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Phospholipase C Activity Assay Kit (Colorimetric)

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(Catalog # K2016-100; 100 assays, Store kit at -20°C)

I. Introduction:

Phospholipase C (PLC) belongs to a family of membrane-associated enzyme that specifically cleaves the phosphoester linkage before the phosphate group in phospholipids. PLC catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG), which are second messengers critical for downstream signal pathways to control diverse cellular processes. PLC family consists of 13 isozymes split between six subfamilies. Members of the PLC family are involved in intra and inter cellular signal transduction pathways that are important for cell proliferation and differentiation. PLC isozymes have been also associated with several diseases such as cancer, inflammation, cardiovascular diseases and neuropathic pain. Therefore, investigation of PLC enzymes and their inhibition may lead to innovative therapeutic strategies. BioVision's Phospholipase C Activity Assay Kit is a direct assay to determine the PLC activity in various biological samples. The assay uses a specific PLC chromogenic substrate to detect PLC activity and the generated final product can be measured colorimetrically at OD 405 nm. The OD signal is proportional to the PLC activity. The assay is rapid, sensitive and is a convenient tool for detecting PLC activity. It can detect as low as 0.1 mU PLC activity under the assay conditions.

Phospholipase C

Colorless PLC Substrate ⟶ Yellow Product (OD 405 nm)

II. Application:

- Detection of PLC activity in various samples

III. Sample Type:

- Organs and tissues such as kidney, brain and muscle etc.

IV. Kit Contents:

Components	K2016-100	Cap Code	Part Number
PLC Assay Buffer	25 ml	WM	K2016-100-1
PLC Substrate	1 vial	Red	K2016-100-2
PLC Standard	100 µl	Yellow	K2016-100-3
PLC Enzyme	1 vial	Blue	K2016-100-4

V. User Supplied Reagents and Equipment:

- 96-well clear flat-bottom plate
- Multi-well spectrophotometer
- 50% glycerol

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C. The kit components are stable for one year when stored as recommended. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **PLC Assay Buffer:** Ready to use as supplied. Warm bottle to room temperature (RT) before use. Store at 4°C.
- **PLC Substrate:** Add 1.2 ml of water into the vial. Vortex for 2 min and let it sit at RT for 5 min before use. Store it -20°C.
- **PLC Standard (100 mM):** Provided as a stock PLC Standard solution. Store at -20°C, protected from light.
- **PLC Enzyme**: Reconstitute with 100 µl of 50% glycerol (not included). Vortex to mix and let it sit at RT for 5 min before use. Aliquot and store at -20°C. Avoid multiple freeze-thaw cycles. Use within 2 months.

VII. Phospholipase C Activity Assay Protocol:

1. **Sample Preparation:** Homogenize ~100 mg of tissue in an eppendorf tube on ice with 200 µl of cold PLC Assay Buffer using a small pestle. Centrifuge at 10,000 x *g* and 4°C for 20 min and collect the supernatant to a new eppendorf tube. For each Sample type, add 2-20 µl of Sample into a well(s) of a clear, flat bottom 96-well plate labeled as Sample. Adjust the volume of each well to 50 µl using PLC Assay Buffer. For background control add 50 µl of PLC buffer in separate well(s).

   For Positive Control well, add 10 µl of the reconstituted PLC enzyme into a desired well in the plate. Adjust the volume to 50 µl/well using PLC Assay Buffer.

2. **Standard Curve Preparation:** Prepare 1 mM PLC Standard solution by adding 10 µl of the 100 mM stock PLC Standard to 990 µl of PLC Assay Buffer. Add 0, 5, 10, 15, 20 and 25 µl of the 1 mM PLC Standard solution into the desired wells to generate 0, 5, 10, 15, 20 and 25 nmole PLC Standard/well respectively. Adjust the volume of all wells to 100 µl/well with PLC Assay Buffer.

3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. Prepare 50 µl of Reaction Mix as indicated in the table below:

	Reaction Mix	Background Mix
PLC Assay Buffer	40 µl	50 µl
PLC Substrate	10 µl	-

Mix well. Add 50 µl of Reaction Mix to the Sample and Positive Control wells and 50 µl of Background Mix to the Background Control well(s) respectively.

Note: For Unknown Samples, we recommend testing several dilutions to ensure the readings are within the linear range of the PLC Standard Curve.

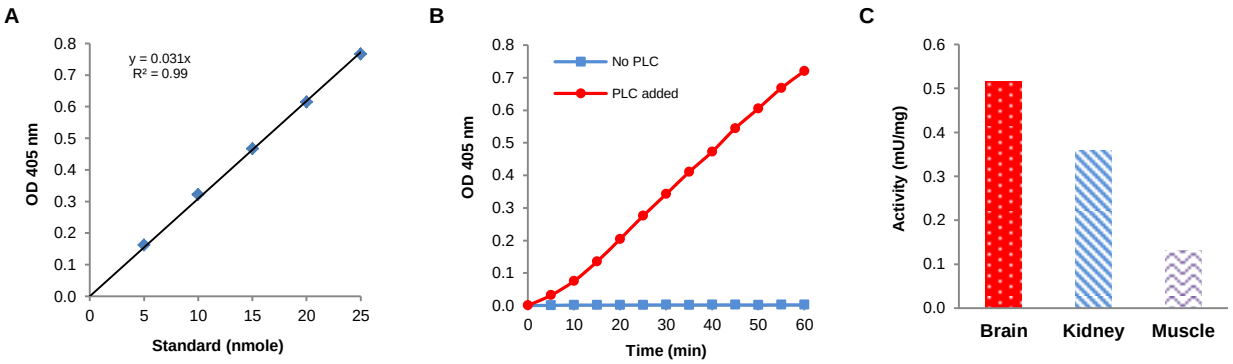
4. **Measurement:** Measure the OD at 405 nm in kinetic mode at 37°C for 60 min. Standard Curve may be read in either kinetic or end point mode.
5. **Calculation:** Subtract the 0 Standard readings from all Standard readings and the Background Control reading(s) from Sample readings respectively. Plot the PLC Standard Curve. Choose any two time points within the linear portion of the curve (t_1 and t_2) for each Sample. Apply the corrected Sample readings to the PLC Standard Curve to get A nmol of product generated during the reaction time ($\Delta t = t_2 - t_1$).

   Calculate the PLC activity of the Sample:

   Sample PLC Activity = A x D / (Δt x M) ((nmol / min x µg)) = mUnit / µg

   Where: **A** is the amount of product generated from the Standard Curve (nmol)
   D is the Sample dilution factor (if applicable, D = 1 for Undiluted Samples)
   Δt is the reaction time (in min)
   M is the Sample added to the well (in µg)

Unit Definition: One unit is 1 µmole of product generated per min at pH 8 and 37°C.



Figures. A. PLC Standard Curve. **B.** Reaction curve of the PLC activity. **C.** PLC activity detected in rat brain, kidney and muscle lysates. Assay was performed according to the kit protocol.

VIII. Related Products:

Phospholipase A2 Activity Assay Kit (Fluorometric) (K400)
Phospholipase D (PLD) Activity Colorimetric Assay Kit (K725)
PAF Acetylhydrolase Activity Assay Kit (Colorimetric) (K765)
PAF Acetylhydrolase (PAF-AH) Inhibitor Screening Kit (Colorimetric) (K766)
Total Phosphodiesterase Activity Assay Kit (Fluorometric) (K927)

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