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ab273343 Phospholipase C Activity Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273343
(use https://www.abcam.cn/ab273343 for china, or
https://www.abcam.co.jp/ab273343 for Japan)

For the determination of Phospholipase C activity in tissue.

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

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1. Overview

Phospholipase C Activity Assay Kit (Colorimetric) (ab273343) is a direct assay to determine the Phospholipase C (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.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standard curve.

Add all samples to the appropriate wells.

Add Reaction Mix and Background Mix to the appropriate wells.

Measure OD in kinetic mode for 60 mins at 37°C.

Determine Phospholipase C activity using equation.

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.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation 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)
Assay Buffer 61	25 mL	-20°C
PLC Enzyme	1 vial	-20°C
p-Nitrophenol Standard	100 µL	-20°C
PLC Substrate	1 vial	-20°C

PLEASE NOTE: Assay Buffer 61 was previously labelled as PLC Assay Buffer, and p-Nitrophenol Standard as PLC Standard. The composition has not changed.

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
- 96-well clear plate with flat bottom
- 50% glycerol

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 61:**
Ready to use as supplied. Bring to room temperature before use. Store at -20°C.

9.2 **PLC Enzyme:**
Reconstitute with 100 µl of 50% glycerol (not included). Vortex to mix and let it sit at RT for 5 mins before use. Aliquot and store at -20°C. Avoid multiple freeze-thaw cycles. Use within 2 months.

9.3 **p-Nitrophenol Standard:**
Provided as a stock p-Nitrophenol Standard solution. Store at -20°C, protected from light.

9.4 **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

10. Sample Preparation

Tissue lysate preparation:

10.1 Homogenize ~100 mg of tissue in an Eppendorf tube on ice with 200 µl of cold Assay Buffer 61 using a small pestle.
10.2 Centrifuge at 10,000 *x g* and 4°C for 20 mins and collect the supernatant to a new Eppendorf tube.
10.3 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.
10.4 Adjust the volume of each well to 50 µl using Assay Buffer 61.
10.5 For background control add 50 µl of PLC buffer in separate well(s).

Δ Note: For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

11. Standard Curve

– Keep standards on ice while in use.

11.1 Prepare 1 mM p-Nitrophenol Standard solution by adding 10 µl of the 100 mM stock p-Nitrophenol Standard to 990 µl of Assay Buffer 61.

11.2 Add 0, 5, 10, 15, 20 and 25 µl of the 1 mM p-Nitrophenol Standard solution into the desired wells to generate 0, 5, 10, 15, 20 and 25 nmole p-Nitrophenol Standard /well respectively.

11.3 Adjust the volume of all wells to 100 µl/well with Assay Buffer 61.

Standard #	1 mM p-Nitrophenol Standard (µl)	Assay Buffer 61 (µl)	PLC (nmol/well)
1	0	100	0
2	5	95	5
3	10	90	10
4	15	85	15
5	20	80	20
6	25	75	25

12. Assay Procedure

– Keep on ice while in use.

12.1 **Positive Control:** Add 10 µl of the reconstituted PLC enzyme into a desired well in the plate. Adjust the volume to 50 µl/well using Assay Buffer 61.

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

	Reaction mix (µl)
Assay Buffer 61	40
PLC Substrate	10

12.3 Add 50 µl of the Reaction mix into Positive Control, Sample and Background control wells.

12.4 Measure the OD at 405 nm in kinetic mode at 37°C for 60 mins. Standard Curve may be read in either kinetic or end point mode.

13. Calculations

13.1 Subtract the 0 Standard readings from all Standard readings and the Background Control reading(s) from Sample readings respectively.
13.2 Plot the p-Nitrophenol Standard Curve.
13.3 Choose any two time points within the linear portion of the curve (t1 and t2) for each Sample.
13.4 Apply the corrected Sample readings to the p-Nitrophenol Standard Curve to get A nmol of product generated during the reaction time ($\Delta t = t2 - t1$).
13.5 Calculate the PLC activity of the Sample:

$$PLC\ activity\ = \frac{A\ x\ D}{(\Delta T\ x\ M)} = nmol/min/\mu g = mU/\mu g$$

A = Amount of product generated from the Standard Curve (nmol)
ΔT = Reaction time ($T_2 - T_1$) (mins)
D = Sample dilution factor (if applicable, D = 1 for Undiluted Samples)
M = Amount of sample added to the well (µg)

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

14. Typical Data

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

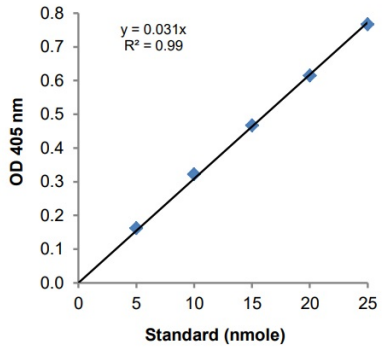

Figure 1. p-Nitrophenol Standard Curve.

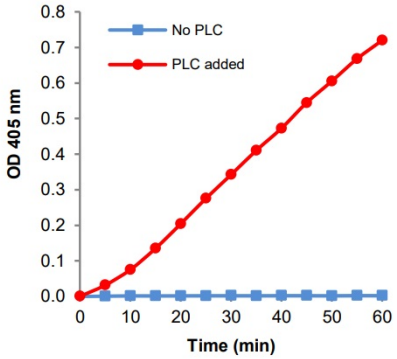

Figure 2. Reaction curve of the PLC activity.

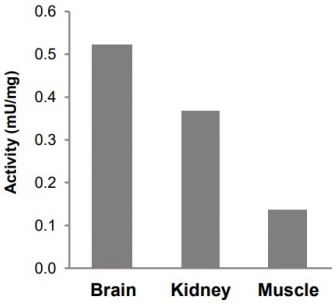

Figure 3. PLC activity detected in rat brain, kidney and muscle lysates.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

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