Phosphatidylethanolamine Assay Kit (Fluorometric) (BN00730)

(Catalog # BN00730; 100 assays; Store at -20°C)

I. Introduction:

Phosphatidylethanolamine (PE), also known as *cephalin* is the second most abundant phospholipid in animal and plant tissues, and is present on the cytoplasmic side of the plasma membrane. Phosphatidylethanolamine is a neutral phospholipid consisting of a phosphatidyl group ester linked to an ethanolamine molecule. Its functions include membrane fission/fusion, maintenance of membrane curvature and stabilization of membrane proteins, since it can form hydrogen bonds with proteins through an ionizable amine group. It acts as a chaperone during assembly of membrane proteins and aids in their translocation from the cytoplasm to the membrane. It is also involved in secretion of very low density lipoproteins in the liver. Assay Genie's Phosphatidylethanolamine assay kit 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.

Phosphatidylethanolamine —(PE Converter)→ Intermediate —(Developer + Enzyme Mix + Probe)→ **Fluorescence (Ex/Em = 535/587 nm)**

II. Applications:

- Measurement of Phosphatidylethanolamine content in lipid extracts from cells and tissues

III. Sample Type:

- Cell lipid extract
- Tissue lipid extract

IV. Kit Contents:

Components	BN00730	Cap Code	Part Number
PE Assay Buffer	25 ml	WM	BN00730-1
PE Converter	1 vial	Purple	BN00730-2
PE Developer	2 vials	Orange	BN00730-3
PE Enzyme Mix	1 vial	Green	BN00730-4
PE Probe	200 µl	Red	BN00730-5
PE Standard (1 mM)	100 µl	Yellow	BN00730-6

V. User Supplied Reagents and Equipment:

- 96-well flat bottom clear plate
- Multi-well spectrophotometer
- Deionized water
- Triton X-100 (peroxide free)
- Water bath/heating plate

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Centrifuge vials prior to opening. Read the protocol before performing the assay.

- **PE Assay Buffer:** Warm to room temperature before use.
- **PE Converter, PE Developer and and PE Enzyme Mix:** Store at -20°C. Lyophilized vials are stable for at least 6 months. Reconstitute each vial in 220 µl assay buffer before use (For Developer reconstitute 1 vial at a time). Aliquot remaining components. Store at -20°C. Reconstituted vials are stable for at least two months.
- **PE Probe:** Store at -20°C. Thaw at room temperature before use.
- **PE Standard:** Store at -20°C. Thaw in a water bath/heat block at 45°C. ***PE Standard should be kept at 45°C for at least 30 minutes to allow proper micellar solubilization***. The solution should look clear. Aliquot and store the remaining at -20°C. Thaw in a water bath at 45°C before next use (allow at least 30 minutes).

VII. Phosphatidylethanolamine Assay Protocol:

Note: 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.

1. **Sample Preparation:** 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*. 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.

   Note:

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

b. *Samples should be diluted using PE Buffer*.

2. **Phosphatidylethanolamine Standard Curve:** Dilute the 1 mM Phosphatidylethanolamine (that has been heated to *45°C* for at least 30 minutes) 1:10 in PE Assay Buffer to obtain 100 µM PE solution. For example, to prepare 200 µl of 100 µM PE solution mix 20 µl of 1 mM PE solution in 180 µl of PE Assay Buffer. *Keep it in a heat block / water bath at 45°C for about 30 minutes to allow solubilization.* 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. Bring up the total volume in these wells to 50 µl with PE Assay buffer.

   Note: 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.

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

	Converter Mix
PE Assay Buffer	18 µl
PE Converter	2 µl

   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**.**

4. **Reaction mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 30 µl:

	Reaction Mix
PE Assay Buffer	22 µl
PE Developer	4 µl
PE Enzyme Mix	2 µl
PE Probe	2 µl

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

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

6. **Calculations:** Subtract 0 PE reading from all PE standard readings. Plot the Phosphatidylethanolamine Standard Curve. Subtract sample background control readings from sample readings. If 0 PE standard readings are higher than sample background control readings, subtract those form sample readings instead. Apply corrected RFU to Standard Curve to get B nmol PE in the sample well.

PE concentration in sample: C = (B / V) X D (nmol/ml or µM)

Where **B** = amount of Phosphatidylethanolamine in the sample well from Standard Curve (nmol)
V = volume of sample added into the well (ml)
D = dilution factor

PE molecular weight: 726 g/mol
PE concentrations can also be expressed as nmol PE per mg protein or nmol PE per mg tissue weight.

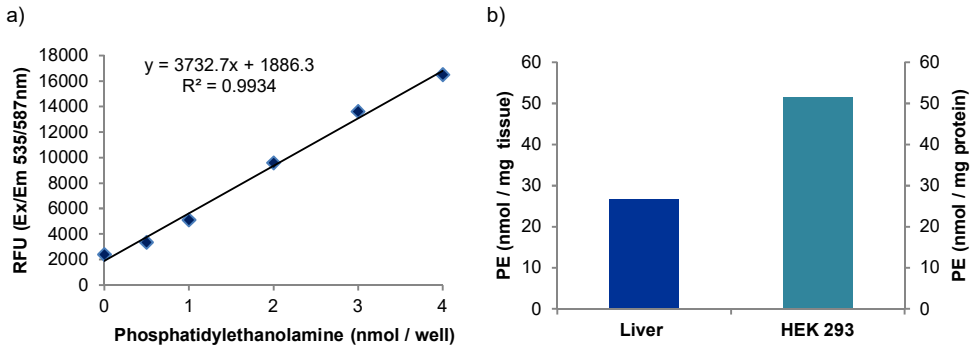

Figure: (a) Phosphatidylethanolamine standard curve. (b) Phosphatidylethanolamine content in rat liver (100 µg wet tissue) and HEK 293 cells (25 µg protein). Sample preparation and assay was carried out according to kit protocol.

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