Phospholipase D

Microplate Assay Kit

User Manual

Catalog # CAK8016

(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.

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 Activity Fluorometric 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, resulting in the generation of an intermediate that reacts with the probe, which can be detected fluorometrically (Ex/Em 535/587).

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Black Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	1 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 ml x 1	4 °C
Standard (100 µmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -20 °C. Use within one month.

Probe: Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 ml Probe Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within one month.

Positive Control: add 1 ml Assay Buffer to dissolve before use. Store at -80 °C. Use within one month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

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 8,000g 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 8,000g 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 the solution to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	160 µl	160 µl	170 µl	170 µl	160 µl
Substrate	10 µl	10 µl	--	--	10 µl
Probe	10 µl	10 µl	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl	10 µl	10 µl
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	10 µl	--
Standard	--	--	10 µl	--	--
Positive Control	--	--	--	--	10 µl
Mix, put it in the oven, 37 °C for 10 minutes, protected from light, record fluorescence measured at Ex/Em = 535/587 nm.					

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

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

$$= 0.01 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.01 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

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

$$= 0.01 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of standard, 100 μmol/L = 0.1 μmol/ml

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

W: the weight of sample, g

N: the quantity of cell or bacteria, $N \times 10^4$

$V_{Standard}$: the volume of the standard, 0.01 ml

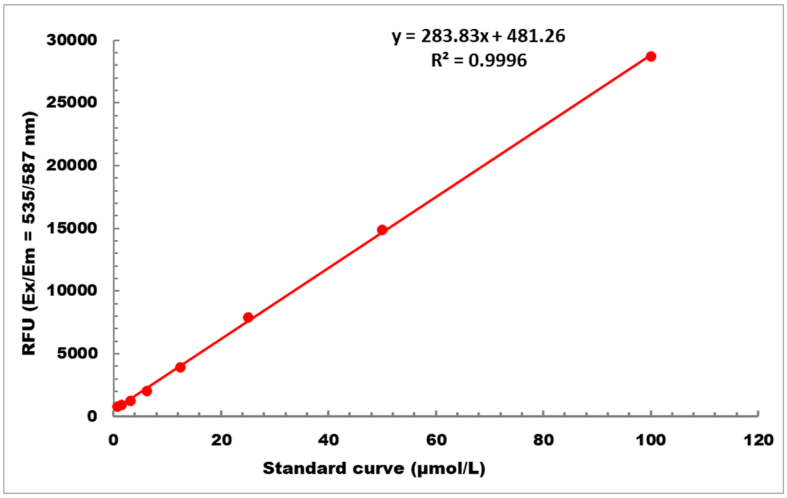
V_{Sample}: the volume of sample, 0.01 ml

V_{Assay}: the volume of Assay buffer, 1 ml

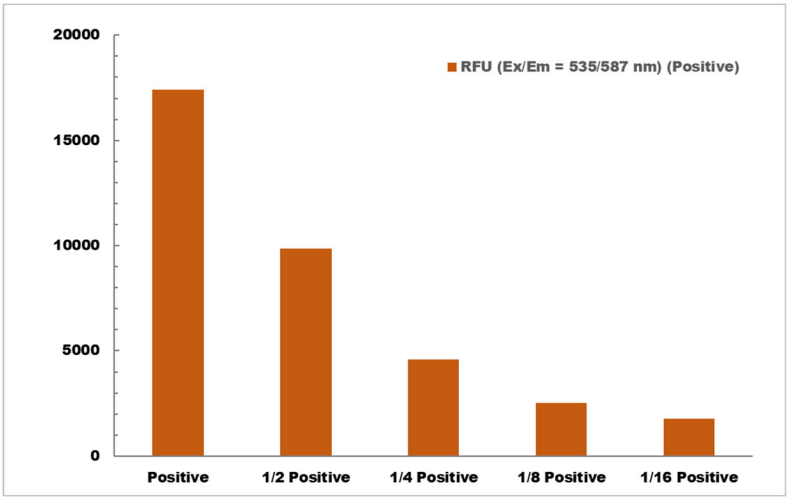
T: the reaction time, 10 minutes

VII. TYPICAL DATA

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



Detection Range: 1 µmol/L - 100 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