EnzyChrom™ Phospholipase D Assay Kit (EPPD-100)

Quantitative Colorimetric/Fluorimetric Phospholipase D Activity Determination

DESCRIPTION

PHOSPHOLIPASE D (PLD) catalyses the hydrolysis of the phosphodiester bond of glycerophospholipids to generate phosphatidic acid and a free headgroup. Abnormalities in PLD expression have been associated with human cancers. BioAssay Systems' method provides a simple and high-throughput assay for measuring PLD activity. In this assay, PLD hydrolyzes phosphatidylcholine to choline which is determined using choline oxidase and a H_2O_2 specific dye. The optical density of the pink colored product at 570nm or fluorescence intensity (530/585 nm) is directly proportional to the PLD activity in the sample.

KEY FEATURES

Sensitive. Use 10 µL samples. Detection range: colorimetric assay 0.06 - 10 U/L, fluorimetric assay 0.04 - 1 U/L.

Simple and High-throughput: the assay involves addition of a single working reagent and can be readily adapted to high-throughput assays for drug screening.

APPLICATIONS

Direct Assays: phospholipase D in biological samples.

Drug Discovery/Pharmacology: effects of drugs on phospholipase D metabolism.

KIT CONTENTS

Assay Buffer:	10 mL	**Dye Reagent:**	120 µL
Enzyme Mix:	120 µL	**Substrate:**	1.5 mL
Calibrator:	400 µL		

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of three months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

COLORIMETRIC ASSAY

Liquid samples can be assayed directly. Solid samples should be homogenized in a suitable enzyme buffer prior to assay.

Note: SH-containing reagents (e.g. β–mercaptoethanol, dithiothreitol, > 5 µM), sodium azide, EDTA, and sodium dodecyl sulfate are known to interfere in this assay and should be avoided in sample preparation. If a sample is known to contain choline, it should be removed by dialysis or membrane filtration.

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.

2. *Calibrator*: mix 33 µL Calibrator with 187 µL dH_2O (final 300 µM choline). Dilute calibrator in dH_2O as follows.

No	300 µM Premix + H_2O	Vol (µL)	Calibrator (µM)
1	100 µL + 0 µL	100	300
2	60 µL + 40 µL	100	180
3	30 µL + 70 µL	100	90
4	0 µL +100 µL	100	0

Transfer 10 µL diluted standards into separate wells of a clear flat-bottom 96-well plate.

Samples: transfer 10 µL of each sample into separate wells of the plate.

3. *Color reaction*. Prepare enough Working Reagent by mixing, for each well, 85 µL Assay Buffer, 1 µL Enzyme Mix, 1 µL Dye Reagent and 12 µL Substrate. Add 90 µL Working Reagent to each well.

Tap plate to mix. Incubate at desired temperature and protect from light. At 10 and 30 min, read optical density 570nm (550-585nm).

FLUORIMETRIC ASSAY

The fluorimetric assay procedure is similar to the colorimetric procedure except that (1) 0, 9, 18 and 30 µM calibrator and (2) a black 96-well plate are used. Read fluorescence intensity at λ_{ex} = 530 nm and λ_{em} = 585 nm.

CALCULATION

Subtract blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the PLD activity of Sample,

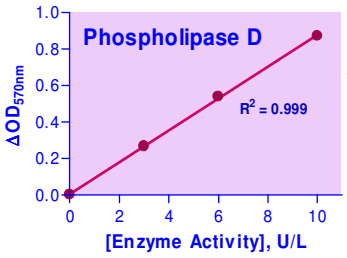
$$[\text{Phospholipase D}] = \frac{R_{30} - R_{10}}{\text{Slope} \times 20} \times n \quad (U/L)$$

R_{30} and R_{10} are optical density or fluorescence intensity readings of the Sample at 30 min and 10 min, respectively. 20 is the enzyme reaction time (30 min - 10 min). *n* is the sample dilution factor. *Note*: if the calculated PLD activity of a sample is higher than 10 U/L in the Colorimetric Assay or 1 U/L in the Fluorimetric Assay, dilute sample in assay buffer and repeat the assay. Multiply result by the dilution factor.

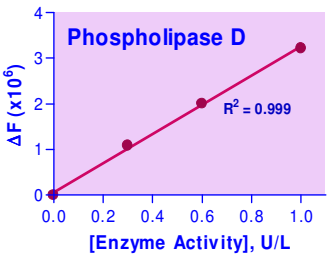
Unit definition: 1 unit of PLD catalyzes formation of 1 µmole of choline per minute under the assay conditions (pH 7.4).

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, optical density plate reader; black flat-bottom uncoated 96-well plates, fluorescence plate reader.



96-well colorimetric assay



96-well fluorimetric assay

LITERATURE

1. Su W, Chen Q, Frohman MA. (2009). Targeting phospholipase D with small-molecule inhibitors as a potential therapeutic approach for cancer metastasis. Future Oncol. 5(9):1477-86.

2. Carman GM, Fischl AS, Dougherty M, Maerker G. (1981). A spectrophotometric method for the assay of phospholipase D activity. Anal Biochem. 110(1):73-6.

3. Imamura S, Horiuti Y. (1978). Enzymatic determination of phospholipase D activity with choline oxidase. J Biochem. 83(3):677-80.