Phospholipase D

Microplate Assay Kit

User Manual

Catalog # CAK1148

(Version 1.1A)

Detection and Quantification of Phospholipase D (PLD) Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Phospholipase D (EC 3.1.4.4) (PLD) is an enzyme of the phospholipase superfamily. Phospholipases occur widely, and can be found in a wide range of organisms, including bacteria, yeast, plants, animals, and viruses. Phospholipase D's principal substrate is phosphatidylcholine, which it hydrolyzes to produce the signal molecule phosphatidic acid (PA), and soluble choline. Plants contain numerous genes that encode various PLD isoenzymes, with molecular weights ranging from 90-125 kDa. Mammalian cells encode two isoforms of phospholipase D: PLD1 and PLD2. Phospholipase D is an important player in many physiological processes, including membrane trafficking, cytoskeletal reorganization, receptor-mediated endocytosis, exocytosis, and cell migration. Through these processes, it has been further implicated in the pathophysiology of multiple diseases: in particular the progression of Parkinson's and Alzheimer's, as well as various cancers.

Phospholipase D Microplate Assay Kit is a sensitive assay for determining Phospholipase D activity in various samples. Phospholipase D activity is determined by the product of choline. The intensity of the product color, measured at 570 nm, is proportional to the Phospholipase D activity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	5 ml x 1	4 °C
Substrate	4 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	10 ml x 1	4 °C
Standard (500 µmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
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Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use; store at -80 °C for a month after reconstitution.

Positive Control: add 1 ml Assay Buffer to dissolve before use; store at -80 °C for a month after reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagents to 37 °C before use.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	40 µl	40 µl	--	--	40 µl
Substrate	40 µl	40 µl	--	--	40 µl
Enzyme	10 µl	10 µl	--	--	10 µl
Sample	10 µl	--	--	--	--
Assay Buffer	--	10 µl	--	--	--
Standard	--	--	100 µl	--	--
Positive Control	--	--	--	--	10 µl
Distilled water	--	--	--	100 µl	--
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, put it in the oven, incubate at 37 °C for 5 minutes, measured at 570 nm and record the absorbance.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of PLD activity is defined as the enzyme generates 1 μmol of H2O2 per minute.

1. According to the protein concentration of sample

$$PLD\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$PLD\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

$$PLD\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 500 μmol/L = 0.5 μmol/ml;

$V_{Standard}$: the volume of standard, 0.1 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

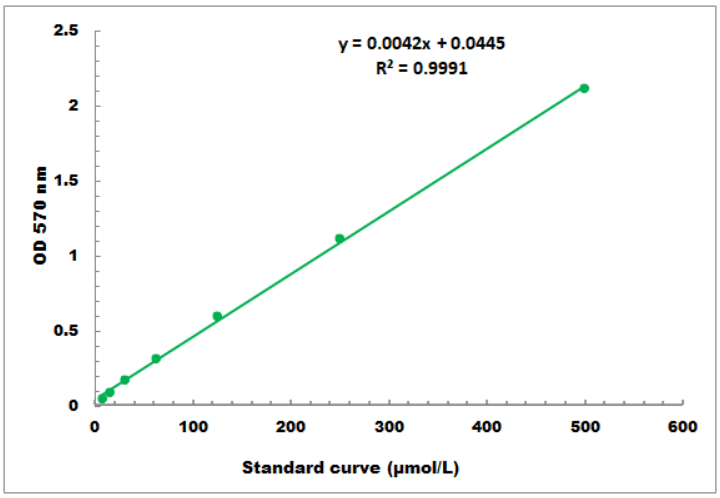
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay Buffer, 1 ml;

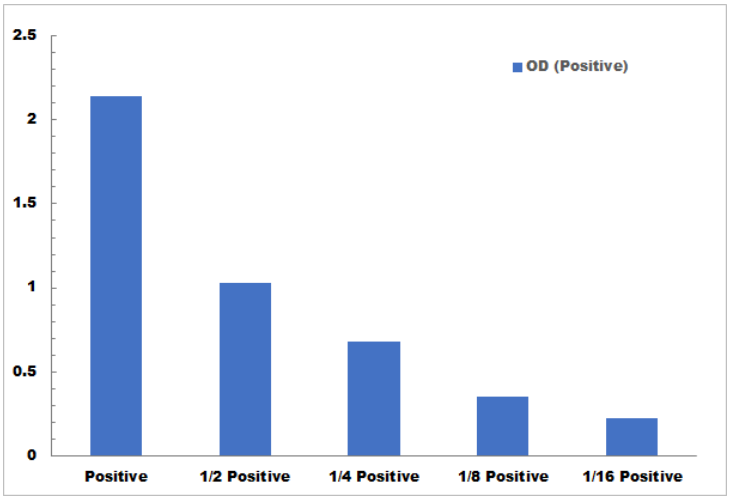
T: the reaction time, 5 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 5 μmol/L - 500 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES