Datasheet for ABIN5067564

Total Phosphatidic Acid Assay Kit (Fluorometric)

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Overview

Quantity:	100 tests
Application:	Biochemical Assay (BCA)

Product Details

Detection Method:	Fluorometric
Sensitivity:	5 µM
Characteristics:	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.
Components:	1. Phosphatidic Acid Standard : One 200 µL vial of 1 mM L-α-Phosphatidic Acid. 2. 10X Assay Buffer : One 1.5 mL vial. 3. Lipase Solution : Three 1.4 mL vials. 4. Enzyme Mixture : Three 1.75 mL vials. 5. Fluorometric Probe : One 110 µL amber vial.

Target Details

Background:	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 metabolised into other membrane lipid components (e.g. phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine). Ultimately, measurement of cellular PA is useful for monitoring lipid synthesis and metabolism.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Detects total phosphatidic acid content (PA and LPA) in cell lysate samples • Detection sensitivity of approximately 5 uM phosphatidic acid • Phosphatidic Acid standard curve included
Reagent Preparation:	• 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 -80 °C to avoid multiple freeze/thaws. • 1X Assay Buffer: 10X Assay Buffer should be thawed/maintained at 4 °C during assay preparation. Dilute the 10X Assay Buffer with deionized water. Stir to homogeneity. The 1X solution is stable for 1 month at 4 °C. For longer term storage, any unused 10X stock material should be aliquoted and frozen at -80 °C to avoid multiple freeze/thaws. 3 • Lipase Solution and Enzyme Mixture: Thaw at 4 °C. Once homogeneous and mixed well, maintain the solution at 4 °C during assay preparation. The solution is stable for 1 week at 4 °C. For longer term storage, the solution should be aliquoted and frozen at -80 °C 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 -80 °C 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.
Sample Preparation:	• 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 107 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 107 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: 4 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 4 °C 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 4 °C. 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 -80 °C for up to a month.

Assay Procedure:

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 4 °C 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 37 °C for 30 minutes.
4. During the step 3 incubation, separately prepare the desired volume of Detection Enzyme Mixture according to Table 2 below, based on the number of tests to be performed. Maintaining all components and mixtures at 4 °C 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 Fluorometric Total Volume of # of Tests in Mixture (mL) Probe (µL) Detection Enzyme 96-well Plate Mixture (mL) (100 µL/test) 5 50 5.05 100 2.5 25 2.525 50 1.25 13 1.263 25 Table
5. Preparation of Detection Enzyme Mixture 5
6. Transfer 50 µL of the above Detection Enzyme Mixture (from step 4) to each well.
7. Cover the plate wells to protect the reaction from light.
8. Incubate at room temperature for 10 minutes.
9. 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.
10. Calculate the concentration of phosphatidic acid within samples by comparing the sample fluorescence to the standard curve. Negative controls (without PA) should be subtracted

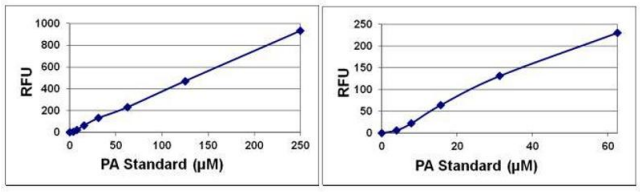
Application Details

	from each PA sample and standard.
Restrictions:	For Research Use only

Handling

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

Images



Biochemical Assay

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