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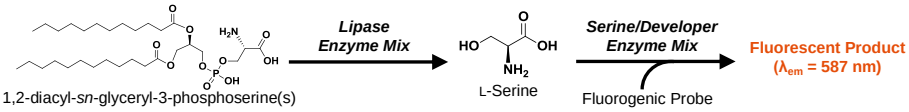
Phosphatidylserine Assay Kit (Fluorometric)

rev 08/19

(Catalog # K565-100; 100 Reactions; Store at -20°C)

I. Introduction:

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. It is 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) cells. BioVision's Phosphatidylserine Assay Kit allows the quantification of PS in lipid extracts of cell or tissue lysates and biological fluids (serum/plasma). The assay is based on the enzymatic cleavage of PS to yield phosphatidic acid and L-serine, which subsequently reacts with a probe to form a stable fluorophore (Ex/Em = 538/587 nm). The assay is selective for PS (other phospholipids such as phosphatidylcholine, phosphatidylethanolamine or phosphatidic acid do not interfere), is high-throughput adaptable and can detect as little as 50 pmole/well of PS (5 µM in a 10 µl sample volume).



II. Application:

- Estimation of phosphatidylserine concentration in lipid extracts of various biological samples

III. Sample Type:

- Soft tissue homogenates (*i.e*. liver, brain, etc.)
- Biological fluids (serum/plasma)
- Cultured cell lysates (adherent or suspension cells)

IV. Kit Contents:

Components	K565-100	Cap Code	Part Number
Phosphatidylserine Assay Buffer	25 ml	WM	K565-100-1
Probe Solution	200 µl	Red	K565-100-2
Lipase Enzyme Mix	1 vial	White	K565-100-3
Serine Enzyme Mix	1 vial	Blue	K565-100-4
Developer Enzyme Mix	1 vial	Green	K565-100-5
Phosphatidylserine Standard (1 mM)	200 µl	Yellow	K565-100-6

V. User Supplied Reagents and Equipment:

- Multiwell fluorescence microplate reader
- Black 96-well plates with flat bottom
- Reagent-grade methanol (MeOH), methyl *tert*-butyl ether (MTBE) and 1x PBS for sample lipid extraction
- Peroxide-free Triton X-100 solution (BV Cat. # 2124)
- 15 ml polypropylene conical centrifuge tubes and 10 ml glass vials
- Vacuum oven/concentrator or dry heat block

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the Phosphatidylserine Assay Buffer to warm to room temperature (RT) prior to use. Read the entire protocol before performing the assay procedure.

- **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 RT. 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 ddH_2O. 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 min and vortex to ensure micellar solubilization. The solution should appear completely transparent.

VII. Phosphatidylserine Assay Protocol:

1. Sample Preparation:

Note: Sample lipid extraction may take several hours and should be completed before preparation of other reagents for the assay. Methanol and methyl *t*-butyl ether (MTBE) vapors are highly flammable and potentially hazardous. Perform the lipid extraction procedure in a fume hood and use appropriate personal protective equipment.

a. Thoroughly homogenize soft tissues (~100 mg wet tissue) or cultured cells (~1 x 10^7 cells) in 1 ml ice cold 1X PBS using a mechanical (Dounce) or ultrasonic probe homogenizer. Serum/plasma may be used directly in the extraction process. Prior to performing lipid extraction, prepare a 1% (w/v) solution of Peroxide-Free Triton X-100 in ddH_2O (store protected from light).

b. Perform sample lipid extraction according to the following protocol: Add 200 µl of the sample homogenate (or serum/plasma) to a 15 ml conical polypropylene centrifuge tube, mix with 1.5 ml MeOH and vortex thoroughly. Add 5 ml of MTBE to the sample/MeOH mix, vortex for 30 sec and incubate the mixture for 30 min at RT with gentle shaking. Following the organic extraction, induce phase separation by adding 1.25 ml of PBS to the mixture (for a final MTBE/MeOH/Saline ratio of 10:3:2.5, v/v/v). Vortex for 30 sec and centrifuge at 3000 x *g* for 10 minutes at RT. At this point, two distinct layers will be visible: an upper (organic) phase containing the solubilized lipids and a lower (aqueous) phase. Carefully collect the upper (organic) phase with a pipette and transfer to a glass tube. Evaporate the organic solvent at ≥60°C in a vacuum oven (or dry heat block within a fume hood) until the solvent evaporates

completely. 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-Free) and vigorously vortex or sonicate to ensure solubilization. *If not being used immediately, resolubilized extracted lipids may be stored at -80°C for up to 1 week.*

c. 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.

Notes:

- Take note of the input sample volume (prior to lipid extraction) and the resuspension volume (following solvent evaporation) in order to properly calculate the relative sample concentration in the lipid extract (µg of tissue/number of cells/µl serum per µl extract).
- PS levels can vary dramatically depending upon the sample type. We recommend performing a pilot experiment to ensure readings are within the standard curve range. Highly concentrated lipid extracts may be diluted in 1% Triton X-100.

2. Standard Curve Preparation: Prepare a 200 µM working solution of PS by adding 20 µl of the 1 mM Phosphatidylserine Standard stock to 80 µl of Phosphatidylserine Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of the 200 µM working solution into a series of wells, generating 0, 400, 800, 1200, 1600 and 2000 pmol of PS per well. Adjust the volume to 30 µl/well with Phosphatidylserine Assay Buffer.

3. Lipase Reaction Mix Preparation:

a. Prepare Lipase Reaction Mix according to the table below. Make a sufficient amount of Lipase Reaction Mix to add 20 µl to each of the test sample and PS standard curve wells.

	Samples & Standards	Sample Background
Phosphatidylserine Assay Buffer	18 µl	20 µl
Lipase Enzyme Mix	2 µl	—

b. Add 20 µl 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 min, **protected from light**.

4. Developer Reaction Mix Preparation:

a. Prepare Developer Reaction Mix according to the table below. Make a sufficient amount of reaction mix to add 50 µl to each well.

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

b. 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.

5. Measurement: Incubate the plate at 37°C for 60 min, **protected from light**. Measure the fluorescence of all sample, background and standard curve wells at Ex/Em = 538/587 nm in endpoint mode.

6. Calculations: For the PS Standard curve, subtract the zero standard (0 pmol/well) reading from all of the standard readings, plot the background-subtracted values and calculate the slope of the standard curve. For test samples, calculate the corrected sample fluorescence (F_S) by subtracting the Sample Background RFU reading from the corresponding sample readings: $F_S = RFU_S - RFU_{BC}$. Apply the F_S values to the standard curve to get *B* pmol of PS in the well.

$$\textbf{Sample Phosphatidylserine Concentration} = \frac{B}{V} \times D = \textbf{pmol/µl = µM}$$

Where: ***B*** is the amount of phosphatidylserine, calculated from the Standard Curve (in pmol)
V is the volume of sample lipid extract added to the well (in µl)
D is the sample dilution factor (if applicable, *D*=1 for undiluted samples)

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.

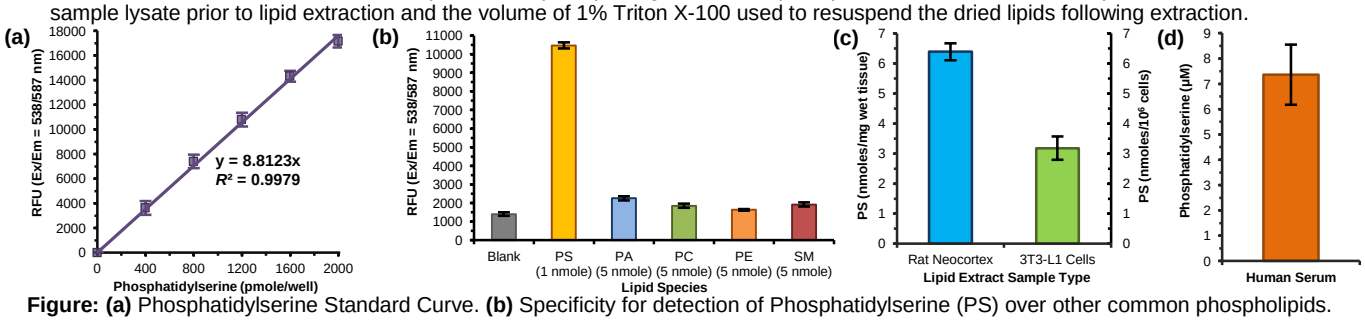


Figure: (a) Phosphatidylserine Standard Curve. **(b)** Specificity for detection of Phosphatidylserine (PS) over other common phospholipids. At a 5-fold molar excess (5 nmoles) versus PS (1 nmole), phosphatidic acid (PA) contributes ≤10% interference while Phosphatidylcholine (PC), Phosphatidylethanolamine (PE) and Sphingomyelin (SM) contribute ≤5%. **(c-d)** Estimation of PS in lipid extracts of lysates from rat neocortex (2 µl extract, equivalent to 200 µg wet brain tissue), 3T3-L1 cells (2 µl extract, equivalent to 200000 cells) and pooled human serum (10 µl extract, equivalent to 20 µl serum). Lipid extraction in MTBE/MeOH/Saline was carried out as described above and the dried extract was re-suspended in 1% Triton X-100 (w/v) solution. Data are mean ± SEM of 3 replicates, assayed according to the kit protocol.

VIII. RELATED PRODUCTS:

PicoProbe™ Phosphatidic Acid Assay Kit (K748)	Phosphatidylethanolamine Assay Kit (K499)	Phosphatidylcholine Assay Kit (K576)
Glycerophosphorylcholine Assay Kit (K433)	Phospholipid Assay Kit (K351)	Sphingomyelin Assay Kit (K600)
Cardiolipin Assay Kit (K944)	Triglyceride Assay Kit (K622)	DL-Serine Assay Kit (K743)

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