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ab273335 Phosphatidic Acid Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273335
(use https://www.abcam.cn/ab273335 for china, or https://www.abcam.co.jp/ab273335 for Japan)

For the determination of Phosphatidic Acid in cell and tissue lipid extract.

This product is for research use only and is not intended for diagnostic use.

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Table of Contents

1.	Overview	1
2.	Protocol Summary	2
3.	Precautions	3
4.	Storage and Stability	3
5.	Limitations	4
6.	Materials Supplied	4
7.	Materials Required, Not Supplied	5
8.	Technical Hints	6
9.	Reagent Preparation	7
10.	Sample Preparation	8
11.	Standard Curve	9
12.	Assay Procedure	10
13.	Calculations	11
14.	Typical Data	12
15.	FAQ / Troubleshooting	14
16.	Notes	15

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1. Overview

Phosphatidic Acid Assay Kit (Fluorometric) (ab273335) is a plate-based enzymatic assay for quantitation of Phosphatidic Acid (PA) in cells and tissues.

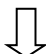
The PA Converter hydrolyzes PA to form an intermediate which, in the presence of a developer and enzyme mix, converts a non-fluorescent probe to a fluorescent product (Ex/Em= 535/587 nm) that can be quantified. The Converter is specific to PA and does not hydrolyze other phospholipids (e.g. those with more complex head groups such as phosphatidylcholine or cardiolipin), allowing direct quantitation of PA.

This kit can detect as low as 40 pmoles of phosphatidic acid per well.

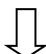
2. Protocol Summary

Prepare all samples (can take several hours/overnight).

Prepare reagents and standards.

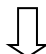


Add all samples to the appropriate wells.



Add Converter Mix to the appropriate wells, incubate then add Reaction Mix and incubate again.

Measure fluorescence in end-point mode for 45 mins at 37°C.



Determine Phosphatidic Acid concentration using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
PA Assay Buffer	100 ml	-20°C
PA Converter	1 vial	-20°C
Developer Mix F	1 vial	-20°C
Developer Mix E	1 vial	-20°C
PA Standard	100 µl	-20°C
PicoProbe I	0.4 ml	-20°C

PLEASE NOTE: PicoProbe I was previously labelled as Probe in DMSO, and Developer Mix F as Development Enzyme Mix VII and PA Developer Lyophilized, and Developer Mix E as Development Enzyme Mix VI and PA Enzyme Mix Lyophilized. The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well flat bottom clear plate
- Multi-well spectrophotometer
- Water bath / heating plate / oven
- Chloroform
- Methanol
- 12 N HCl and 1 M NaCl
- Triton X-100
- 10 ml conical vials
- Glass test tubes (5 ml / 10 ml)

8. Technical Hints

- **This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **PA Assay Buffer:**

Warm to room temperature before use.

9.2 **PA Converter:**

Store at -20°C. Lyophilized vials are stable for at least 6 months. Reconstitute PA Converter in 1760 µl PA assay buffer before use. Aliquot remaining components and store at -20°C. Reconstituted vials are stable for at least two months.

9.3 **Developer Mix F:**

Store at -20°C. Lyophilized vials are stable for at least 6 months. Reconstitute Developer Mix F in 220 µl PA assay buffer before use. Aliquot remaining components and store at -20°C. Reconstituted vials are stable for at least two months.

9.4 **Developer Mix E:**

Store at -20°C. Lyophilized vials are stable for at least 6 months. Reconstitute PA Enzyme in 220 µl PA assay buffer each before use. Aliquot remaining components and store at -20°C. Reconstituted vials are stable for at least two months.

9.5 **PA Standard (1 mM):**

Store at -20°C. Thaw in a water bath at 37°C for 15-20 minutes. Aliquot and store at -20°C.

9.6 **PicoProbe I:**

Store at -20°C. Thaw at room temperature before use. Aliquot and store at -20°C.

10. Sample Preparation

- Sample preparation **may take several hours / overnight**.
- Complete sample preparation before preparing other reagents for the assay.
- Chloroform that is used for sample preparation is toxic, therefore sample preparation should be done under a fume hood.

10.1 Homogenize tissue (~100 mg; non-perfused) or cells (~1 million) in 1 ml PA assay buffer.

10.2 Protein content of the sample may be determined at this stage if desired. We recommend using a BCA protein assay kit.

10.3 Carry out lipid extraction according to the following protocol: Combine chloroform/methanol/12N HCl in a ratio of 2 : 4 : 0.1 v/v. Mix thoroughly and add 1.25 ml of this mixture to 1 ml of cell/tissue homogenate. Vortex for 30 secs and add 1.25 ml of 1 M NaCl. Mix well and centrifuge at 3000 x *g* for 10 mins at room temperature. Two distinct layers will be visible at this point. Collect the lower organic layer (containing solubilized lipids) with a pipette and transfer to a glass tube. Allow the chloroform to evaporate in a vacuum oven or a regular oven (37°C) overnight or until the chloroform evaporates completely. Extracted lipids will stick to the walls of the tube and should be solubilized in 50 - 500 µl 5% Triton X-100 solution. If not being used immediately, the lipid extract may be stored at -80°C for 3-4 days.

10.4 Add 2 to 10 µl of samples into wells of a 96-well clear plate.

10.5 For each sample prepare two wells; Sample background control and Sample. Bring the volume of Sample wells to 50 µl and Sample background control wells to 70 µl with PA Assay buffer.

Δ **Note:** For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

11. Standard Curve

– Keep standards on ice while in use.

11.1 Dilute the provided 1 mM PA standard 1:10 in PA Assay Buffer to obtain 100 µM PA.

11.2 Add 0, 2, 4, 6, 8, and 10 µl of the 100 µM PA standard to wells of the 96 well plate to obtain 0, 0.2, 0.4, 0.6, 0.8 and 1 nmol of Phosphatidic acid per well.

11.3 Bring up the total volume in these wells to 50 µl with PA Assay buffer.

Standard #	100 µM PA Standard (µL)	PA Assay Buffer (µL)	1 mM PA (nmol/well)
1	0	50	0
2	2	48	0.2
3	4	46	0.4
4	6	44	0.6
5	8	42	0.8
6	10	40	1.0

12. Assay Procedure

– Keep on ice while in use.

12.1 **Converter Mix:** Mix enough reagents for the number of assays to be performed. For each sample and standard well, prepare 20 µl:

	Converter Mix (µl)
PA Assay Buffer	4
PA Converter	16

Add 20 µl the converter mix to wells containing the samples and standards. Mix well. Do not add the converter mix to Sample background control wells. Incubate at 45 °C for 1 hour.

12.2 **Reaction Mix:**

12.3 Prepare enough reagents for the number of assays to be performed. For each well, prepare 30 µl of the Reaction mix. The total reaction volume after addition of reaction mix is 100 µl:

	Reaction mix (µl)
PA Assay Buffer	20
Developer Mix F	4
Developer Mix E	2
PicoProbe I	4

12.4 Add 30 µl of the reaction mix to all wells. Mix well. Incubate at 37°C for 45 mins.

12.5 Record fluorescence in end-point mode at Ex/Em= 535/587 nm.

13. Calculations

13.1 Subtract 0 PA reading from all PA standard readings. Plot the Phosphatidic acid Standard Curve.

13.2 Subtract sample background control readings from sample readings.

13.3 If 0 PA readings are higher than sample background control readings, subtract those form sample readings instead.

13.4 Apply corrected RFU to Standard Curve to get B nmol PA in the sample well.

$$[PA] = \frac{B}{(\mathrm{V})} \; X \; D = nmol/ml$$

B = Amount of Phosphatidic acid in the sample well from Standard Curve (nmol)
V = Volume of sample added into the well (ml)
D = Dilution factor, D=1 is undiluted

PA molecular weight: 711 g/mol.
PA concentrations can also be expressed as nmol PA per mg protein or nmol PA per mg tissue weight.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

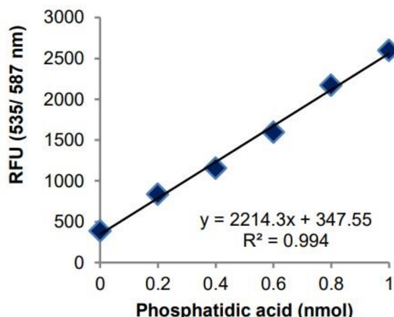


Figure 1. Phosphatidic acid standard curve.

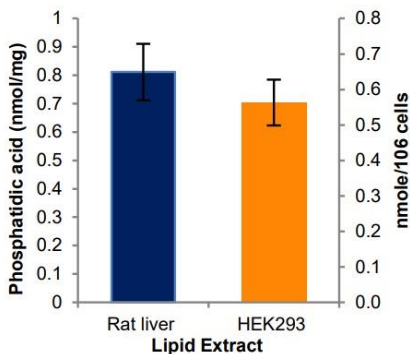


Figure 2. Phosphatidic acid content in rat liver and HEK 293 cells. For rat liver extract, 100 mg tissue was homogenized in 1 ml PA assay buffer. Lipid extraction was carried out and was re-suspended in 200 µl 5% Triton X-100 solution. For HEK293, cells were grown to confluency and harvested using a rubber policeman.

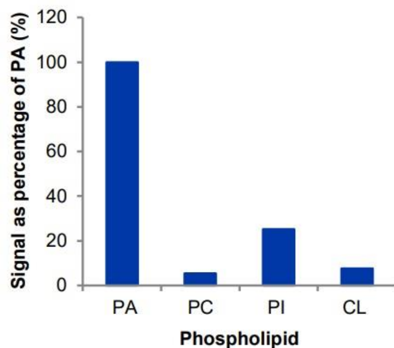


Figure 3. Assay Specificity: Equimolar amounts (20 nmol) of phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylinositol (PI) and cardiolipin (CL) show interference less than 20% with respect of Phosphatidic Acid. Assay shows specificity for phosphatidic acid.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

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