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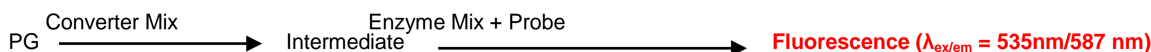
BioVision
BioVision Incorporated

Phosphatidylglycerol Assay Kit (Fluorometric)

(Catalog # K488-100; 100 assays; Store at -20°C)

I. Introduction:

Phospholipids are physiologically critical amphiphilic molecules that are involved in regulation of numerous cellular processes. Phosphatidylglycerol (PG) is a glycerophospholipid consisting of a glycerol backbone with two acyl chains and a phosphatidyl group that carries another glycerol moiety. Lung surfactant is an important lipid-protein mixture that aids in expansion and prevents total collapse of the lung during respiration. PG is a prominent phospholipid in lung surfactant, and its presence can be monitored as a measure of lung development in the maturing fetus. In addition, most bacteria utilize PG in their membrane, and plants utilize this component as the major phospholipid in the photosynthetic membranes of leaf tissue. In HPLC analysis of phospholipids, phosphatidic acid can interfere with and complicate determination of PG content. BioVision's Phosphatidylglycerol Assay Kit utilizes specific enzymes to generate an intermediate that reacts with a probe, yielding a signal that can be quantified fluorometrically, and is proportional to the amount of 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 (see figure below) and capable of detecting as little as 20 pmoles of phosphatidylglycerol.



II. Applications:

- Measurement of PG content of various tissue/cell extracts
- Determination of PG concentration in biological fluids

III. Sample Type:

- Lipid extracts from tissue and cell lysates
- Lipid extracts from various biological fluids (*e.g.* serum, plasma)

IV. Kit Contents:

Components	K488-100	Cap Code	Part Number
PG Assay Buffer	25 ml	WM	K488-100-1
PG Converter Mix	1 vial	Purple	K488-100-2
PG Enzyme Mix	1 vial	Green	K488-100-3
PG Probe (in DMSO)	200 µl	Red	K488-100-4
PG Standard (0.5 µmol)	1 vial	Yellow	K488-100-5

V. User Supplied Reagents and Equipment:

- Black Opaque 96-well plate with flat bottom
- Multi-well spectrophotometer
- Dounce Tissue Homogenizer (BV Cat. # 1998 or similar)
- 15 ml conical tubes
- 100% Methanol, Chloroform

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **PG Assay Buffer:** Allow to warm to room temperature prior to use. Store at 4°C, protected from light.
- **PG Converter Mix and PG Enzyme Mix:** Reconstitute each vial with 220 µl PG Assay Buffer. Aliquot and store at -20°C. Once reconstituted, use within two months.
- **PG Probe (in DMSO):** 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.
- **PG Standard:** Reconstitute with 0.5 ml Assay Buffer, heat to 37°C, and mix thoroughly to generate a 1.0 mM PG Standard solution.
- **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**.

VII. PG Assay Protocol:

a. Sample Preparation:

Tissues and cell pellets: (Can be scaled appropriately) Thoroughly homogenize soft tissues (~10 mg wet tissue) or cultured cells (~2 x 10^6 cells) in 200 µl ice cold PG Assay Buffer using a mechanical (Dounce) homogenizer (BV Cat. #1998 or similar). 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 min. Add 250 µl chloroform to the sample/chloroform/methanol mix, vortex for 30 sec. Add 250 µl dH_2O to the tube, and again vortex to thoroughly mix and centrifuge sample at 1500 x *g* for 10 minutes at room temperature (25°C).
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 1500 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. Re-suspend the dried lipid film in 20-200 µl of PG Assay Buffer 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.*

Prepare duplicate wells, one sample and one sample background, by adding same volume (2-20 µl) of the lipid extract to the duplicate wells of a black 96-well plate and adjust the volume in each well to 50 µl with Assay Buffer.

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

2. **Standard Curve Preparation:** Generate a 100 µM PG standard solution by diluting 50 µl of the PG Standard (1.0 mM) with 450 µl of PG Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of the 100 µM PG standard to a series of wells in an opaque black 96-well plate; Bring the total volume of each well to 50 µl with PG Assay Buffer to generate 0, 200, 400, 600, 800, and 1000 pmoles PG per well.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well (Sample and Standard), prepare 50 µl Reaction Mix/well and 50 µl Sample Background Mix for wells designated as Sample Background:

	Reaction Mix	**Sample Background Mix**
PG Assay Buffer	45.6 µl	47.6 µl
PG Converter Mix	2 µl	--
PG Enzyme Mix	2 µl	2 µl
PG Probe*	0.4 µl	0.4 µl

Add the reaction mix to each well. Mix and incubate at 37°C for 30 minutes.

4. **Measurement:** Record Fluorescence in endpoint mode at Ex/Em = 535/587 nm.
5. **Calculation:** Subtract the 0 PG Standard reading from all standard curve readings, plot the background-subtracted PG Standard Curve and calculate the slope. For each sample well, subtract the background control reading from its paired sample reaction reading. Calculate the corrected absorbance/fluorescence of the test samples $\Delta RFU = RFU_{sample} - RFU_{background}$. Apply the corrected ΔRFU value to the PG Standard Curve to get *B* pmoles PG in the well.

Sample PG Concentration = (*B* / *V*) x *D* = pmoesl/µl ≡ µM

Where: ***B*** = PG amount from Standard Curve (in /pmoles)
V = sample volume added into the reaction well (in µl)
D = sample dilution factor (if applicable)

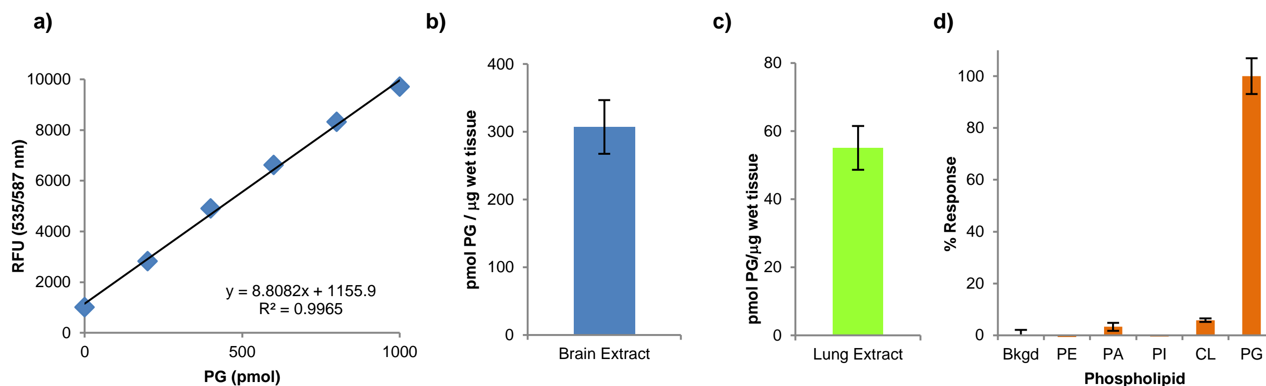


Figure: (a) PG standard curve, fluorometric **(b)** Determination of total PG concentration in lipid extracted from brain lysate **(c)** Determination of Phosphatidylglycerol in lipid extracted from lung tissue. **(d)** Specificity of assay, when used as described, for Phosphatidylglycerol (PG) versus Phosphatidylethanolamine (PE), Phosphatidic Acid (PA), Phosphatidylinositol (PI), and cardiolipin (CL). The amount of phospholipid for each sample was 1 nmole.

VIII. RELATED PRODUCTS:

Phospholipase D Activity Colorimetric Assay Kit (K725)
Cardiolipin Assay Kit (Fluorometric) (K944)
PicoProbe™ Phosphatidic Acid Assay Kit (K748)
PicoProbe™ Phosphatidylinositol Assay Kit (K750)
Phosphatidylcholine Colorimetric/Fluorometric Assay Kit (K576)
Phospholipid Assay Kit (Colorimetric/Fluorometric) (K351)
Lysophosphatidylcholine Assay Kit (K735)
Glycerophosphorylcholine Assay Kit (C/F) (K433)

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