended: U-bottom, black Thermo Electron cat. #7205 or VWR cat. #25227-304)
37°C water bath / incubator
PLTP source: plasma or serum (fresh or frozen)

Assay Method
1. Using the assay buffer provided with the kit, reconstitute the buffer with distilled water (5 ml 10X assay buffer plus 45 ml dH₂O) to yield 50 ml of 1X assay buffer (150 mM NaCl, 10 mM Tris, 2 mM EDTA, pH 7.4).
2. 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 reconstituted assay buffer to the wells. Then, add 50 μl acceptor to the wells. Note: the total assay volume should be 0.1 ml.
3. Incubate for 8 – 20 minutes at 37°C
4. Read assay in a fluorescence spectrometer 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. **Do not incubate the standard curve.**

1. Spectrally pure (HPLC grade or better) isopropanol is utilized 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.


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 pmoles amounts read (80, 40, 20, 10, 5, 0 pmoles).

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 in wet ice and let it chill and add the components while chilling.
3. The donor can be left at room temperature during the assay set-up - **you should store the donor at room temp.**

Good results may be obtained if all of the components are pre-mixed if they are chilled and kept cold before adding the mixture to the plate. Temperature and volume are critical factors with these components and the assay.

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.

Roar Biomedical, Inc., Audubon Biomedical Center, 3960 Broadway, New York, NY 10032 USA
Tel: +1 (212) 280-2983 • Fax: +1 (212) 280-2968 • info@roarbiomedical.com • www.roarbiomedical.com
©2002-2010 Roar Biomedical, Inc. All rights reserved. This information is subject to change without notice.