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ab273288 Phosphatidylglycerol Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273288
(use https://www.abcam.cn/ab273288 for china, or
https://www.abcam.co.jp/ab273288 for Japan)

For the measurement of Phosphatidylglycerol content in cell and tissue extracts and biological fluids.

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

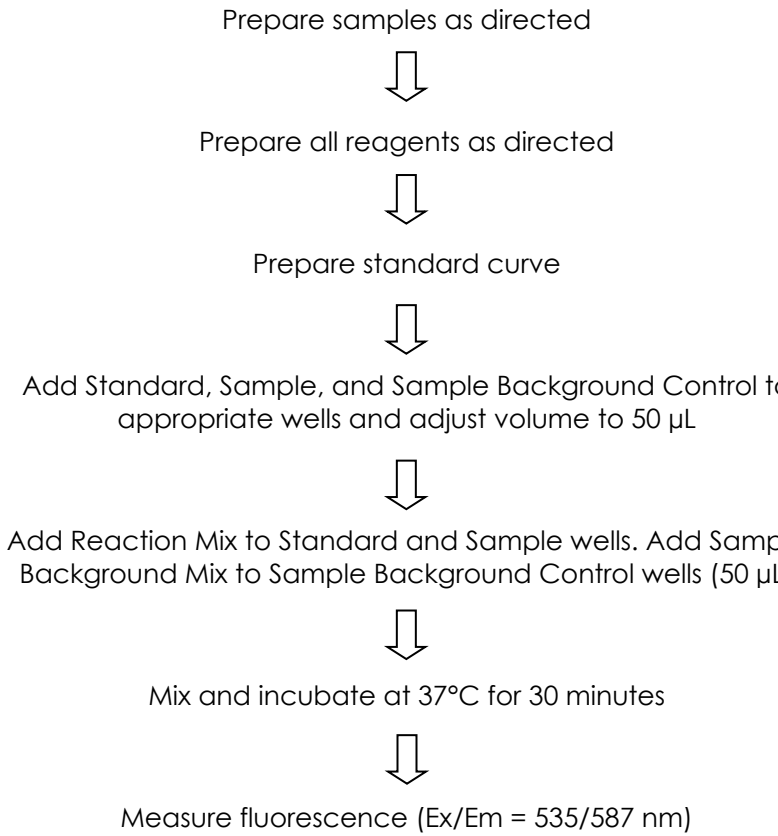
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to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

Table of Contents

1. Overview

Phosphatidylglycerol Assay Kit (Fluorometric) (ab273288) utilizes specific enzymes to generate an intermediate that reacts with a probe. This yields a signal that can be quantified fluorometrically and is proportional to the amount of Phosphatidylglycerol (PG) present in the sample, unaffected by the presence of phosphatidic acid, cardiolipin, or other lipids. When used as described, the assay is highly selective against other phospholipids and capable of detecting as little as 20 pmoles of phosphatidylglycerol.

2. Protocol Summary



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. Kit has a storage time of 6 months from 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)
Assay Buffer I/PG Assay Buffer	25 mL	-20°C	+4°C
PG Converter Mix/PG Converter Mix (Lyophilized)	1 vial	-20°C	-20°C
Enzyme Mix VI/PG Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
OxiRed Probe/PG Probe (in DMSO)	200 µL	-20°C	-20°C
Phosphatidylglycerol Standard/PG Standard (0.5 µM) (Lyophilized)	1 vial	-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= 535/587 nm
- Black Opaque 96-well plate with flat bottom
- Dounce Tissue Homogenizer
- 15 ml conical tubes
- 100% Methanol
- Chloroform

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 **Assay Buffer I/PG Assay Buffer:**
Ready to use as supplied. Store at 4°C, protected from light. Bring to room temperature before use.

9.2 **PG Converter Mix:**
Reconstitute with 220 µL Assay Buffer I/PG assay buffer. Aliquot and store at -20°C. Use within two months.

9.3 **Enzyme Mix VI/PG Enzyme Mix:**
Reconstitute with 220 µL Assay Buffer I/PG assay buffer. Aliquot and store at -20°C. Use within two months.

9.4 **OxiRed Probe/PG Probe:**
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.

9.5 **Phosphatidylglycerol Standard/PG Standard:**
Reconstitute with 0.5 mL Assay Buffer, heat to 37°C, and mix thoroughly to generate a 1.0 mM Phosphatidylglycerol Standard/PG Standard solution.

9.6 **Upper Wash Layer (for washing lipid extract):**
Combine 5 mL methanol (not provided) with 5 mL chloroform (not provided) and 4.5 mL dH_2O, shake vigorously and allow mixture to separate into a lower layer and an aqueous upper wash layer.

10. Sample Preparation

Tissues and cell pellets: (Can be scaled appropriately)

10.1 Thoroughly homogenize soft tissues (~10 mg wet tissue) or cultured cells (~2 x 10^6 cells) in 200 µL ice cold Assay Buffer I/PG Assay Buffer using a mechanical (Dounce) homogenizer.

10.2 Once homogenized, perform sample lipid extraction according to the following protocol: add 200 µL of the sample homogenate to a 15 mL conical polypropylene centrifuge tube, mix with 750 µl of a 1:2 mixture of chlorofom:methanol (both not provided). Vortex thoroughly for 1 minute. Add 250 µL chloroform to the sample/chloroform/methanol mix, vortex for 30 second. Add 250 µL dH_2O to the tube, and again vortex to thoroughly mix and centrifuge sample at 1,500 x *g* for 10 minutes at room temperature (25°C).

10.3 Once the sample/chloroform/methanol centrifugation has finished, three distinct layers will be visible: an upper phase containing methanol and aqueous fractions, a thin layer of precipitated protein, and the solubilized lipids in a lower organic phase. Aspirate and discard the upper aqueous phase, being careful not to remove the lower phase. Add 500 µl of the upper wash layer (Section VI) to the sample. Mix vigorously and spin down at 1,500 *x g* for 10 minutes at room temperature. Remove upper phase. This wash step may be repeated to further enrich lipids. After final wash(es), collect the lower phase through the thin precipitated protein layer with a pipette and transfer to a fresh tube. Evaporate the organic solvent at ≥37°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 20-200 µl of Assay Buffer I/PG Assay Buffer and vigorously vortex or sonicate to ensure solubilization.

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

Δ Note: Once extracted, the lipid extracts can be stored up to two months at -80°C for future experiments.

11. Standard Curve

11.1 Prepare a 100 µM solution of PG-Standard by diluting 50 µL of 1.0 mM Phosphatidylglycerol Standard/PG Standard with 450 µL of Assay Buffer I/PG Assay Buffer.

11.2 Add 0, 2, 4, 6, 8 and 10 µL of the 100 µM Phosphatidylglycerol Standard/PG standard into a series of wells in an opaque black 96-well plate to obtain 0, 200, 400, 600, 800 and 1000 pmol/well.

11.3 Adjust the volume of each well to 50 µL with Assay Buffer I/PG Assay Buffer.

Standard #	Phosphatidylglycerol Standard/PG Standard (µL)	Assay Buffer I/PG Assay Buffer (µL)	Phosphatidylglycerol Standard/PG Standard (pmol/well)
1	10	40	1000
2	8	42	800
3	6	44	600
4	4	46	400
5	2	48	200
6	0	50	0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1 For Sample (S), prepare two wells for each sample labeled "Sample Background Control" (SBC), and "Sample" (S). Add 2 -20 µL sample of the lipid extract into each of these wells.

12.2 Adjust the volume of Sample and Sample Background Control to 50 µL/well with Assay Buffer I/PG Assay Buffer. Mix well.

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

Component	Reaction Mix	Sample Background Mix
Assay Buffer I/PG Assay Buffer	45.6 µL	47.6 µL
PG Converter Mix	2 µL	--
Enzyme Mix VI/PG Enzyme Mix	2 µL	2 µL
OxiRed Probe/PG Probe	0.4 µL	0.4 µL

12.4 Add Reaction Mix to Standard Curve and Sample wells. Add Sample Background Mix to wells designated as Sample Background.

12.5 Mix and incubate at 37°C for 30 minutes.

12.6 Record Fluorescence in endpoint mode at Ex/Em = 535/587 nm.

13. Calculations

13.1 Subtract the 0 Phosphatidylglycerol Standard/PG Standard reading from all standard curve readings.
13.2 Plot the background-subtracted PG Standard Curve and calculate the slope.
13.3 For each sample well, subtract the background control reading from its paired sample reaction reading.
13.4 Calculate the corrected absorbance/fluorescence of the test samples $\Delta RFU = RFU_{sample} - RFU_{background}$.
13.5 Apply the corrected ΔRFU value to the PG Standard Curve to get B pmoles PG in the well.

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

B = PG amount from Standard Curve (pmol)
V = sample volume added into the reaction well (µL).
D = Sample dilution factor (if applicable)

14. Typical Data

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

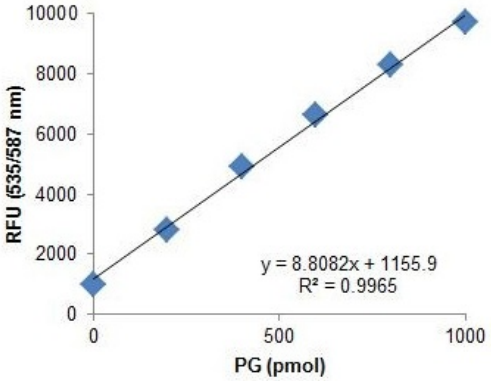


Figure 1. PG standard curve.

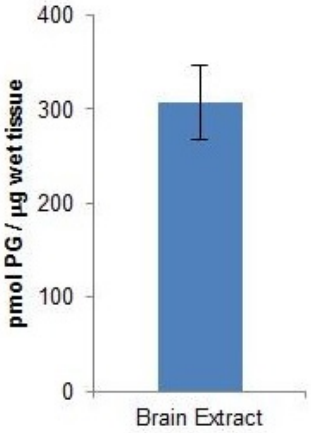


Figure 2. Determination of total PG concentration in lipid extracted from brain lysate.

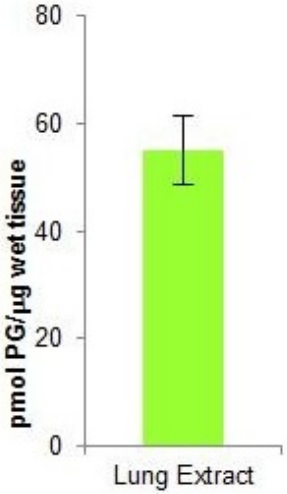


Figure 3. Determination of Phosphatidylglycerol in lipid extracted from lung tissue.

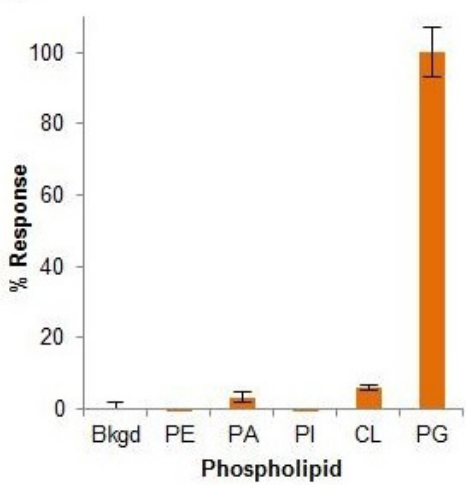


Figure 4. Specificity of assay, when used as described, for Phosphatidylglycerol (PG) versus Phosphatidylethanolamine (PE), Phosphatidic Acid (PA), Phosphatidylinositol (PI), and cardiolipin (CL). The amount of phospholipid for each sample was 1 nmole.

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)