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# Product Information

**Phosphatidylethanolamine Assay Kit**

Catalog Number **MAK361**
Storage Temperature –70 °C

## TECHNICAL BULLETIN

### Product Description

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.

The Phosphatidylethanolamine Assay Kit is a microplate based enzymatic assay for the quantitation of PE in cells and tissues. PE Converter hydrolyzes PE to an intermediate, which converts a colorless probe to a fluorescent product via enzymatic reaction ($\lambda_{ex}$ = 535 nm/$\lambda_{em}$ = 587 nm). The intermediate formed through PE converter hydrolysis is specific to phosphatidylethanolamine. No other phospholipids (i.e., phosphatidylcholine, phosphatidylinositol, or phosphatidic acid) will be detected, making the kit highly specific. The assay kit can detect as low as 0.2 nmol per well.

The kit is suitable for the measurement of phosphatidyl-ethanolamine content in lipid extracts from cells and tissues.

### Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

| | |
|---|---|
| PE Assay Buffer<br>Catalog Number MAK361A | 25 mL |
| PE Converter<br>Catalog Number MAK361B | 1 vial |
| PE Developer<br>Catalog Number MAK361C | 600 µL |
| PE Enzyme Mix<br>Catalog Number MAK361D | 1 vial |
| PE Probe<br>Catalog Number MAK361E | 200 µL |
| PE Standard (1 mM)<br>Catalog Number MAK361F | 100 µL |

### Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Clear flatbottom 96 well plates
- Fluorescence multiwell plate reader
- Water bath/heating plate capable of 80 °C
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1)
- Triton™ X-100, peroxide- and carbonyl-free (Catalog Number X100PC)

### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

The kit is shipped on dry ice. Upon arrival, store PE Developer at –70 °C, protected from light. Store the remaining components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

**Preparation Instructions**

PE Assay Buffer and PE Probe: Warm to room temperature prior to use.

PE Converter and PE Enzyme Mix: Reconstitute each vial in 220 μL of PE Assay Buffer before use. Aliquot remaining components. Store at –20 °C. Reconstituted vials are stable for at least two months.

PE Developer: Store at –70 °C. Thaw on ice before use. Aliquot and store the remaining solution at –70 °C. Avoid repeated freeze that cycles.

PE Standard: Before each use, thaw in a water bath at 45 °C for 15-20 minutes. The solution should look clear. Aliquot and store the remaining at –20 °C.

5% Triton X-100: Prepare a 5% (v/v) solution by diluting peroxide free Triton X-100 20-fold in ultrapure water.

**Procedure**

Sample Preparation

1. Homogenize tissue (~100 mg; non-perfused) or cells (~1 million) in 1 mL of 5% Triton X-100.
2. Protein content in the sample may be determined at this stage if desired (use BCA method).
3. Heat the samples to 80 °C in a water bath or on a heat plate for 5-10 minutes or until the solution becomes cloudy, then cool down to room temperature.
4. Repeat the heating step once more to solubilize all lipids then allow the solution to cool to room temperature.
5. Centrifuge at 10,000 × *g*, for 10 minutes at 4 °C and collect supernatant, which contains solubilized lipids. If not being used immediately, store supernatant at –70 °C.
6. 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". Note: Different dilutions of sample should be tested to make sure that phosphatidylethanolamine concentration falls in the linear range of the assay. Samples should be diluted using PE Assay Buffer.
7. Bring the volume in "Sample" wells to 50 μL and in "Sample background control" to 70 μL using PE Assay Buffer.

Standard Curve Preparation

Dilute the 1 mM PE Standard 10-fold with 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 Standard in 180 μL of PE Assay Buffer. Incubate at 45 °C for 30 minutes. Prepare Phosphatidylethanolamine (PE) Standards in desired wells of a clear 96 well plate according to Table 1. Mix well.

**Table 1.**
Preparation of Phosphatidylethanolamine (PE) Standards

| Well | 100 μM Premix | PE Assay Buffer | PE (nmol/well) |
|---|---|---|---|
| 1 | 0 μL | 50 μL | 0 |
| 2 | 5 μL | 45 μL | 0.5 |
| 3 | 10 μL | 40 μL | 1 |
| 4 | 20 μL | 30 μL | 2 |
| 5 | 30 μL | 20 μL | 3 |
| 6 | 40 μL | 10 μL | 4 |

Converter Mix

Mix enough reagent for the number of assays to be performed. For each sample and standard well, prepare 20 μL by mixing:

18 μL PE Assay Buffer
2 μL PE Converter

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.

Reaction Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare 30 μL by mixing:

21 μL PE Assay Buffer
5 μL PE Developer
2 μL PE Enzyme Mix
2 μL PE Probe

Add 30 μL of the reaction mix to all wells. Mix well. Incubate at 45 °C for 3 hours.

Measurement

Record fluorescence in end-point mode at $\lambda_{ex}$ = 535 nm/$\lambda_{em}$ = 587 nm.

**Results**

1. Subtract 0 PE reading from all PE standard readings.
2. Plot the Phosphatidylethanolamine Standard Curve.
3. Subtract sample background control readings from sample readings. If 0 PE standard readings are higher than sample background control readings, subtract those from sample readings instead.
4. Apply corrected RFU to Standard Curve to get B nmol PE in the sample well.

PE concentration in sample (nmol/mL or μM) =

$$(B/V) \times D$$

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 1.**
Typical Phosphatidylethanolamine (PE) Standard Curve

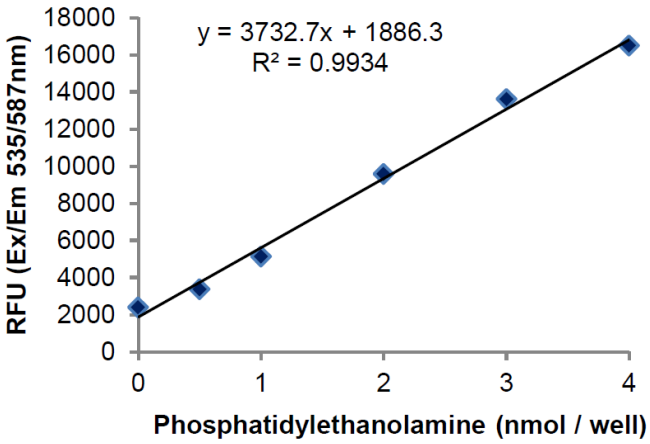
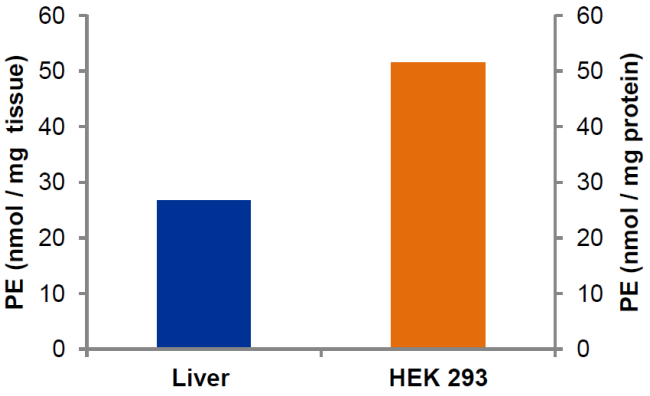



**Figure 2.**
Phosphatidylethanolamine content




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 procedure.

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