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Product Information

Phosphatidylcholine Assay Kit

Catalog Number **MAK049**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Phosphatidylcholine (PC) accounts for more than 50% of the phospholipids composing mammalian plasma membranes and is particularly enriched in the extracellular leaflet. PC is synthesized in the liver and is required for lipoprotein assembly and secretion. PC is cleaved by phospholipase D, which hydrolyses the choline head, producing the signaling intermediate phosphatidic acid.

In this assay, PC concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the PC present.

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

PC Assay Buffer Catalog Number MAK049A	25 mL
Fluorescent Peroxidase Substrate, in DMSO Catalog Number MAK049B	0.2 mL
PC Hydrolysis Enzyme Catalog Number MAK049C	1 vl
PC Development Mix Catalog Number MAK049D	1 vl
PC Standard, 10 µmole Catalog Number MAK049E	1 vl

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
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

PC Assay Buffer – Allow buffer to come to room temperature before use.

Fluorescent Peroxidase Substrate – Thaw at room temperature to melt the solution prior to use. Aliquot and store protected from light and moisture at –20 °C. Upon thawing, the Fluorescent Peroxidase Substrate is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Fluorescent Peroxidase Substrate 5 to 10-fold with PC Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

PC Hydrolysis Enzyme and PC Development Mix – Reconstitute each with 220 µL of PC Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

PC Standard – Reconstitute in 200 µL of water to 0generate a 50 mM (50 nmole/µL) PC Standard solution. Mix well by pipetting until it forms a uniform suspension, then aliquot and store at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

PC Standards for Colorimetric Detection
Dilute 10 µL of the 50 mM (50 nmole/µL) PC Standard Solution with 990 µL of water to prepare a 0.5 mM (0.5 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, 10 µL of the 0.5 mM PC standard solution into a 96 well plate, generating 0 (blank), 1, 2, 3, 4, and 5 nmole/well standards. Add PC Assay Buffer to each well to bring the volume to 50 µL.

PC Standards for Fluorometric Detection
Dilute 10 µL of the 50 mM (50 nmole/µL) PC Standard Solution with 990 µL of water to prepare a 0.5 mM (0.5 nmole/µL) standard solution. Dilute 10 µL of the 0.5 mM standard solution with 90 µL of water to generate a 0.05 mM (0.05 nmole/µL). Add 0, 2, 4, 6, 8, 10 µL of the 0.05 mM PC standard solution into a 96 well plate, generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 nmole/well standards. Add PC Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (10 mg) or cells (2×10^6) should be rapidly homogenized in 4 volumes of cold PC Assay buffer. Centrifuge at 13,000 × *g* for 10 minutes at 4 °C to remove insoluble material.

Serum samples may be assayed directly.

Bring samples to a final volume of 50 µL with PC Assay Buffer.

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

Choline present in the sample can generate background. To control for choline background, include a blank sample for each sample by omitting the PC Hydrolysis Enzyme in the Reaction Mix.

Assay Reaction
1. Set up the Master Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Master Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Sample Blank	Samples and Standards
PC Assay Buffer	46 µL	44 µL
PC Hydrolysis Enzyme	–	2 µL
PC Development Mix	2 µL	2 µL
Fluorescent Peroxidase Substrate	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) PC Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate PC standards to plot a standard curve.

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

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of PC present in the sample may be determined from the standard curve

Concentration of PC

$$S_a/S_v = C$$

S_a = Amount of PC in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of PC in sample

PC molecular weight: 768 g/mole

Sample Calculation

Amount of PC (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of PC in sample

$$5.84 \text{ nmole}/50 \ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 768 \text{ ng/nmole} = 89.7 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice 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 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 samples will be used 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 tubes
	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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