



Phospholipase D Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1148

(Version 1.2A)

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 Activity Colorimetric 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 I	30 ml x 4	4 °C
Assay Buffer II	Powder x 1	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
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 500/250/125/62.5/31.2/15.6/7.8 $\mu\text{mol/L}$. Keep in dark for immediate use.

Assay Buffer II: Briefly centrifuge prior to opening. Add 1.2 ml ethanol to dissolve before use. Keep in dark for immediate use or store at -20°C for 3-4 months after reconstitution.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use. Prepare for immediate use or store at -80°C for 1 month after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use; store at -80°C for 1 month after reconstitution.

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

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.99 ml Assay Buffer I and 10 μ l Assay Buffer II 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 0.99 ml Assay Buffer I and 10 μ l Assay Buffer II 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.

VI. 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, measure at 570 nm and record the absorbance.					

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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of PLD activity is defined as the enzyme generates 1 μmol of H_2O_2 per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2 According to the weight of sample

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

2.3 According to the volume of sample

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

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

C_{Standard} : the concentration of standard, $\mu\text{mol/mL}$

V_{Standard} : the volume of standard in assay procedure, mL

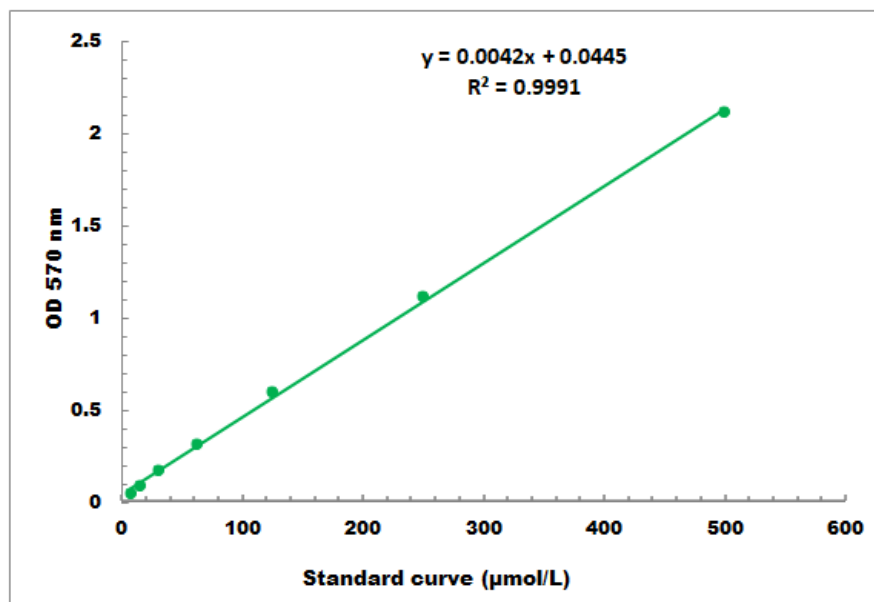
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of total Assay Buffer in sample preparation, mL

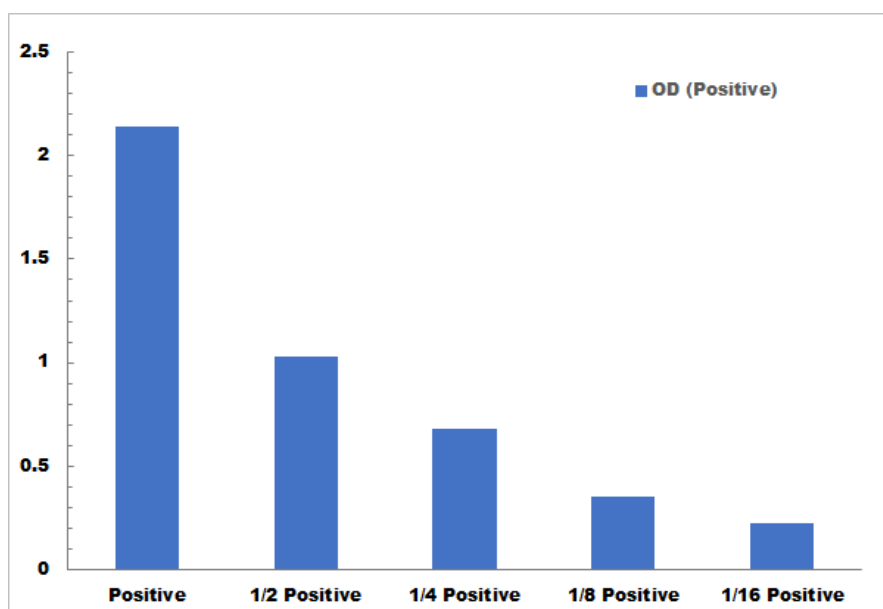
T: the reaction time, minute

VIII. 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