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ab252900 Phosphatidylinositol Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab252900
(use https://www.abcam.cn/ab252900 for china, or
https://www.abcam.co.jp/ab252900 for Japan)

For the measurement/determination of Phosphatidylinositol content/concentration in cell/tissue extracts and biological fluids.

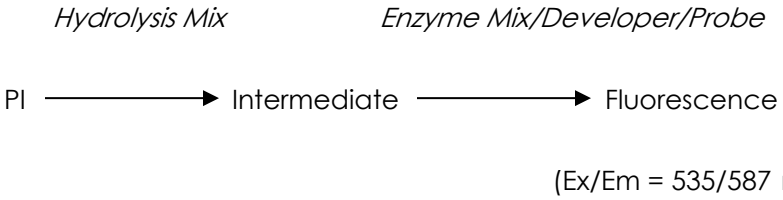
This product is for research use only and is not intended for diagnostic use.

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

The Phosphatidylinositol Assay Kit (Fluorometric) (ab252900) utilizes specific enzymes to generate an intermediate that then reacts with a PicoProbe I/Probe, yielding a signal that can be quantified fluorometrically, and is proportional to the amount of Phosphatidylinositol (PI) present in the sample. The assay is capable of detecting as little as 15 pmols of phosphatidylinositol.



2. Protocol Summary

Prepare all samples, standards and controls as instructed.

Prepare the chromophore standard curve dilutions and plate into a 96-well plate.

Add 2 µL of the Phosphatidylinositol Hydrolysis Mix/PI Hydrolysis mix to each standard well and sample well. Add 2 µL Assay Buffer I/PI Assay Buffer to the sample Background wells. Incubate for 2 hours at 45°C.

Prepare the Reaction Mix and add 50 µL to each well. Incubate for 30°C for 1 hour.

Record Fluorescence in endpoint mode at EX/EM=535/587 nm.

Use the RFU values to calculate the Sample PI concentration in µM.

3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer I/PI Assay Buffer	25 mL	4°C	4°C
Phosphatidylinositol Hydrolysis Mix/PI Hydrolysis Mix	1 vial	-20°C	-20°C
Inositol Enzyme Mix/PI Enzyme Mix	1 vial	-20°C	-20°C
Development Enzyme Mix VI/PI Developer	1 vial	-20°C	-20°C
Sample Clean-Up-Mix	1 vial	-20°C	-20°C
PicoProbe I/Probe (in DMSO)	300 µL	-20°C	-20°C
Phosphatidylinositol Standard/PI Standard (0.2 µmol)	1 vial	-20°C	-20°C

4. Materials Required, Not Supplied

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

- White opaque 96-Well plate with flat bottom.
- Multi-well spectrophotometer.
- Dounce Tissue Homogenizer
- 100% Methanol
- Chloroform.

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 Assay Buffer I/PI Assay Buffer:

Warm to room temperature before use. Store at 4°C protected from the light.

6.2 Phosphatidylinositol Hydrolysis Mix/PI Hydrolysis Mix:

Reconstitute each vial with 220 µL Assay Buffer I/PI Assay Buffer. Aliquot and
store at -20°C. Once reconstituted, use within two months.

6.3 Inositol Enzyme Mix/PI Enzyme Mix:

Reconstitute each vial with 220 µL Assay Buffer I/PI Assay Buffer. Aliquot and
store at -20°C. Once reconstituted, use within two months.

6.4 Clean-up Mix:

Reconstitute each vial with 220 µL Assay Buffer I/PI Assay Buffer. Aliquot and
store at -20°C. Once reconstituted, use within two months.

6.5 Development Enzyme Mix VI/PI Developer:

Reconstitute each vial with 220 µL Assay Buffer I/PI Assay Buffer. Aliquot and

store at -20°C. Once reconstituted, use within two months.

6.6 PicoProbe I/Probe:

Provided as a solution in DMSO. Store at -20°C, protected from the light. Prior to use, warm solution to room temperature. After use, promptly retighten cap to minimize adsorption of airborne moisture.

6.7 Phosphatidylinositol Standard/PI Standard:

Reconstitute with 200 µL Assay Buffer I/Assay Buffer, heat to 37°C, and mix thoroughly to generate a 1.0 mM Phosphatidylinositol Standard/PI Standard solution. This

can be aliquoted and evaporated in a vacuum oven for long term storage (> 2 weeks).

6.8 Upper Wash Layer (For washing lipid extract):

Combine 5 mL methanol (not provided) with 5 mL chloroform (not provided) and 4.5 mL dH2O, 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.

7. Sample preparation

7.1 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., and then centrifuge for 5 minutes, 10,000 x *g*, RT in a microcentrifuge.
- A pellet will be visible; discard the pellet. Collect the supernatant and transfer to a new tube without disturbing this 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 Assay Buffer I/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.

7.2 Tissues and cell pellets:

- (Can be scaled appropriately) Thoroughly homogenize soft tissues (~10 mg wet tissue) or cultured cells (~2 x10^6 cells) in 200 µL ice cold Assay Buffer I/PI Assay Buffer using a mechanical (Dounce) homogenizer.
- 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 seconds. 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, being careful not to remove the lower phase. Add 500 µL of the upper wash layer (Section 6.8) 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 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 Assay Buffer I/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 black 96-well plate and adjust the volume in each well to 50 µL for background wells, and 48 µL for sample/standard wells with Assay Buffer I/PI Assay Buffer.

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

8. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

8.1 Generate a 50 µM PI standard solution by diluting 50 µL of the PI Standard (1.0 mM) with 950 µL of Assay Buffer I/PI Assay Buffer.

8.2 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.

8.3 Bring the total volume of each well to 48 µL with Assay Buffer I/PI Assay Buffer to generate 0, 100, 200, 300, 400, and 500 pmol PI per well.

Standard #	Phosphatidylinositol Standard/50 µM PI Standard (µL)	Assay Buffer I/PI Assay Buffer (µL)	Volume in well (µL)	End amount of PI in well (pmol/well)
1	0	48	48	0
2	2	46	48	100
3	4	44	48	200
4	6	42	48	300
5	8	40	48	400
6	10	38	48	500

9. Assay Procedure

9.1 Hydrolysis Step:

1. Add 2 µL of the Phosphatidylinositol Hydrolysis Mix/PI Hydrolysis mix to each standard well and each sample well. To the sample background wells add 2 µL Assay Buffer I/PI Assay Buffer. Incubate the plate at 45°C for 2 hours.

9.2 Reaction mix:

1. Mix enough reagents for the number of assays to be performed.
2. For each Reaction, Background and Standard well, create the 50µL Reaction Mix shown Below:

	Reaction Mix
Assay Buffer I/PI Assay Buffer	44 µL
Inositol Enzyme Mix/PI Enzyme Mix	2 µL
Development Enzyme Mix VI/PI Developer Mix	2 µL
PicoProbe I/PI probe	2 µL

3. Mix well. Add the reaction Mix to the wells of the 96-well plate, and incubate for 1 hour at 30°C.

9.3 Measurement:

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

9.4 Calculation:

1. Subtract the 0 Phosphatidylinositol Standard/PI Standard reading from all standard curve readings, plot the background-subtracted Phosphatidylinositol Standard/PI Standard Curve and calculate the slope.
2. For each sample, subtract the sample background reading from its paired sample reading.
3. Calculate the corrected absorbance/fluorescence of the test samples $\Delta RFU = RFU_{sample} - RFU_{background}$. Apply the corrected ΔRFU value to the Phosphatidylinositol Standard/PI Standard Curve to get B pmole PI in the well.

$$\text{Sample PI Concentration} = (B / V) \times D = \text{pmol}/\mu L = \mu M$$

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)

10. Typical Data

Data provided **for demonstration purposes only.**

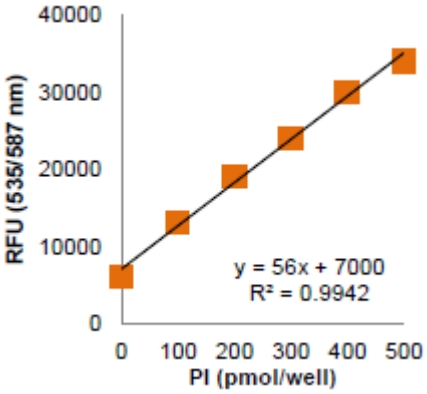


Figure 1. PI Standard curve

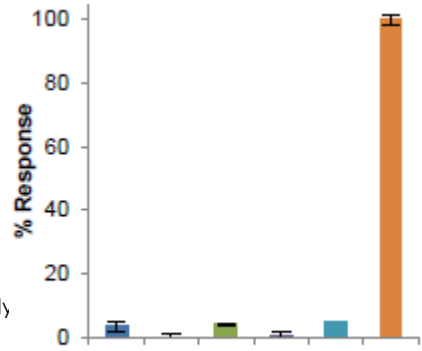


Figure 2. Specificity of the assay. Various phospholipids were assayed according to protocol: Phosphatidic Acid (PA; 5 nmol), Phosphatidylcholine (PC; 2.5 nmol), Sphingomyelin (SM; 5 nmol), Phosphatidylserine (PS; 5 nmol), and Cardiolipin (CL, 1 nmol) and 500 pmol phosphatidylinositol (PI).

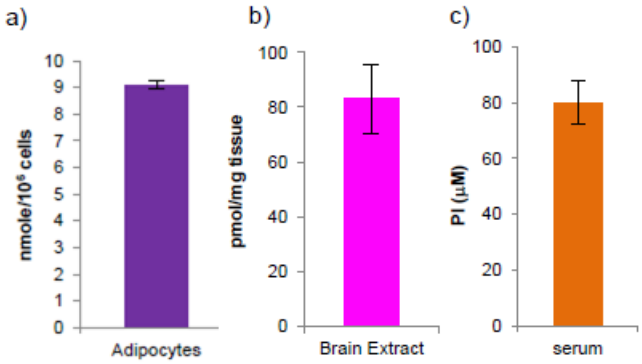


Figure 3. **(a)** PI concentration in lipid extract of differentiated adipocyte cell lysate. **(b)** PI concentration in lipid extract of brain tissue. **(c)** PI concentration in lipid extracted from pooled human serum.

11. Notes

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