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Product Information

Phosphatidylserine Assay Kit

Catalog Number **MAK371**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Phosphatidylserine (PS) is a glycerophospholipid consisting of a phosphatidyl group attached to L-serine via a phosphodiester linkage. PS is a critical component of the cellular plasma membrane and accounts for 2-15% of plasma membrane lipid composition, depending on the cell or tissue type. The highest concentrations of PS are found in neuronal tissues, which are critical for maintaining conduction velocity in myelinated neurons, as well as for higher order cognitive skills such as learning and memory. In normal, healthy cells, PS is held in the inner membrane surface (facing the cytosol) by the lipid transporter protein flippase. However, in apoptotic cells, PS molecules 'shuffle' between the inner and outer plasma membrane monolayers. When PS molecules flip to the extracellular (outer) surface of the cell membrane, they act as a signal for macrophages to engulf and digest the (apoptotic) cell.

The Phosphatidylserine Assay Kit allows for quantification of PS in lipid extracts of cell and tissue lysates. The assay is based on the enzymatic cleavage of PS to yield phosphatidic acid and L-serine, which is subsequently metabolized and reacts with a probe to form a stable fluorophore at λ_{ex} = 538 nm/λ_{em} = 587 nm. The assay is selective for PS (other phospholipids such as phosphatidylcholine, phosphatidylethanolamine, or phosphatidic acid do not interfere). The kit is high-throughput ready and highly sensitive, detecting as little as 50 pmole/well of PS (5 μM in a 10 μL sample volume).

The kit is suitable for the measurement of phosphatidylserine concentration in lipid extracts such as soft tissue homogenates (i.e. liver, brain, etc.) and cultured cell lysates (adherent or suspension cells).

Components

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

Phosphatidylserine Assay Buffer Catalog Number MAK371A	25 mL
Probe Solution Catalog Number MAK371B	200 μL
Lipase Enzyme Mix Catalog Number MAK371C	1 vial
Serine Enzyme Mix Catalog Number MAK371D	1 vial
Developer Enzyme Mix Catalog Number MAK371E	1 vial
Phosphatidylserine Standard (1 mM) Catalog Number MAK371F	200 μL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Black flatbottom 96 well plates
- Fluorescence multiwell plate reader
- Centrifuge capable of RCF ≥3,000 × *g*
- Methanol (MeOH) (Catalog Number M1775)
- *tert*-Butyl methyl ether (MTBE) (Catalog Number 306975)
- Phosphate Buffered Saline (Catalog Number P3813)
- Triton™ X-100 (peroxide- and carbonyl-free) (Catalog Number X100PC)
- 15 mL polypropylene conical centrifuge tubes
- 10 mL glass vials
- Vacuum oven/concentrator or dry heat block
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)

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 wet ice. Store components at –20 °C, protected from light. Allow the Phosphatidylserine Assay Buffer to warm to room temperature prior to use. Briefly centrifuge small vials prior to opening.

Preparation Instructions.
Reagent Preparation
Probe Solution: Provided as a solution in DMSO. Divide into aliquots and store at –20 °C, protected from light. Prior to use, warm solution to room temperature. After use, promptly retighten cap to minimize adsorption of airborne moisture.

Lipase Enzyme Mix: Reconstitute contents with 220 µL of Phosphatidylserine Assay Buffer. Divide into aliquots and store at –20 °C (avoid repeated freeze/thaw cycles). Upon reconstitution, use within two months.

Serine Enzyme Mix and Developer Enzyme Mix: Reconstitute contents of each vial with 220 µL of ultrapure water. Divide into aliquots and store at –20 °C. Protect from light and avoid repeated freeze/thaw cycles. Upon reconstitution, use within two months.

Phosphatidylserine Standard (1 mM): Store at –20 °C, stable for at least 3 freeze/thaw cycles. Prior to use, thaw in a water bath or heat block at 45 °C for 15 minutes and vortex to ensure micellar solubilization. The solution should appear completely transparent.

Procedure
Sample Preparation
Notes:
- Sample lipid extraction may take several hours and should be completed before preparation of other reagents for the assay.
- **Methanol and *tert*-Butyl methyl ether (MTBE) vapors are highly flammable and potentially hazardous**. Consult institutional safety regulations regarding appropriate precautions for the lipid extraction procedure and consult the Safety Data Sheet regarding use of proper personal protective equipment.
- Take note of the input sample volume (prior to lipid extraction) and the resuspension volume (following organic solvent evaporation) in order to properly calculate the relative sample concentration in the lipid extract (µg of tissue/number of cells per µL of extract).

1. Thoroughly homogenize soft tissues (~100 mg of wet tissue) or cultured cells (~1×10^7 cells) in 1 mL of ice cold 1× PBS using a mechanical (Dounce) or ultrasonic probe homogenizer. Prior to performing lipid extraction, prepare a 1% (w/v) solution of Triton X-100 (peroxide- and carbonyl-free) in ultrapure water. Store protected from light.
2. Perform sample lipid extraction according to the following protocol:
   a. Add 200 µL of the sample homogenate to a 15 mL conical polypropylene centrifuge tube.
   b. Add 1.5 mL of MeOH and vortex thoroughly.
   c. Add 5 mL of MTBE to the sample/MeOH mixture.
   d. Vortex for 30 seconds and then incubate the mixture for 30 minutes at room temperature with gentle shaking.
   e. Following the organic extraction, induce phase separation by adding 1.25 mL of 1× PBS to the mixture (final MTBE/MeOH/PBS ratio of 10:3:2.5, [v/v/v]).
   f. Vortex for 30 seconds then centrifuge at 3,000 × *g* for 10 minutes at room temperature.
   g. At this point, two distinct layers will be visible: an upper (organic) phase containing the solubilized lipids and a lower (aqueous) phase.
   h. Carefully collect the upper (organic) phase with a pipette and transfer to a glass tube.
   i. Evaporate the organic solvent at ≥60 °C in a vacuum oven (or dry heat block within a fume hood) until the solvent evaporates completely.
   j. Once fully dried, the extracted lipids will form a thin translucent film stuck to the walls of the tube. Resuspend the dried lipid film in 50-200 µL of 1% Triton X-100 (peroxide- and carbonyl-free) and vigorously vortex or sonicate to ensure solubilization.
   k. If not being used immediately, resolubilized extracted lipids may be stored at –80 °C for up to 1 week.

3. Add 2-10 µL of sample lipid extract to desired well(s) in a black, flat bottom 96 well plate. For each test sample, prepare two parallel wells: one for determination of PS and one to serve as a sample background control. Adjust the volume of all sample wells to 30 µL/well with Phosphatidylserine Assay Buffer. PS levels can vary dramatically depending upon the sample type. Perform a pilot experiment to ensure readings are within the standard curve range. Highly concentrated lipid extracts may be diluted in 1% Triton X-100.

Standard Curve Preparation

Prepare a 200 µM Phosphatidylserine standard by adding 20 µL of the 1 mM Phosphatidylserine Standard stock to 80 µL of Phosphatidylserine Assay Buffer. Prepare Phosphatidylserine (PS) Standards in desired wells of a black 96 well plate according to Table 1.

Table 1.
Preparation of Phosphatidylserine (PS) Standards

Well	200 µM Premix	Phosphatidylserine Assay Buffer	PS (pmol/well)
1	0 µL	30 µL	0
2	2 µL	28 µL	400
3	4 µL	26 µL	800
4	6 µL	24 µL	1,200
5	8 µL	22 µL	1,600
6	10 µL	20 µL	2,000

Lipase Reaction Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µL of Lipase Enzyme Mix according to Table 2.

Table 2.
Preparation of Lipase Enzyme Mix

Reagent	Samples & Standards	Sample Background
Phosphatidylserine Assay Buffer	18 µL	20 µL
Lipase Enzyme Mix	2 µL	–

Add 20 µL of the Lipase Reaction Mix to wells containing the samples and standards. For sample background control wells, add 20 µL of Phosphatidylserine Assay Buffer without Lipase Enzyme Mix, bringing the volume of all wells to 50 µL. Incubate the plate at 45 °C for 90 minutes, **protected from light.**

Developer Reaction Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Developer Reaction Mix according to Table 3.

Table 3.
Preparation of Developer Reaction Mix

Reagent	Developer Reaction Mix
Phosphatidylserine Assay Buffer	45 µL
Probe Solution	1 µL
Serine Enzyme Mix	2 µL
Developer Enzyme Mix	2 µL

Add 50 µL of Developer Reaction Mix to all test sample, standard curve, and sample background control wells, bringing the final reaction volume to 100 µL per well.

Measurement

Incubate the plate at 37 °C for 60 minutes, protected from light. Measure the fluorescence of all sample, background, and standard curve wells at λ_{ex} = 538 nm/ λ_{em} = 587 nm in endpoint mode.

Results

1. For the PS Standard curve, subtract the zero standard (0 pmol/well) reading from all of the standard readings.
2. Plot the background-subtracted values and calculate the slope of the standard curve.
3. For test samples, calculate the corrected sample fluorescence (F_s) by subtracting the Sample Background RFU reading from the corresponding sample readings: $F_s = RFU_S - RFU_{BC}$.
4. Compare the F_s values to the standard curve to get pmol of PS (B) in the well.

Phosphatidylserine Concentration (pmol/µL or µM) =

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

where:
B = amount of phosphatidylserine in the sample well from Standard Curve (pmol)
V = volume of sample lipid extract added to the well (in µL)
D = dilution factor

Note: PS concentrations can also be expressed as pmol per mg of tissue or pmol per number of cells, based upon the concentration of sample lysate prior to lipid extraction and the volume of 1% Triton X-100 used to resuspend the dried lipids following extraction.

Figure 1.
Typical Phosphatidylserine Standard Curve

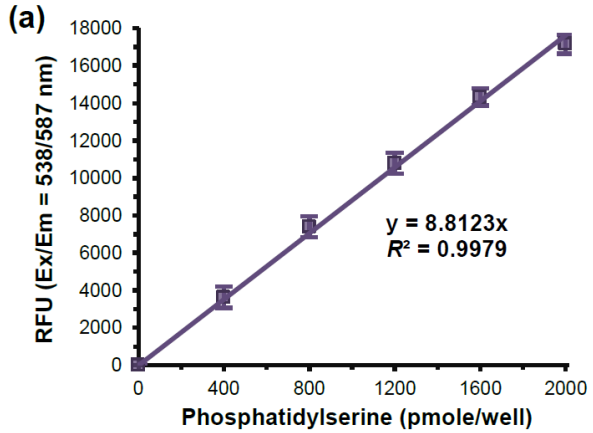
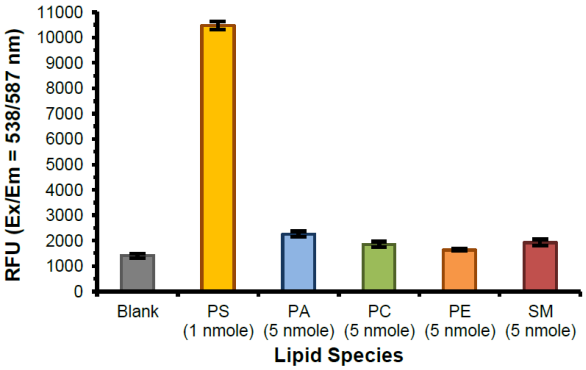
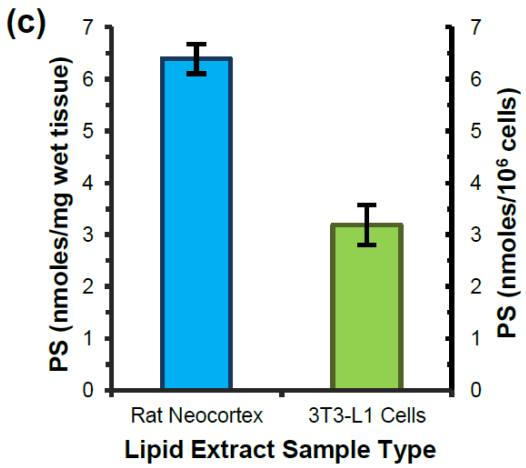


Figure 2.
Specificity for Detection of Phosphatidylserine (PS)



Specificity for detection of phosphatidylserine (PS) over other common phospholipids. At a 5-fold molar excess (5 nmoles) versus PS (1 nmole), phosphatidic acid (PA) contributes ≤10% interference while phosphatidyl-choline (PC), phosphatidylethanolamine (PE), and sphingomyelin (SM) contribute ≤5%.

Figure 3.
Estimation of PS in Various Lipid Extracts



Estimation of PS in lipid extracts of lysates from rat neocortex (2 µL extract, 200 µg/µL wet brain tissue) and 3T3-L1 cells (2 µL extract, 100,000 cells/µL). Lipid extraction was carried out as described above. Data are mean ± SEM of 3 replicates, assayed according to the kit procedure.

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