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BioVision
BioVision Incorporated

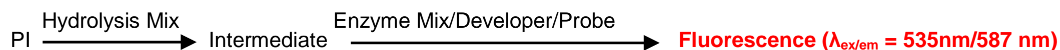
PicoProbe™ Phosphatidylinositol Assay Kit (Fluorometric)

rev 03/19

(Catalog # K750-100; 100 assays; Store at -20°C)

I. Introduction:

Phosphoinositides are critical second messengers in a multitude of cellular processes. The most fundamental phosphoinositide, phosphatidylinositol (PI), can be phosphorylated at multiple sites around its inositol ring leading to downstream activation. For instance, phosphatidylinositol 4-[mono]phosphate is involved in membrane trafficking. But cleavage of phosphatidylinositol triphosphate yields inositol triphosphate, a molecule involved in calcium signaling. Signaling is conveyed in many signaling proteins (including Protein Kinase B/Akt) via their domain referred to as a Pleckstrin homology, or PH domain, which is capable of binding to specific phosphoinositides. Biosynthesis of PI involves the action of phosphatidylinositol synthase on cytidine diacylglycerol and *myo*-inositol. The critical signal transducer enzymes, Phosphatidylinositolinositol-3-kinases phosphorylate PI at the 3 position hydroxyl of inositol leading to modulation in pathways including as cell growth, survival and motility. PI is typically at concentrations in the low- to mid-micromolar range in human serum and plasma and is capable of activating second messengers such as Ca^{2+} and cyclic AMP. It is through these pathways that PI has been found to affect such biological events as the pro-inflammatory response and intestinal uptake. BioVision's Phosphatidylinositol Assay Kit utilizes specific enzymes to generate an intermediate that then reacts with PicoProbe™ yielding a signal that can be quantified fluorometrically and is proportional to the amount of PI present in the sample. The assay is capable of detecting as little as 15 pmoles of phosphatidylinositol.



II. Applications:

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

III. Sample Type:

- Tissue and cell lysates
- Biological fluids (*e.g.* serum, plasma)

IV. Kit Contents:

Components	K750-100	Cap Code	Part Number
PI Assay Buffer	25 ml	WM	K750-100-1
PI Hydrolysis Mix	1 vial	Purple	K750-100-2
PI Enzyme Mix	1 vial	Green	K750-100-3
PI Developer	1 vial	Red	K750-100-4
Sample Clean-Up Mix	1 vial	Orange	K750-100-5
PicoProbe™ (in DMSO)	300 µl	Blue	K750-100-6
PI Standard (0.2 µmol)	1 vial	Yellow	K750-100-7

V. User Supplied Reagents and Equipment:

- White opaque 96-well plate with flat bottom
- Multi-well spectrophotometer
- Dounce Tissue Homogenizer (BV Cat.# 1998 or similar)
- 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.

- **PI Assay Buffer:** Allow to warm to room temperature prior to use. Store at 4°C, protected from light.
- **PI Hydrolysis Mix, PI Enzyme Mix, Clean-Up Mix and PI Developer:** Reconstitute each vial with 220 µl PI Assay Buffer. Aliquot and store at -20°C. Once reconstituted, use within two months.
- **PicoProbe™:** Provided as a solution 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.
- **PI Standard:** Reconstitute with 200 µl Assay Buffer. Heat to 37°C and mix thoroughly to generate a 1.0 mM PI Standard solution. This can be aliquoted and evaporated in a vacuum oven for long term storage (> 2 weeks).
- **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. The organic phase will form the lower layer and the methanol will be saturated with water in an **upper wash layer**.

VII. PI Assay Protocol:

1. Sample Preparation:

Serum and plasma: Pre-treat serum or plasma by adding Sample Clean-Up Mix (1 µl Sample Clean-Up Mix per 50 µl Sample). Incubate at 37°C for 2 hr. Then proceed to lipid extraction according to the following protocol: To 50 µl Sample, add 500 µl 100% methanol. Vortex for 1 min. Place the Sample on ice for 10 min. Centrifuge for 5 min, 10,000 x *g* at RT in a microcentrifuge. A pellet will be visible. Discard the pellet. Collect the supernatant and transfer to a new tube without disturbing the pellet. Evaporate the organic solvent (supernatant) 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 50 µl of PI Assay Buffer (or less if concentrating is desired) 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.

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 PI 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 (250 µl Chloroform:500 µl Methanol). 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 RT (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 phase. Be 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 the upper phase. This wash step may be repeated to further enrich lipids. After final washes, collect the lower phase through the 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 PI 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 white 96-well plate. Adjust the volume in each well to 50 µl for background wells and 48 µl for Sample/Standard wells with PI Assay Buffer.

NOTE: Once extracted and solubilized in assay buffer, the lipid extracts can be stored at -80°C for future experiments.

2. **Standard Curve Preparation:** Generate a 50 µM PI Standard solution by diluting 50 µl of the PI Standard (1.0 mM) with 950 µl of PI Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of the 50 µM PI Standard to a series of wells in an opaque white 96-well plate. Bring the total volume of each well to 48 µl with PI Assay Buffer to generate 0, 100, 200, 300, 400, and 500 pmol PI per well.
3. **Hydrolysis Step:** Add 2 µl of the PI Hydrolysis mix to each Standard well and each Sample well. To the Sample Background wells add 2 µl PI Assay Buffer. Incubate the plate at 45°C for 2 hours.
4. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well (Reaction, Background, and Standard):

	Reaction Mix
PI Assay Buffer	44 µl
PI Enzyme Mix	2 µl
PI Developer Mix	2 µl
PI Probe*	2 µl

Add the reaction mix to each well. Mix and incubate at 30°C for 1 hour.

5. **Measurement:** Record Fluorescence in endpoint mode at Ex/Em= 535/587 nm.
6. **Calculation:** Subtract the 0 PI Standard reading from all Standard curve readings. Plot the Background-subtracted PI Standard Curve and calculate the slope. For each Sample, subtract the Sample Background reading from its paired Sample reading. Calculate the corrected absorbance/fluorescence of the Test Samples $\Delta RFU = RFU_{sample} - RFU_{background}$. Apply the corrected ΔRFU value to the PI Standard Curve to get *B* pmole PI in the well.

Sample PI Concentration = (*B* / *V*) x D = pmol/µl = µM

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

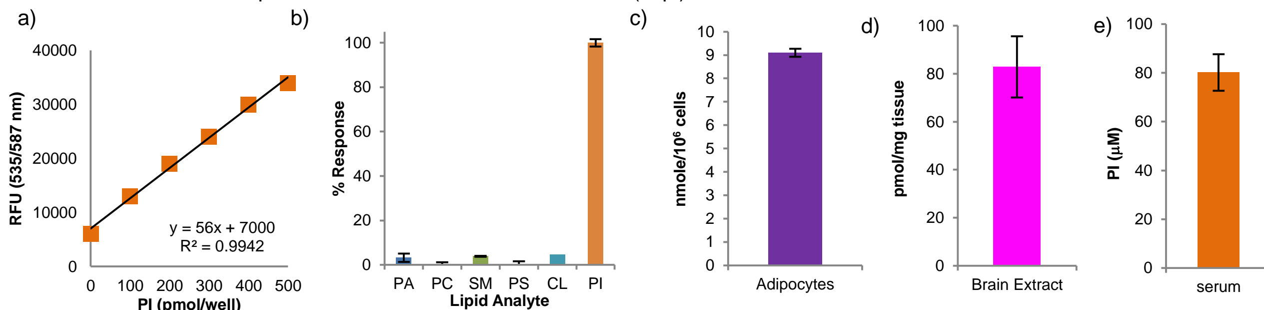


Figure: (a) PI Standard Curve **(b)** Specificity of the assay. Various phospholipids were assayed according to protocol: Phosphatidic Acid (PA; 5 nmol), Phosphatidylcholine (PC; 2.5 nmol), Sphingomyelin (SM; 5 nmol), Phosphatidylserine (PS; 5 nmol), and Cardiolipin (CL, 1 nmol) and 500 pmol phosphatidylinositol (PI). **(c)** PI concentration in lipid extract differentiated adipocyte cell lysate. **(d)** PI concentration in lipid extracted (brain tissue). **(e)** PI concentration in lipid extracted from pooled human serum.

VIII. RELATED PRODUCTS:

Phospholipase D Activity Colorimetric Assay Kit (K725)
Cardiolipin Assay Kit (Fluorometric) (K944)
Phosphatidic Acid Assay Kit (K748)
Lysophosphatidylcholine (C/F) Assay Kit (K735)
Phosphatidylcholine Colorimetric/Fluorometric Assay Kit (K576)
Phospholipid Assay Kit (Colorimetric/Fluorometric) (K351)
Glycerophosphorylcholine Assay Kit (C/F) (K433)
Alkaline Sphingomyelinase Activity Assay Kit (Colorimetric) (K987)

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