Product Manual

Total Phosphatidic Acid Assay Kit

Catalog Number

MET-5019 **100 assays**

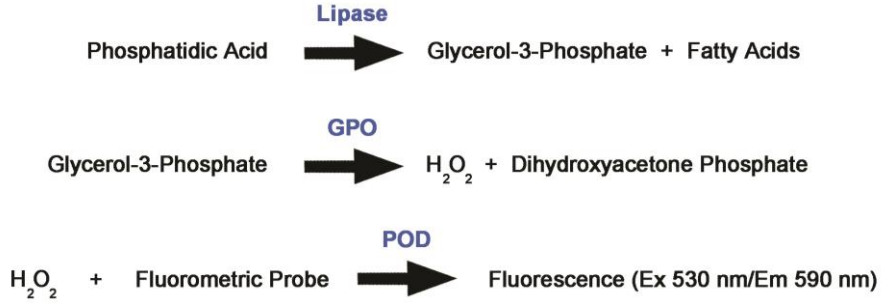
FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Phosphatidic Acid (PA) is a critical precursor for the biosynthesis of many lipids in the cell. PA plays a critical role in membrane structure and acts as a signaling lipid, recruiting cytosolic proteins to the cell membrane. Within the cell, PA concentrations are maintained at extremely low levels by the activity of potent phospholipid phosphatases, converting phosphatidic acid to diacylglycerol (DAG). Because DAG is another important lipid precursor, it too is rapidly metabolized into other membrane lipid components (e.g., phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine). Ultimately, measurement of cellular PA is useful for monitoring lipid synthesis and metabolism.

Cell Biolabs' Total Phosphatidic Acid Assay Kit measures total phosphatidic acid content, including lysophosphatidic acid (LPA), in samples by a coupled enzymatic reaction system. First, lipase is used to hydrolyze phosphatidic acid in samples to glycerol-3-phosphate. Next, the glycerol-3-phosphate product is oxidized by glycerol-3-phosphate oxidase (GPO), producing hydrogen peroxide which reacts with the kit's Fluorometric Probe (Ex. 530-560 nm/Em. 585-595 nm).



The Total Phosphatidic Acid Assay Kit is a simple, fluorometric assay that quantitatively measures total PA (PA and LPA) in samples using a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, standards and unknown samples. The kit contains an L-α-Phosphatidic Acid Standard and has a detection sensitivity limit of ~5 µM.

Note: This kit is not intended for urine, plasma, or serum samples.

Related Products

1. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
2. STA-399: Free Glycerol Assay Kit (Fluorometric)
3. STA-600: Phosphatidylcholine Assay Kit
4. STA-619: Free Fatty Acid Assay Kit (Fluorometric)
5. MET-5129- Lysine Assay Kit (Fluorometric)

Kit Components

1. Phosphatidic Acid Standard (Part No. 50191D): One 200 µL vial of 1 mM L-α-Phosphatidic Acid.
2. 10X Assay Buffer (Part No. 50192D): One 1.5 mL vial.
3. Lipase Solution (Part No. 50193D): Three 1.4 mL vials.
4. Enzyme Mixture (Part No. 50194D): Three 1.75 mL vials.
5. Fluorometric Probe (Part No. 261901): One 110 µL amber vial.

Materials Not Supplied

1. Standard 96-well fluorescence black microtiter plate
2. PBS (containing Magnesium and Calcium)
3. Extraction reagents for cellular lipids (methanol, chloroform, 1M NaCl)

Storage

Store the entire kit at -80ºC. Avoid multiple freeze/thaws by aliquoting. The Fluorometric Probe is light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- Phosphatidic Acid Standard: Thaw at room temperature. Once homogeneous and mixed well, maintain the standard at room temperature during assay preparation. The solution is stable for 1 week at room temperature. For longer term storage, the solution should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- 1X Assay Buffer: 10X Assay Buffer should be thawed/maintained at 4ºC during assay preparation. Dilute the 10X Assay Buffer with deionized water. Stir to homogeneity. The 1X solution is stable for 1 month at 4ºC. For longer term storage, any unused 10X stock material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- Lipase Solution and Enzyme Mixture: Thaw at 4ºC. Once homogeneous and mixed well, maintain the solution at 4ºC during assay preparation. The solution is stable for 1 week at 4ºC. For longer term storage, the solution should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
  Note: These components are provided in multiple tubes to minimize multiple freeze/thaws.
- Fluorometric Probe: Thaw and maintain at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- PEU (pre-equilibrated upper phase) Solution: Mix 50 mL of chloroform, 50 mL of methanol, and 45 mL of 1M NaCl in a glass container. Shake or mix the solution well, then allow it to separate into 2 phases. Use the upper phase for washing during the extraction.

Preparation of Phosphatidic Acid Standard

Thaw the Phosphatidic Acid Standard at room temperature. Mix well by vortexing to ensure the solution is homogeneous. Freshly prepare a dilution series of standard in the concentration range of 0 µM – 250 µM by diluting the standard stock solution (provided at 1 mM) in 1X Assay Buffer (see Table 1). Standards should be prepared fresh, vortexed well and used immediately.

Standard Tubes	1 mM Phosphatidic Acid Standard (µL)	1X Assay Buffer (µL)	Final Phosphatidic Acid Standard (µM)
1	25	75	250
2	50 of Tube #1	50	125
3	50 of Tube #2	50	62.5
4	50 of Tube #3	50	31.2
5	50 of Tube #4	50	15.6
6	50 of Tube #5	50	7.8
7	50 of Tube #6	50	3.9
8	0	50	0

Table 1. Preparation of Phosphatidic Acid Standards

Preparation of Samples

- Urine, plasma and serum: This kit is not recommended for these samples**.**
- Cell Lysates:
  For adherent cells, remove media and wash cells twice with cold PBS. Harvest ~1x 10^7 cells by using a rubber policeman. Do not use proteolytic enzymes. Centrifuge at 1500 x g for 10 minutes. Carefully remove the supernatant and resuspend in 1 mL of cold PBS. Proceed to step 1 of the extraction procedure below.
  For suspension cells, collect ~1 x 10^7 cells by centrifugation at 1500 x g for 10 minutes. Carefully remove the supernatant and wash the cell pellet with cold PBS. Repeat PBS wash/centrifugation once more. Carefully discard the supernatant and resuspend in 1 mL of cold PBS. Proceed to step 1 of the extraction procedure below:

Extraction Procedure

1. Sonicate the 1 mL of cell suspension on ice.
2. Add 1.5 mL of methanol to the sonicated sample.
3. Add 2.25 mL of 1 M NaCl and 2.5 mL of chloroform to the sample. Vortex well.
4. Centrifuge at 1500 x g for 10 minutes at 4ºC to separate the phases.
5. Carefully remove the upper aqueous phase and discard.
6. Wash the lower chloroform phase 2 times with 2 mL of pre-equilibrated upper phase (PEU) (see Preparation of Reagents Section). Separate the phases each time by centrifuging at 1500 x g for 10 minutes at 4ºC. Carefully remove the upper phase and discard each time.
7. After the final wash, carefully transfer the lower organic phase to a glass vial or tube (a syringe works well). Avoid transferring any remaining upper, aqueous phase.
8. Dry the lower phase in a speedvac or under a gentle stream of nitrogen.
9. Resuspend the dried sample with 50 µL of 1X Assay Buffer (1:20 of the original volume). Samples may be stored at -80ºC for up to a month.

Assay Protocol

Note: Each PA standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed. Maintain the Lipase Solution and Enzyme Mixture at 4ºC during assay preparation.

1. Add 10 µL of the PA standards, samples or blanks to the 96-well microtiter plate.
2. Add 40 µL of Lipase Solution to each well.
3. Incubate at 37ºC for 30 minutes.
4. During the step 3 incubation, separately prepare the desired volume of Detection Enzyme Mixture according to Table 2, based on the number of tests to be performed. Maintaining all components and mixtures at 4ºC throughout this step, add components in the following sequence:
   a. In a tube, add the appropriate volume of Enzyme Mixture.
   b. To the Enzyme Mixture, add the corresponding volume of Fluorometric Probe. Mix well and immediately use.

Note: Detection Enzyme Mixture may appear slightly pink in color. This is normal background and should be subtracted from all absorbance values (see step 9 for calculation).

Enzyme Mixture (mL)	Fluorometric Probe (µL)	Total Volume of Detection Enzyme Mixture (mL)	**# of Tests in 96-well Plate (100 µL/test)**
5	50	5.05	**100**
2.5	25	2.525	**50**
1.25	13	1.263	**25**

Table 2. Preparation of Detection Enzyme Mixture

5. Transfer 50 µL of the above Detection Enzyme Mixture (from step 4) to each well.
6. Cover the plate wells to protect the reaction from light.
7. Incubate at room temperature for 10 minutes.
8. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-560 nm range and for emission in the 585-595 nm range.
9. Calculate the concentration of phosphatidic acid within samples by comparing the sample fluorescence to the standard curve. Negative controls (without PA) should be subtracted from each PA sample and standard.

Example of Results

The following figures demonstrate typical Total Phosphatidic Acid Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

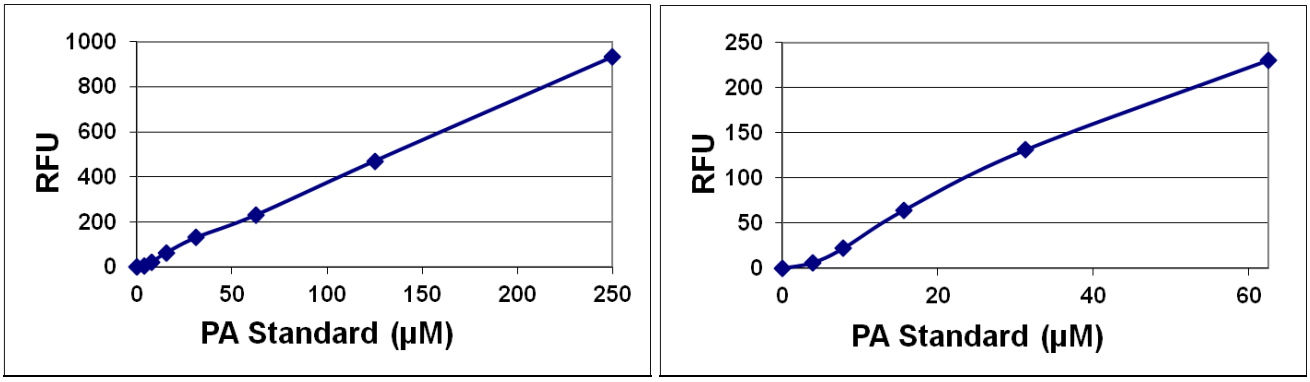


Figure 1: Total Phosphatidic Acid Assay Standard Curve. PA standard curve was performed according to the Assay Protocol. Background has been subtracted.

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Recent Product Citations

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Warranty

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