3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

PLTP Activity Assay Kit
Supplied by Roar Biomedical, Inc

Catalog Number **MAK108**
Storage Temperature 2–8 °C, except where indicated

TECHNICAL BULLETIN

Product Description
Phospholipid transfer protein (PLTP) is a protein present in normal human plasma. PLTP transfers phospholipids among lipoproteins in plasma. The PLTP Activity Assay Kit includes proprietary substrates to detect PLTP mediated transfer of a fluorescent substrate. Transfer activity results in increased fluorescent emission intensity from the assay.

Components
The kit is sufficient for 100 assays in 100 µL total assay volume.

Donor Particle (concentration on label) Catalog Number MAK108A	0.3 mL
Acceptor Particle Catalog Number MAK108B	5 mL
PLTP Assay Buffer Catalog Number MAK108C	5 mL

Reagents and Equipment Required but Not Provided.
- 96 well U-bottom black plates for fluorescence assays.
- 37 °C water bath incubator
- Fluorescence multiwell plate reader
- 2-propanol (isopropanol, Catalog Number 34863)
- Thimerosal (Catalog Number T8784), for assay validation

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening.

Donor Particle – May be left at room temperature during assay set up.

Acceptor Particle and PLTP Assay Buffer – Store on ice during assay set up.

Storage/Stability
The kit is shipped on wet ice. Store the Acceptor Particle mixture and PLTP Assay Buffer at 2–8 °C, protected from light. Upon receipt, store the Donor Particle mixture at room temperature. DO NOT FREEZE.

Components are stable for 1 year, if stored properly.

Procedure
All samples and standards should be run in duplicate.

Standards for Fluorometric Detection

1. Prepare six test tubes labeled from T0 to T5 each containing 1 mL of 2-propanol. Add an additional 1 mL of 2-propanol to tube T5.

2. Pipette 5 µL of PLTP Donor Particle mixture to tube T5 and thoroughly mix (vortex) to adequately disperse the Donor Particle mixture in the 2-propanol.
   Note: The concentration of fluorescent substrate in the Donor Particle mixture is listed on the vial label.

3. Make four serial 2-fold dilutions (transfer 1 mL of previous standard solution to a tube with 1 mL of 2-propanol). For example transfer 1 mL of the mixture in tube T5 to tube T4 and vortex. Use tube T0 with isopropanol only as the 0 (Blank) Standard.
   Note: DO **NOT** incubate the standards.

4. Measure the fluorescence intensity (λ_{ex} = 465/ λ_{em} = 535) of each standard. For example, pipette 100 µL of each tube to a separate well of a plate and read the plate.

5. Create a standard curve by plotting the fluorescence intensity units of each standard versus the pmole amounts of Donor Particle in each standard based on the concentration listed on the label.

Sample Preparation

1. Chill the microplate by placing in a tray on wet ice. Add the components while chilling.

2. Set up the Master Reaction Mix, on ice, according to the scheme in Table 1. Add 45 µL of the Master Reaction Mix to each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
PLTP Assay Buffer	42 µL
Donor Particle	3 µL

3. Add 5 µL of desired PLTP source (plasma or serum, fresh or frozen) to the appropriate wells. To prepare a sample blank, add 5 µL of PLTP Assay Buffer in place of the PLTP sample.
   Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction

1. Add 50 µL of Acceptor Particle to each well. Total assay volume should be 100 µL in each well.

2. Incubate sample plate for 8–20 minutes at 37 °C.
   Note: The microplate incubator must be able to rapidly raise the assay temperature to 37 °C. Large, humidified air incubators may cause problems by slowly increasing the temperature from 25 °C to only 34 °C after three hours. Floating the plate in a water bath is recommended, rather than using an air incubator.

3. Measure the increase in fluorescence of samples using a fluorometer (λ_{ex} = 465/λ_{em} = 535). Determine the fluorescence intensity in the plasma or serum samples by subtracting the fluorescence intensity of the sample blank from each sample.

Assay Validation Procedure

Thimerosal is a phospholipid transfer protein (PLTP) inhibitor.

The steps to validate the assay were adapted from a published procedure,[1] using thimerosal (ethyl-mercurithiosalicylate) to inhibit PLTP activity.

Plasma (10-fold dilution) was pre-incubated with different concentrations of thimerosal (0–100 mM) at room temperature for 30 minutes.[1] 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 fluorometer (λ_{ex} = 465/λ_{em} = 535).

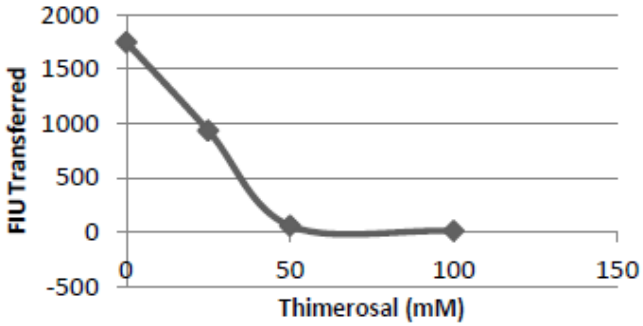
Results

Assay results may be expressed in terms of pmoles of fluorescent substrate transferred. The substrate concentration of the Donor Particle solution (nmoles/mL) is printed on the label of the donor particle vial.

Calculate the pmoles of fluorescent substrate transferred from the standard curve using the fluorescence intensity values in the assay. Be sure to subtract the sample blank fluorescence intensity from the sample fluorescence intensity before attempting to enter the values into the regression or the values from the assay will be higher than the standard.

Figure 1.

Plasma PLTP Activity Inhibition by Thimerosal



References

1. J. Lipid Res. 40(4), 654-664 (1999).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

This product is supplied by Roar Biomedical, Inc. and covered by several patents including U.S. Pat. Nos. 7,618,784; 7,851,223 and related US and foreign patents.

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