

Technical Manual

ColorFluor Phospholipid Assay Kit

Catalogue Code: BA0143

Pack Size: 100 assays

Research Use Only

DESCRIPTION

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. The Assay Genie ColorFluor Phospholipid Assay Kit provides a simple, direct and high-throughput assay for measuring choline-containing phospholipids in biological 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 or fluorescence intensity (530/585 nm) is directly proportional to the phospholipid concentration in the sample.

KEY FEATURES

Sensitive. Use 20 μ L samples. Linear detection range: colorimetric assay 3 - 200 μ M, fluorimetric assay 0.6 - 20 μ M phospholipid.

APPLICATIONS

Assays: phospholipid in biological samples such as serum and non-EDTA plasma.

Drug Discovery/Pharmacology: effects of drugs on choline-containing phospholipid metabolism.

KIT CONTENTS

Assay Buffer: 10 mL **PLD Enzyme:** 120 μ L
Enzyme Mix: Dried **Dye Reagent:** 120 μ L
Standard: 400 μ L 2 mM phosphatidylcholine

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

COLORIMETRIC ASSAY

Liquid samples such as serum and plasma can be assayed directly. Solid samples can be homogenized in the assay buffer.

Note: SH-containing reagents (e.g. β -mercaptoethanol, dithiothreitol, > 5 μ M), sodium azide, EDTA, and sodium dodecyl sulfate are known to interfere in this assay and should be avoided in sample preparation.

- Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay. Reconstitute Enzyme Mix with 120 μ L Assay Buffer. Reconstituted Enzyme Mix is stable for 1 month when stored at -20°C.
- Standards:** mix 24 μ L 2 mM Standard with 216 μ L dH₂O (final 200 μ M). Dilute standard in dH₂O as follows.

No	200 μ M STD + H ₂ O	Vol (μ L)	Standard (μ M)
1	100 μ L + 0 μ L	100	200
2	60 μ L + 40 μ L	100	120
3	30 μ L + 70 μ L	100	60
4	0 μ L + 100 μ L	100	0

Transfer 20 μ L diluted standards into separate wells of a clear flat-bottom 96-well plate. *Samples:* transfer 20 μ L of each sample into separate wells of the plate.

Note: if a sample is known to contain choline, prepare an extra sample blank well with 20 μ L of the sample.

3. *Color reaction.* Prepare enough Working Reagent by mixing, for each well, 85 μL Assay Buffer, 1 μL PLD Enzyme, 1 μL Enzyme Mix and 1 μL Dye Reagent. Add 80 μL Working Reagent to each well.

For samples that contain choline, prepare a blank control reagent with no PLD Enzyme (i.e., 85 μL Assay Buffer, 1 μL Enzyme Mix and 1 μL Dye Reagent). Add 80 μL of the Control Reagent to the Sample Blank well.

Tap plate to mix. Incubate 30 min at room temperature.

Note: if precipitation occurs with certain samples, carry out the reaction in centrifuge tubes. After the 30 min incubation, centrifuge 5 min at 14,000 rpm. Transfer the supernatant into the wells for OD reading.

4. Read optical density at 570nm (550-585nm).

FLUORIMETRIC ASSAY

The fluorimetric assay procedure is similar to the colorimetric procedure except that (1) 0, 6, 12 and 20 μM phospholipid standards and (2) a black 96-well plate are used. Read fluorescence intensity at $\lambda_{\text{ex}} = 530 \text{ nm}$ and $\lambda_{\text{em}} = 585 \text{ nm}$.

Note: if the calculated phospholipid concentration of a sample is higher than 200 μM in the Colorimetric Assay or 20 μM in the Fluorimetric Assay, dilute sample in 0.5% Triton X-100 and repeat the assay. Multiply result by the dilution factor n .

CALCULATION

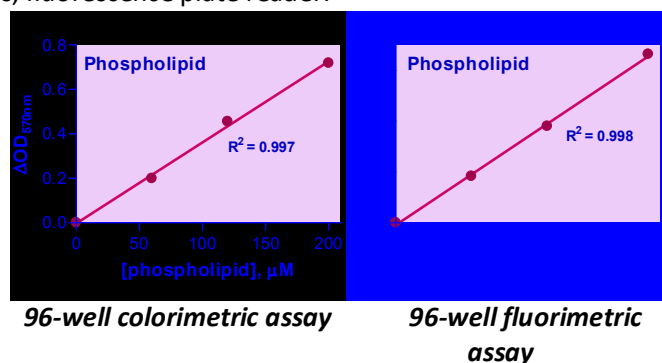
Subtract blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the phospholipid concentration of Sample,

$$[\text{Phospholipid}] = \frac{R_{\text{SAMPLE}} - R_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and H_2O Blank (or Sample Blank if sample contains choline), respectively. n is the sample dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, optical density plate reader; black flat-bottom uncoated 96-well plates, fluorescence plate reader.



PUBLICATIONS

- Kim S et al (2012) Aberrant upregulation of astroglial ceramide potentiates oligodendrocyte injury. Brain Pathol 22(1):41-47
- Mukherjee A et al (2012) Lysophosphatidic acid activates lipogenic pathways and de novo lipid synthesis in ovarian cancer cells. J Biol Chem 287(3):24990-50000.

3. Stoll C et al (2011). Liposomes alter thermal phase behavior and composition of red blood cell membranes. *Biochim Biophys Acta* 1808(1):474-481.

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