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ab241005 Phosphatidylethanolamine Assay Kit

For the measurement Phosphatidylethanolamine content in lipid extracts from cells and tissues.

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

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1. Overview

The Phosphatidylethanolamine assay kit (ab241005) is a microplate based enzymatic assay for the quantitation of PE in cells and tissues. PE Converter hydrolyses PE to an intermediate, which converts a colorless probe to a fluorescent product via enzymatic reaction (Ex/Em: 535/587).

The intermediate formed through PE converter hydrolysis is specific to phosphatidylethanolamine. Thus no other phospholipids (i.e. phosphatidylcholine, phosphatidylinositol or phosphatidic acid) will be detected, making the kit highly specific. This assay kit can detect as low as 0.2 nmol per well.

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Prepare the standard curve using the 1 mM Phosphatidylethanolamine standard. Dilute 1:10 using the PE Assay Buffer.

Create the converter Mix using PE Assay Buffer and PE converter. Add to wells and incubate for 1 hour at 45°C.

Create the Reaction Mix, add to desired wells and incubate for 3 hours at 40°C.

Read the fluorescence in end point mode at Ex/Em 535/587 nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
PE Assay Buffer	25 mL	-20°C
PE Converter	1 vial	-20°C
PE Developer	600 µL	-20°C
PE Enzyme Mix	1 vial	-20°C
PE Probe	200 µL	-20°C
PE Standard (1 mM)	100 µL	-20°C

5. Materials Required, Not Supplied

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

- Clear or Black (Fluorometric only) 96-well plate with flat bottom.
- Multi-well spectrophotometer.
- Triton X-100 (peroxide free).

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 PE Assay Buffer:

Allow to warm to room temperature prior to use.

6.2 PE Converter:

Store at -20°C, protected from light. Lyophilized vials are stable for at least 6 months. Reconstitute each vial in 220 µL assay buffer before use. Aliquot remaining components. Store at -20°C. Reconstituted vials are stable for at least two months.

6.3 PE Developer:

Store -20°C. Thaw on ice before use. Aliquot and store the remaining at -20°C. Product is stable for at least six months.

6.4 PE Enzyme Mix:

Store at -20°C, protected from light. Lyophilized vials are stable for at least 6 months. Reconstitute each vial in 220 µL assay buffer before use. Aliquot remaining components. Store at -20°C. Reconstituted vials are stable for at least two months.

6.5 PE Probe:

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

6.6 PE Standard (1 mM):

Store at -20°C. Thaw in a water bath/heat block at 45°C for 15-20 minutes. The solution should look clear. Divide into aliquots and store the remaining at -20°C. Thaw in a water bath at 45°C before next use.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Dilute the 1 mM PE Standard (that has been heated to 45°C for at least 30 minutes) 1:10 in PE Assay Buffer to obtain 100 µM PE solution.

7.2 Add 0, 5, 10, 20, 30, and 40 µl of 100 µM standard to wells of the 96 well plate to obtain 0, 0.5, 1, 2, 3 and 4 nmol, of Phosphatidylethanolamine per well.

7.3 Bring the total volume of each well to 50 µL with PE Assay Buffer.

ΔNote: The 100 µM PE solution should be warm when being dispensed into wells of the 96 well plate. This is crucial for proper solubilization of the lipid.

Standard #	100 µM PE standard (µL)	PE Assay Buffer (µL)	PE concentration Per well (nmol)
1	40	10	4
2	30	20	3
3	20	30	2
4	10	40	1
5	5	45	0.5
6	0	50	0

Table 1: Standard curve preparation for a fluorometric assay.

8. Sample Preparation

ΔNote: Allow at least 5-6 hours for the entire assay including sample preparation. Put PE standard at 45°C while preparing samples in order to save time.

8.1 For tissues and cultured cells:

- Prepare a 5% (v/v) solution of peroxide free triton X-100 in water. Homogenize tissue (~100 mg; non-perfused) or cells (~1 million) in 1 mL solution containing 5% Triton X-100 in water.
- Protein content in the sample may be determined at this stage if desired. We recommend *BCA protein assay kit* (ab102536)
- Heat the samples to 80°C in a water bath for 5 - 10 minutes or until the solution becomes cloudy, then cool down to room temperature. Repeat the heating step once more to solubilize all lipids and allow the solution to cool to room temperature again.
- Centrifuge (10000 X g, 10 min, 4°C) and collect supernatant, which contains solubilized lipids. If not being used immediately, store supernatant at -80°C.
- Add 2 to 10 µL of samples into wells of a 96-well clear plate. For each sample prepare two wells; "Sample background control" and "Sample". Bring the volume in "Sample" wells to 50 µL and in "Sample background control" to 70 µL using PE Assay buffer respectively.

  ΔNote: Different dilutions of sample should be tested to make sure that Phosphatidylethanolamine concentration falls in the linear range of the assay.

9. Assay Procedure

9.1 **Converter Mix:** Mix enough reagents for the number of assays to be performed, including PE standard curve wells. For each test sample well, prepare 20 µL Converter Mix Shown in the table below:

	Converter Mix
PE Assay Buffer	18 µL
PE Converter	2 µL

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

9.3 **Reaction Mix:** Mix enough reagents for the number of assays to be performed, including PE standard curve wells. For each test sample well, prepare 30 µL Reaction Mix Shown in the table below:

	Reaction Mix
PE Assay Buffer	22 µL
PE Developer	4 µL
PE Enzyme Mix	2 µL
PE probe	2 µL

9.4 Add the reaction mix to all wells. Mix well. Incubate at 40°C for 3 hours.

9.5 **Measurement:** Record the fluorescence in end point mode at Ex/Em 535/587 nm.

10. Data Analysis

10.1 Subtract the 0 PE Standard reading from all Standard curve readings. Plot the background-subtracted PE Standard Curve and calculate the slope.

10.2 If sample background control slope is significant, then subtract sample background control reading from sample readings.

10.3 Apply the corrected ΔRFU value to the PE Standard Curve to get B nmol PE in the sample well.

$$\text{Sample PE Concentration} = (B/V) \times D \text{ nmol/mL} = \text{nmol/}\mu\text{M}$$

Where:

B is the amount of PE in the sample well from Standard Curve (nmol)
V is the sample volume added into the reaction well (mL)
D is the sample dilution factor

11. Typical Data

Typical data provided **for demonstration purposes only**.

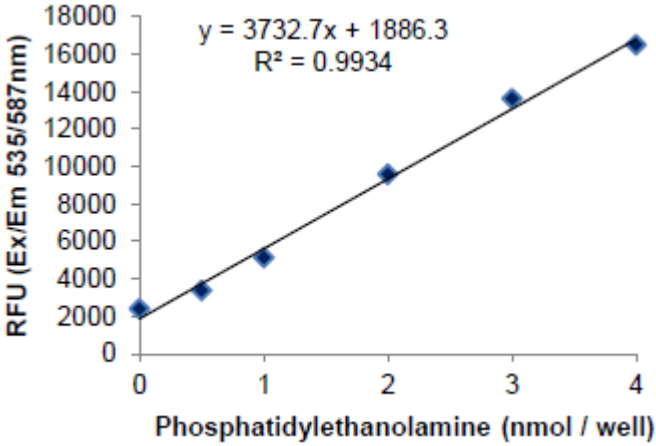

Figure 1. PE Standard Curve.

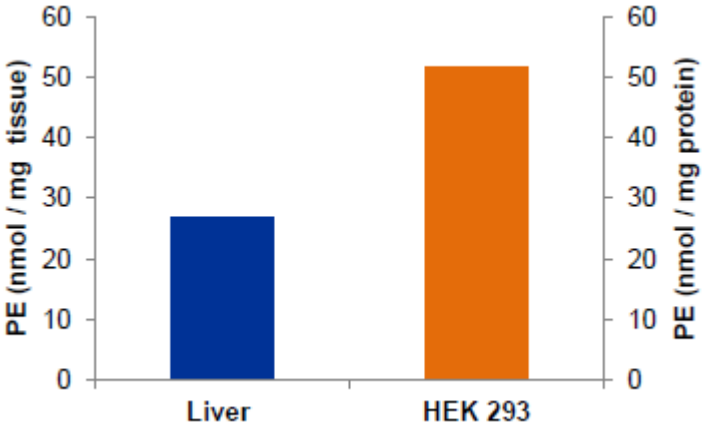

Figure 2. Phosphatidylethanolamine content in rat liver (100 µg wet tissue) and HEK 293 cells (25 µg protein). Sample preparation and assay was carried out according to kit protocol.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)