Technical Bulletin

Phospholipase C Activity Kit (Colorimetric)

CS0011

Storage Temperature –20 °C

Product Description

Phosphatidylcholine-specific phospholipase C (PC-PLC) is an important member of the phospholipase family. PC-PLC specifically hydrolyzes the third phosphate linker of glycerophospholipid molecules, to release phosphocholine and diacylglycerols (DAG).[1] PC-PLC has diverse biological functions and participates in various cell signaling pathways, such as apoptosis, proliferation, differentiation, motility, gene expression and metastasis.[2] As well, PC-PLC is reported to play a crucial role in the development of atherosclerosis, which makes PC-PLC a target of interest for cardiovascular drug discovery and development. Identifying novel inhibitors of PC-PLC and their influence on PC-PLC is thus of interest.[2]

The Phospholipase C (PLC) Activity Assay Kit provides a simple, rapid and sensitive procedure for measuring PLC activity in various sample types. The lowest limit of detection of this kit is 0.03 mU of PC-PLC activity. This kit can detect PC-PLC activity of purified enzymes and PC-PLC activity in cell extracts. This kit can be used to screen for PC-PLC inhibitors.

p-Nitrophenylphosphorylcholine (NPPC) is a chromogenic substrate that is used in this kit to measure the activity of PC-PLC. Hydrolysis of NPPC by PC-PLC results in the liberation of *p*-nitrophenol, which can be measured at 405 nm at pH 7.2-7.5. The colorimetric signal is proportional to the PLC activity.

The PLC Positive Control, which is included in the kit, can be used to screen for PLC inhibitors.

This kit does not require weighing.

Unit definition

One unit of PLC is defined as the amount of enzyme required to produce 1 µmol of product in 1 minute, under the assay conditions, at 37 °C.

Components

This kit contains sufficient reagents for 100 colorimetric tests in 96-well plates.

Component	Component Number	Amount	Cap Color/ Container Information
Assay Buffer	CS0011A	30 mL	White cap/bottle
Substrate	CS0011B	2 vials	Red cap/vial
Standard	CS0011C	500 µL	Brown cap/vial
PLC Positive Control	CS0011D	500 µL	Yellow cap/vial

Component Information

- Assay Buffer (CS0011A): Ready-to-use. Upon thawing, store at 2–8 °C. Equilibrate to room temperature before use.
- Substrate (CS0011B): Reconstitute each vial with 1.5 mL of water. Mix by vortexing, until no particulates are observed. To avoid freeze/thaw cycles, it is recommended to prepare aliquots, and store the aliquots at –20 °C.
- Standard (CS0011C): Upon thawing, store at 2–8 °C, protected from light.
- PLC Positive Control (CS0011D): Ready-to-use. Keep on ice while in use. To avoid freeze/thaw cycles, it is recommended to prepare aliquots, and store the aliquots at –20 °C.

Equipment Required, But Not Provided

- 96-well clear flat-bottom plate
- Spectrophotometric (405 nm) multi-well plate reader

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Upon receipt, store components at –20 °C, protected from light. Upon thawing, the Assay Buffer and Standard can be stored at 2–8 °C. The unopened kit is stable for 2 years as supplied.

Procedure

General notes:

- All samples and standards should be run in duplicate.
- A fresh set of standards should be prepared for each set of assays.
- Briefly centrifuge vials before opening.
- **For convenience, an Excel-based calculation sheet is available on the Product Detail Page.** Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.

Sample preparation

Add 0.2 mL of ice-cold Assay Buffer to 100 mg of the dissociated tissue (for example, kidney, brain, etc.), and homogenize using a Dounce homogenizer. The dissociated tissue can be also sonicated on ice for several sonication cycles, to achieve a homogeneous preparation. If sonication is performed, it is important to keep samples chilled during the sonication procedure.

Centrifuge the homogenate at 10,000 × g for 20 minutes at 4 °C. Transfer the supernatant to a new microfuge tube. All samples should be in a final volume of 50 µL. Therefore, adjust the volume to 50 µL when needed, with Assay Buffer.

Note: Always include a sample blank, where the sample is replaced with 50 µL of Assay Buffer.

Note: If desired, the PLC Positive Control can be included, either as a positive control, and/or to screen for PLC inhibitors. Add 50 µL of PLC Positive Control into each positive control well.

Standard curve preparation:

1. Dilute the Standard 10-fold to a final concentration of 1 mM: 50 µL of the Standard with 450 µL of Assay Buffer.
2. Add 0, 5, 10, 15, 20, 25, 30 and 35 µL of the 10-fold diluted solution into a 96-well plate, generating 0 (blank), 5, 10, 15, 20, 25, 30 and 35 nmol/well standards.
3. Complete the volume to 100 µL with Assay Buffer (see Table 1).

Table 1.
Standard curve preparation, per well*

1 mM Standard (µL)	Assay Buffer (µL)	Final standard amount per well (nmol)
0	100	0 (blank)
5	95	5
10	90	10
15	85	15
20	80	20
25	75	25
30	70	30
35	65	35

* Work in duplicate

Reaction Mix

Set up the Reaction mix according to Table 2.

50 µL of Reaction mix is required for each reaction (well). Multiply the volumes in Table 2 according to the number of wells in the assay.

Table 2:
Reaction mix, per one well

Reagent	Cap color	Volume (µL)
Assay Buffer	White cap	20
Substrate	Red cap	30

Assay reaction:

1. Add samples directly to the sample wells of the 96-well plate. Bring the samples to a final volume of 50 µL with Assay Buffer. For the sample blank, prepare only 50 µL of Assay Buffer.
2. Add 50 µL of reaction mix to each of the sample wells, sample blank and PLC Positive Control (if relevant). Mix by pipetting.
   Note: Do not add reaction mix to standard wells.
3. Measure the absorbance at 405 nm (A_{405}) in kinetic mode at 37 °C for 60 minutes, taking readings every 5 minutes.
4. Standard curve may be read in either kinetic or end point mode.

Results

Calculations

An Excel-based calculation sheet is available at the Product Detail Page. Use this sheet to calculate the test results.

If the Excel-based calculation sheet at the Product Detail Page is not used, calculations should be performed as follows:

1. Average the O.D. readings of each duplicate.
2. Subtract the blank value (no standard) from all standard values.
3. Subtract the mean sample blank (no sample) of each time point from its respective mean sample time point. For example, the mean sample blank of the 5-minute reading should be subtracted from each mean sample reading of 5 minutes.

Construct a standard curve:

1. Plot the absorbance (O.D.) measured for each standard against the amount of the standard (in nmol).
2. Determine the linear regression equation from all standard readings.
3. Construct a sample kinetic curve:
4. Plot the absorbance (O.D.) measured for each sample against time (in minutes).
5. Determine the linear regression equation for each sample.
6. Use the standard curve linear regression slope and the sample kinetic curve linear regression slope to transform the values of the samples from O.D./min to nmol/min (mU):

Sample enzymatic activity (nmol/min) = $[Slope_{Sample}] / [Slope_{Std}] \times DF$

Where:

$Slope_{Sample}$ =
The slope of the blank-subtracted sample curve (O.D./min)

$Slope_{Std}$ =
The slope of the blank-subtracted standard curve (O.D./nmol)

DF =
Dilution factor
(if the sample is not diluted, the DF value is 1)

Calculation example:

$Slope_{Sample}$ =
0.01 O.D./min (from sample kinetic curve)

$Slope_{Std}$ =
0.023 O.D./nmol (from standard curve)

Sample enzymatic activity =
(0.01 O.D./min)/(0.023 O.D./nmol) =
0.43 nmol/min = 0.43 mU

References

1. Zhao, Y. *et al*., *Chem. Biol. Drug. Des.* **95,** 380– 387 (2020).
2. Eurtivong, C. *et al*., *Eur. J. Med. Chem.* **187,** 111919 (2020).

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