Technical Bulletin

Phospholipid Assay Kit

Catalogue number MAK122

Product 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 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 release choline, which is determined using choline oxidase and a H_2O_2 specific dye. This results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 530/λ_{em} = 585 nm) product directly proportional to the phospholipid concentration in the sample. The range of linear detection is 3-200 µM for colorimetric assays and 0.6-20 µM for fluorometric assays.

Components

The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer Catalog Number MAK122A	10 mL
Enzyme Mix Catalog Number MAK122B	1 vl
Phosphatidylcholine Standard, 2 mM Catalog Number MAK122C	400 µL
PLD Enzyme Catalog Number MAK122D	120 µL
Dye Reagent Catalog Number MAK122E	120 µL

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate - It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Use ultrapure water for the preparation of reagents. Equilibrate all components to room temperature before use. Briefly centrifuge vials before opening. Keep thawed tubes on ice during assay.

Enzyme Mix – Reconstitute in 120 µL of Assay Buffer. Mix well by pipetting, then aliquot and store at -20 °C. Use within 1 month of reconstitution and keep cold while in use.

A precipitate may be present in the reconstituted aliquot. Gently centrifuge the vial and use the supernatant in the Reaction Mixes.

Storage/Stability

The kit is shipped on dry ice and storage at -20 °C, protected from light, is recommended.

Use within 1 month of reconstitution and keep cold while in use.

Procedure

All Samples and Standards should be run in duplicate

Standards for Colorimetric Detection

Add 24 µL of the 2 mM Phosphatidylcholine Standard to 216 µL of water to prepare a 200 µM Standard working solution. Add 0, 30, 60, and 100 µL of the 200 µM Standard working solution into tubes. Add water to each tube to bring the volume to 100 µL, generating 0 (blank), 60, 120, and 200 µM Standards. Transfer 20 µL of Standards into separate wells of 96 well plate.

Standards for Fluorometric Detection

Prepare Standards as described for the Colorimetric Detection. Further dilute each Standard 10-fold with water, generating 0 (blank), 6, 12, and 20 µM Standards. Transfer 20 µL of Standards into separate wells of 96 well plate.

Sample Preparation

Thiol (SH) group containing reagents (e.g., DTT, mercaptoethanol), sodium azide, EDTA, and sodium dodecyl sulfate may interfere with this assay and should be avoided in Sample preparation.
Liquid Samples such as serum and plasma can be assayed directly. Solid Samples can be homogenized in the assay buffer. Aliquot 20 µL of each Sample into two separate wells of a 96 well plate.
Notes: If a Sample is known to contain choline, prepare a Sample blank well with 20 µL of the Sample.

For unknown Samples, it is suggested to test several dilutions to ensure the readings are within the linear range of the Standard curve.

Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1. 80 µL of the appropriate Reaction Mix is required for each reaction (well). Allow the Reaction Mixes to equilibrate to room temperature.

Table 1.

Reaction Mixes

Reagent	Sample and Standards	Sample Blank
Assay Buffer	85 µL	86 µL
Enzyme Mix	1 µL	1 µL
PLD Enzyme	1 µL	-
Dye Reagent	1 µL	1 µL

2. Add 80 µL of the appropriate Reaction Mix to each well. Mix well using a horizontal shaker or by pipetting and incubate the reaction 30 minutes at room temperature. Protect the plate from light during the incubation.

   Note: If precipitation occurs with certain Samples, carry out the reaction in centrifuge tubes. After the 30 minutes incubation, centrifuge for 5 minutes at 14,000 rpm. Transfer the supernatant into the wells to measure absorbance.

3. Measure the absorbance of the Samples and Standards at 570 nm for the colorimetric assay or the fluorescence intensity (λex = 530/λem = 585 nm) for the fluorometric assay.

   Note: If the calculated phospholipid concentration of a Sample is higher than 200 µM in the colorimetric assay or 20 µM in the fluorometric assay, dilute Sample in 0.5% Triton™ X-100 and repeat the assay. Multiply result by the dilution factor.

Results

Calculations

Subtract blank value (0 standard) from the standard values and plot the absorbance or fluorescence measured for each standard against the standard concentrations. Determine the slope and use to calculate the phospholipid concentration of the sample.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Phospholipid

The concentration of phospholipid in the sample can be calculated using the equation below.

$$\mu M = \frac{M_{sample} - M_{blank}}{Slope} \times N$$

M_{sample} = Absorbance or fluorescent intensity measured in unknown sample

M_{blank} = Absorbance or fluorescent intensity measured in blank (0 Standard, or sample blank if sample contains choline)

Slope = Determined from standard curve (μM^{-1})

N = Dilution factor (if used)

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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