Phosphatidic Acid Assay kit

ARG83365 Phosphatidic Acid Assay kit is an assay kit for total phosphatidic acid in cell lysate.

Catalog number: ARG83365

Package: 100 assay

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INTRODUCTION

Phosphatidic acid (PA) is a crucial lipid in cell membranes, serving as a precursor for various lipid molecules. In mammals and yeast, PA is synthesized through glycerol 3-phosphate or dihydroxyacetone phosphate pathways. PA's conversion to diacylglycerol (DAG) is pivotal for phospholipid production. Maintained at low levels by lipid phosphate phosphatases (LPPs), PA's biophysical properties influence membrane curvature, potentially impacting vesicle dynamics. Functionally, PA plays a role in signaling, activating proteins like TREK-1 channels. Despite interconversion with DAG, distinct signaling pathways may exist, with specific acyl chains influencing lipid-protein interactions. This intricate interplay of biosynthesis, biophysics, and signaling highlights PA's multifaceted roles in cellular processes.

PRINCIPLE OF THE ASSAY

ARG83365 Phosphatidic Acid Assay Kit measures total phosphatidic acid content (PA and LPA) in cell lysate samples by a coupled enzymatic reaction system. First, lipase is used to hydrolyze phosphatidic acid to glycerol-3-phosphate. Next, the glycerol-3-phosphate product is oxidized by glycerol-3-phosphate oxidase (GPO), producing hydrogen peroxide. The hydrogen peroxide released from this reaction reacts specifically with the kit's Fluorometric Probe and is detected at ex. 530-560 nm/em. 585-595 nm. Phosphatidic Acid levels in unknown sampled are determined based on the provided phosphatidic acid standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Standard (1.0 mM)	200 µL	-80°C
10X Assay Buffer	1.5 ml	-80°C
Probe	110 µL	-80°C
Lipase Solution	1.4 ml	-80°C
Enzyme Mixture	1.75 ml	-80°C

The kit is shipped with blue-ice. Store the all reagent -80°C.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 nm
- Flat bottomed 96-well black microplate
- Pipettes and pipette tips
- Extraction reagents (methanol, chloroform and 1M NaCl)
- 1 X PBS (pH 7.4)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Store the kit at -80°C at all times, avoid freeze/thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Lysates-

Adherent Cell: Remove media and wash cells twice with cold PBS. Harvest 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.

Suspension Cell: Collect 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.

REAGENT PREPARATION

- **1X Assay Buffer:** Thaw and maintain the solution at 4°C during assay preparation. Dilute the 10X Assay Buffer with deionized water.
  The 1X solution is stable for 1 month at 4°C. For longer term storage, the 10X stock material should be aliquoted and frozen at -80°C and avoid multiple freeze/thaws.
- **Probe:** Thaw and maintain the solution at room temperature during assay preparation. Prepare the **working probe** at a 1:100 dilution with Enzyme Mixture at each assay (e.g., 5ml Enzyme Mixture + 50µl Probe).
  Any unused material should be aliquoted and frozen at -80°C to avoid multiple freeze/thaws.

- **Lipase Solution and Enzyme Mixture:** Thaw and 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 and avoid multiple freeze/thaws.

  ***** Note: These components are provided in multiple tubes to avoid freeze/thaws.

- **pre-Equilibrated Upper Phase Solution**: Mix 50 mL of chloroform, 50 mL of methanol, and 45 mL of 1M NaCl. Mix the solution for allow it to separate into 2 phases. Use the upper phase for washing during the extraction.

- **Standards**: Add 100 µl of 1.0 mM stock standard into 300 µl 1X Assay Buffer to generate a standard with 250 µM of Phosphatidic Acid. Dilute the standards with 1X Assay Buffer serves as zero standard (blank standard, 0 µM). The example of the standards dilution table is as below:

Standard#	Phosphatidic Acid (µM)	Volume of 1X Assay Buffer (µl)	Volume of Phosphatidic Acid (µM)
S1	250	300	100(1 mM stock)
S2	125	200	200(S1)
S3	62.5	200	200(S2)
S4	31.25	200	200(S3)
S5	15.625	200	200(S4)
S6	7.8	200	200(S5)
S7	3.9	200	200(S6)
S8	0	200	0

- Samples:

Cell Extraction

1. Sonicate the 1 mL of cell suspension on ice.
2. Add 1.5 mL of methanol in to the sonicated sample.
3. Add 2.25 mL of 1 M NaCl and 2.5 mL of chloroform in to the sample and vortex. Centrifuge at 1500 x g for 10 minutes at 4°C.
4. Carefully remove the upper aqueous phase and discard.
5. Wash the lower chloroform phase 2 times with 2 mL of pre-equilibrated upper phase. 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.
6. After the final wash, carefully transfer the lower organic phase to a glass vial or tube. Avoid transferring any remaining upper, aqueous phase.
7. Dry the lower phase in a speedvac or under a gentle stream of nitrogen.
8. 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

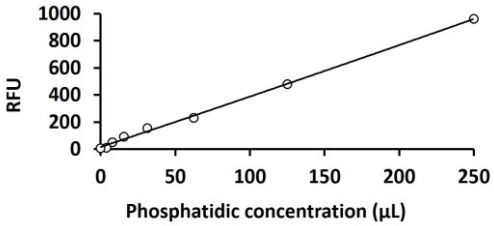
Standards and samples should be assayed in at least duplicates.

Assay Procedure:

1. Add 10 µl of sample and standard to each wells.
2. Add 40 µL of Lipase Solution to each well.
3. Mix well and Incubate for 30 min at 37°C.
4. Add 50 µL of fresh working probe to each well.
5. Incubate for 10 min at room temperature protect from light.
6. Read O.D. with a microplate reader at 540 nm (500-570) nm immediately.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Phosphatidic Acid Assay kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Nitric Oxide ranged from 3.9-250 µM. The mean MDD was 1.9 µM