Phospholipase D Activity Fluorometric Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6905

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 .

1. 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 Phospholip ase 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).

2. Kit Components

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

3. 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

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Per form 2 -fold serial dilutions of the top standardsolution using distilled water to make the standard curve. The concentration of standard curve could be 100/50/25/12.5/6.25/3.12/1.56μmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 mL Reaction Buffer to dissolve before use. Aliquot & store at -20 °C. Use within 1 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 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 1 mL Assay Buffer to dissolve before use.Store at -80°C. Use within 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. 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.

6. Assay Procedure

Warm thesolution to room temperature before use . Add following reagents in to 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

7. Calculation

Unit Definition:

One unit of PL D activity is defined as the enzyme generates 1 μmol of H_2O_2 per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{Standard}}{V_{Sample}} \times n \ (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \ (U/10^4)$$

2.3 According to the weight of sample

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

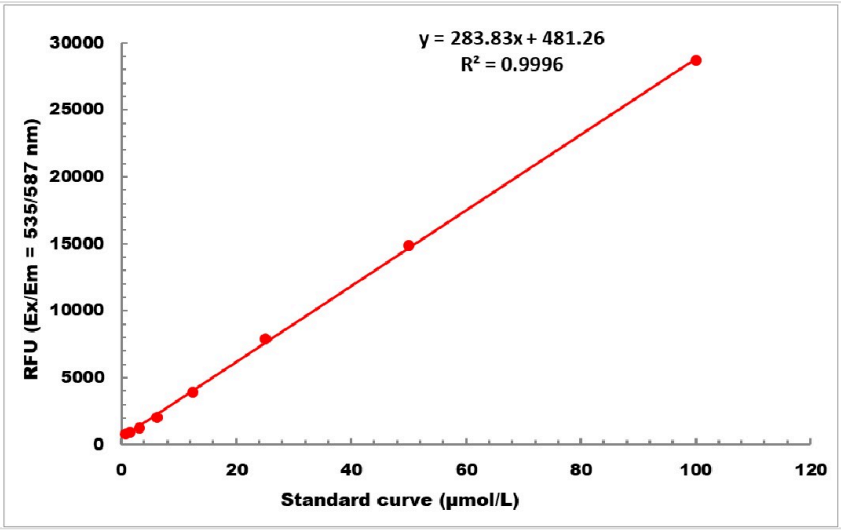
2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \ (U/mL)$$

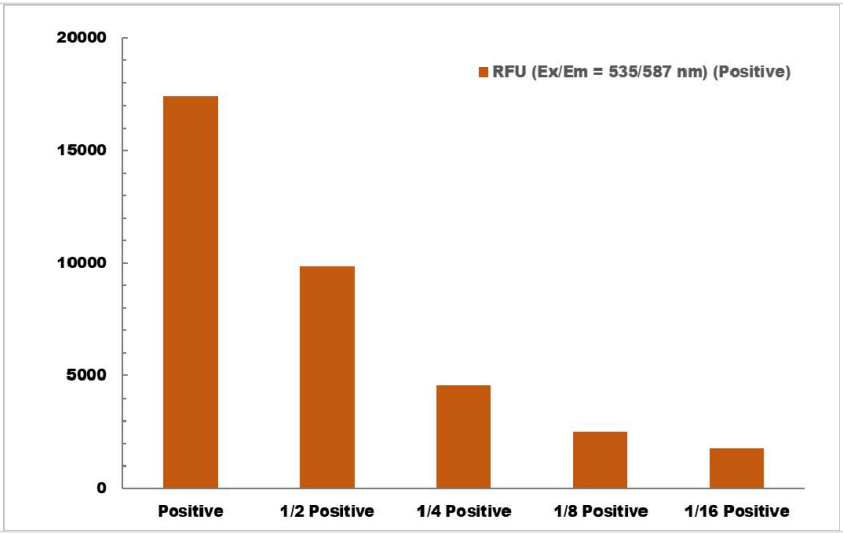
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration of sample, mg/mL
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, μmol/mL
$V_{Standard}$	the volume of standard in assay procedure, mL
V_{Sample}	the volume of sample in assay procedure, mL
V_{Assay}	the volume of Assay Buffer in sample preparation , mL
T	the reaction time , minute

8. 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