

Phospholipase D (PLD) Assay Kit (Colorimetric/Fluorometric)

LS-K190-100 (100 Tests) • Store at -20°C



Introduction

Phospholipase D (PLD) catalyzes the hydrolysis of the phosphodiester bond of glycerophospholipids to generate phosphatidic acid and a free head-group. Abnormalities in PLD expression have been associated with human cancers. This 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 H₂O₂ 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, fluorometric 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.

Components

Component	K190-100
	100 Tests
Assay Buffer	10 mL
Enzyme Mix	Dried
Dye Reagent	120 µL
Substrate	1.5 mL
Calibrator	400 µL

Materials Not Supplied

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.

Storage

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

FOR RESEARCH USE ONLY! Not for use in humans.

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Assay Procedure

Colorimetric Procedure

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 μ M), 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. Reconstitute Enzyme Mix with 120 μ L Assay Buffer. Reconstituted Enzyme Mix is stable for 1 month when stored at -20°C. Note: a yellow precipitate may form after thawing reconstituted Enzyme Mix. If a precipitate forms, pellet it by centrifuging for 2 min at 14000 rpm and use the clear supernatant.
2. Calibrator: mix 33 μ L Calibrator with 187 μ L dH₂O (final 300 μ M choline). Dilute calibrator in dH₂O as follows.

No	300 μ M Premix + H ₂ O	Vol (μ L)	Calibrator (μ M)
1	100 μ L + 0 μ L	100	300
2	60 μ L + 40 μ L	100	180
3	30 μ L + 70 μ L	100	90
4	0 μ L + 100 μ L	100	0

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

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

3. Color reaction. Prepare enough Working Reagent by mixing, for each well, 85 μ L Assay Buffer, 1 μ L Enzyme Mix, 1 μ L Dye Reagent and 12 μ L Substrate. Add 90 μ L 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 570 nm (550-585 nm).

Fluorometric Procedure

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

Calculations

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

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

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 Fluorometric 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 μ mole of choline per minute under the assay conditions (pH 7.4).

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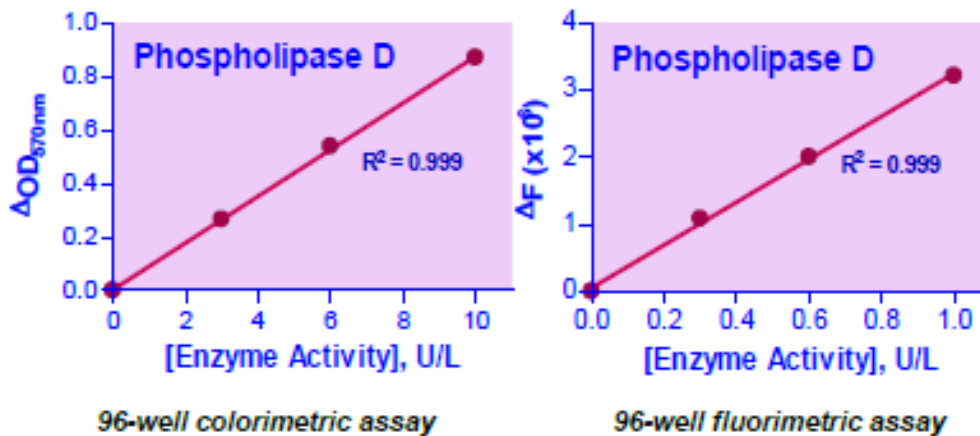
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Sample Data



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