Phospholipid Microplate Assay Kit

User Manual

Catalog # CAK1267

(Version 1.2B)

Detection and Quantification of Phospholipid Content 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

Phospholipids are a class of lipids which constitute a major component of cell membranes and play important roles in signal transduction. Most phospholipids contain one diglyceride, a phosphate group, and one choline.

Phospholipid Microplate Assay Kit provides a simple and direct procedure for measuring phospholipid content in a variety of samples. In this assay, phospholipids (such as lecithin, lysolecithin and sphingomyelin) are enzymatically hydrolyzed to choline which is determined using choline oxidase and a H2O2 specific dye. The optical density of the pink colored product at 570nm is directly proportional to the phospholipid concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Enzyme I	Powder x 1	-20 °C
Enzyme II	Powder x 1	-20 °C
Dye Reagent	Powder x 1	-20 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

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

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

Dye Reagent: add 10 ml distilled water to dissolve before use, mix; store at -20 °C for 1 month after reconstitution.

Standard: add 0.5 ml Assay Buffer to dissolve before use, the concentration will be 20 mmol/L; store at -20 °C for 1 month after reconstitution. Perform 2-fold serial dilutions with Assay Buffer.

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 0.5 ml distilled water for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); then add 0.5 ml Assay Buffer, mix, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Standard	Blank	Sample
Reaction Buffer	70 µl	70 µl	70 µl
Enzyme I	10 µl	10 µl	10 µl
Enzyme II	10 µl	10 µl	10 µl
Standard	10 µl	--	--
Distilled water	--	10 µl	--
Sample	--	--	10 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, put it in the oven, incubate at 37 °C for 10 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) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Phospholipid (μmol/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 20 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Phospholipid (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 20 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$\text{Phospholipid (μmol/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 20 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\text{Phospholipid (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 20 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the standard concentration, 20 mmol/L = 20 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

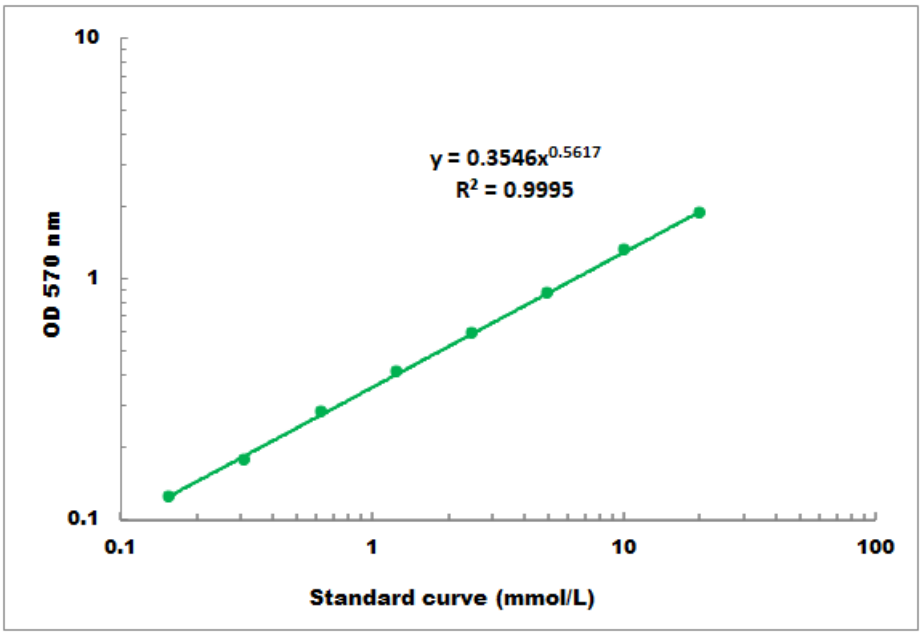
V_{Sample}: the volume of sample, 0.01 ml;

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

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

VII. TYPICAL DATA

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



Detection Range: 0.2 mmol/L - 20 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES