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ab273295 Phosphatidylserine Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273295
(use https://www.abcam.cn/ab273295 for china, or
https://www.abcam.co.jp/ab273295 for Japan)

For the estimation of Phosphatidylserine concentration in lipid extracts of various biological samples.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Phosphatidylserine Assay Kit (Fluorometric) (ab273295) allows the quantification of Phosphatidylserine (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).

2. Protocol Summary

Prepare samples as directed

⇩

Prepare all reagents as directed

⇩

Prepare standard curve

⇩

Add Standard, Sample, and Sample Background Control to appropriate wells and adjust volume to 30 µL

⇩

Add Reaction Mix (20 µL)to Standard and Sample wells. Add Sample Background Mix (20 µL) to Sample Background Control wells

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Mix and incubate at 45°C for 90 minutes protected from light

⇩

Add Developer Reaction Mix to Standard, Sample and Sample Background Control wells (50 µL)

⇩

Mix and incubate at 37°C for 60 minutes protected from light

⇩

Measure fluorescence (Ex/Em = 538/587 nm) in end point mode

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Phospholipid Assay Buffer/Phosphatidylserine Assay Buffer	25 mL	-20°C	+4°C
OxiRed Probe/Probe Solution	200 µL	-20°C	-20°C
Converter Enzyme IV/Lipase Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
Serine Racemase Enzyme Mix/Serine Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
PS Developer Mix/Developer Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
Phosphatidylserine Standard (1 mM)	200 µL	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Multi-well spectrophotometer capable of measuring fluorescence at Ex/Em= 538/587 nm
- Black 96-well plate 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
- 15 ml polypropylene conical centrifuge tubes
- 10 ml glass vials
- Vacuum oven/concentrator or dry heat block
- Dounce homogenizer or ultrasonic probe homogenizer

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Phospholipid Assay Buffer/Phosphatidylserine Assay Buffer:**
Ready to use as supplied. Store at -20°C. Bring to room temperature before use.

9.2 **OxiRed Probe/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 absorption of airborne moisture.

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

9.4 **Serine Racemase Enzyme Mix/Serine Enzyme Mix:**
Reconstitute 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.

9.5 **PS Developer Mix/Developer Enzyme Mix:**
Reconstitute with 220 µL of ddH2O. Divide into aliquots and store at -20°C. Protect from light and avoid repeated freeze/thaw cycles. Upon reconstitution, use within two months.

9.6 **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.

10. Sample Preparation

Δ 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.

10.1 **Soft tissues and cultured cells:**

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.

10.2 **Serum and plasma:**

Serum/plasma may be used directly in the lipid extraction process.

10.3 **Lipid extraction:**

10.3.1 Prior to performing lipid extraction, prepare a 1% (w/v) solution of Peroxide-Free Triton X-100 in ddH_2O (store protected from light).

10.3.2 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.

10.3.3 Add 5 mL of MTBE to the sample/MeOH mix, vortex for 30 seconds and incubate the mixture for 30 minutes at room temperature with gentle shaking.

10.3.4 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 room temperature.

10.3.5 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.

10.3.6 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.

10.3.7 Resuspend the dried lipid film in 50-200 µL of 1% Triton X-100 (Peroxide-Free) and vigorously vortex or sonicate to ensure solubilization.

Δ Note: If not being used immediately, resolubilized extracted lipids may be stored at -80°C for up to 1 week.

Δ Note: 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).

11. Standard Curve

11.1 Prepare a 200 µM solution of Phosphatidylserine-Standard by diluting 20 µL of 1 mM Phosphatidylserine Standard with 80 µL of Phospholipid Assay Buffer/Phosphatidylserine Assay Buffer.

11.2 Add 0, 2, 4, 6, 8 and 10 µL of the 200 µM Phosphatidylserine standard into a series of wells in a black96-well plate to obtain 0, 400, 800, 1200, 1600 and 2000 pmol/well.

11.3 Adjust the volume of each well to 30 µL with Phospholipid Assay Buffer/Phosphatidylserine Assay Buffer

Standard #	Phosphatidylserine Standard (µL)	Phospholipid Assay Buffer/Phosphatidylserine Assay Buffer (µL)	Phosphatidylserine Standard (pmol/well)
1	10	20	2000
2	8	22	1600
3	6	24	1200
4	4	26	800
5	2	28	400
6	0	30	0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1.1 For Sample (S), prepare two wells for each sample labeled "Sample Background Control" (SBC), and "Sample" (S). Add 2 -10 µL sample of the lipid extract into each of these wells.

12.1.2 Adjust the volume of Sample and Sample Background Control to 30 µL/well with Phospholipid Assay Buffer/Phosphatidylserine Assay Buffer. Mix well.

12.1.3 **Reaction Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 20 µL of Reaction Mix. Mix well.

Component	Reaction Mix	Sample Background Mix
Phospholipid Assay Buffer/Phosphatidylserine Assay Buffer	18 µL	20 µL
Converter Enzyme IV/Lipase Enzyme Mix	2 µL	--

12.1.4 Add Reaction Mix to Standard Curve and Sample wells. Add Sample Background Mix (Phospholipid Assay Buffer/Phosphatidylserine Assay Buffer without Converter Enzyme IV/Lipase Enzyme Mix) to wells designated as Sample Background.

12.1.5 Mix and incubate the plate at 45°C for 90 minutes, protected from light.

12.1.6 **Developer Reaction Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Developer Reaction Mix. Mix well.

Component	Reaction Mix
Phospholipid Assay Buffer/Phosphatidylserine Assay Buffer	45 µL
OxiRed Probe/Probe Solution	1 µL

Serine Racemase Enzyme Mix/Serine Enzyme Mix	2 µL
PS Developer Mix/Developer Enzyme Mix	2 µL

12.1.7 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.

12.1.8 Incubate the plate at 37°C for 60 minutes, protected from light.

12.1.9 Measure the fluorescence of all sample, background and standard curve wells at Ex/Em = 538/587 nm in end-point mode.

13. Calculations

13.1 Subtract the 0 Phosphatidylserine Standard reading from all standard curve readings.
13.2 Plot the background-subtracted values and calculate the slope of the standard curve.
13.3 For test samples, calculate the corrected sample fluorescence (Fs) by subtracting the Sample Background RFU reading from the corresponding sample readings: Fs = RFU_S – RFU_{BC}.
13.4 Apply the Fs values to the standard curve to get B pmol of PS in the well.

$$Sample\ Phosphatidylserine\ Concentration = \left(\frac{B}{V}\right) x\ D = pmol/\mu L = \ \mu M$$

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

Δ 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.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

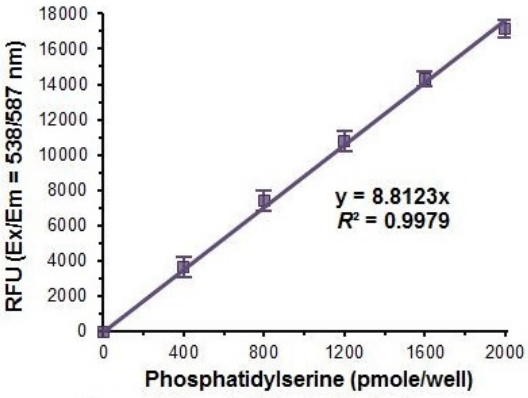

Figure 1. Phosphatidylserine standard curve.

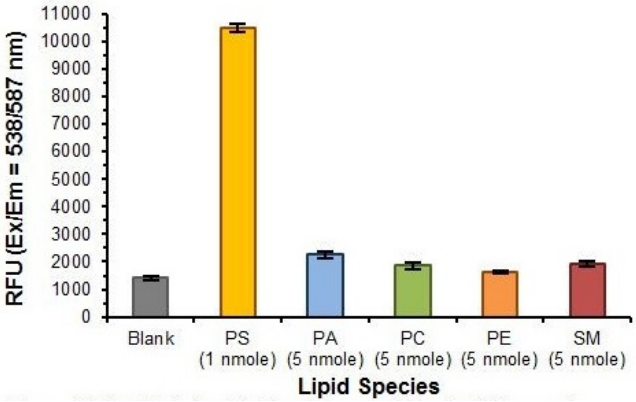

Figure 2. Specificity for detection of Phosphatidylserine (PS) over other common phospholipids. At a 5-fold molar excess (5 nmoles) versus PS (1 nmole), phosphatidic acid (PA) contributes ≤10% interference while Phosphatidylcholine (PC), Phosphatidylethanolamine (PE) and Sphingomyelin (SM) contribute ≤5%.

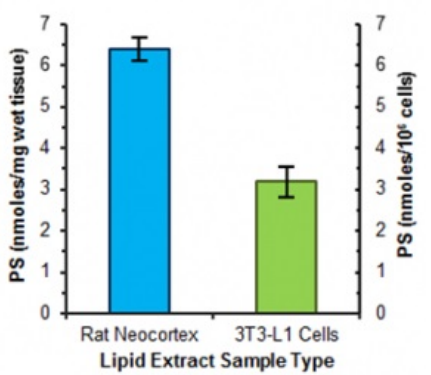

Figure 3. Estimation of PS in lipid extracts of lysates from rat neocortex (2 µL extract, equivalent to 200 µg wet brain tissue) and 3T3-L1 cells (2 µL extract, equivalent to 200000 cells). Lipid extraction in MTBE/MeOH/Saline was carried out as described above and the dried extract was re-suspended in 1% Triton X-100 (w/v) solution. Data are mean ± SEM of 3 replicates, assayed according to the kit protocol.

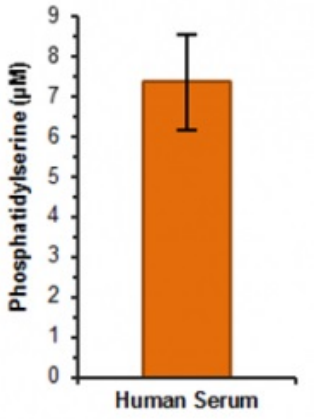

Figure 4. Estimation of PS in lipid extracts of lysates from pooled human serum (10 µl extract, equivalent to 20 µl serum). Lipid extraction in MTBE/MeOH/Saline was carried out as described above and the dried extract was re-suspended in 1% Triton X-100 (w/v) solution. Data are mean ± SEM of 3 replicates, assayed according to the kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. 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)