Phosphatidylcholine Assay Kit

Catalog Number KA0827

100 assays

Version: 01

Intended for research use only

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Introduction

Background

Phosphatidylcholine (PC) is a phospholipid which incorporates choline as the headgroup of the lipid. PC is a major constituent of biological membranes and is involved in cell signaling through release of choline by phospholipase D leaving the second messenger phosphatidic acid. The Phosphatidylcholine Assay Kit is a simple convenient means of measuring Phosphatidylcholine in a variety of biological samples. The kit utilizes an enzyme-coupled assay in which PC is hydrolyzed, releasing choline which is subsequently oxidized resulting in development of the OxiRed probe to generate fluorescence (Ex/Em = 535/587 nm) and absorbance (O.D. = 570 nm). The kit measures PC in the range of 0.1 to 10 nmol per sample. PC is present in serum at ~ 0.2-2.5 mM (~50-200 mg/dL).

General Information

Materials Supplied

List of component

Component	Amount
PC Assay Buffer	25 ml
OxiRed™ Probe	0.2 ml
PC Hydrolysis Enzyme	lyophilized
PC Development Mix	lyophilized
PC Standard (10 µmol)	lyophilized

Storage Instruction

Store the kit at -20℃, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

Assay Protocol

Reagent Preparation

- PC Probe: Ready to use as supplied. Warm to >18℃ to melt frozen DMSO prior to use. Protect from light and moisture. Stable for 2 months at -20℃
- PC Hydrolysis Enzyme, Development Mix: Dissolve with 220 µl PC Assay Buffer separately. Pipette up and down to dissolve. Keep the Enzyme and Development Mix on ice during use. Aliquot and store at -20℃ if they will not all be used at once. Avoid repeated freeze/thaw cycles. Use within two months.
- PC Standard: Dissolve in 200 µl dH_2O to generate 50 mM (50 nmol/µl) PC Standard solution. Keep on ice while in use. Store at -20℃.
- Ensure that the Assay Buffer is warmed to room temperature before use.

Assay Procedure

1. Standard Curve:
   For the Colorimetric Assay: Dilute 10 µl of the 50 mM PC Standard with 990 µl dH_2O to generate 0.5 mM Standard Phosphatidylcholine. Add 0, 2, 4, 6, 8, 10 µl of the diluted PC Standard into a 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well Standard. Bring the volume to 50 µl with Assay Buffer.
   For the Fluorometric Assay: Dilute the standard to 0.05 mM (0.05 nmol/µl), then follow the same protocol as colorimetric assay, to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Standard.

2. Sample Preparation:
   Add samples to sample wells in a 96-well plate and bring the volume to 50µl/well with Assay buffer. We suggest testing several doses of your samples to make sure the reading are within the standard curve range.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

	Phosphatidylcholine Measurement	Background Control*
Assay Buffer	44 µl	46 µl
PC Hydrolysis Enzyme	2 µl	--
Development Mix	2 µl	2 µl
PC Probe **	2 µl	2 µl

**Choline can generate background in samples. If Choline is suspected to be in your sample, perform a background control without the PC Converting Enzyme. Subtract the background readings from sample readings.*

*** For the fluorescent assay, dilute the probe 10X to reduce background reading. Add 50 µl of the Reaction Mix to each well containing the PC Standard and test samples. Mix well. Incubate the reaction for 30 min at room temperature, protect from light.*

4. Measure O.D. at 570 nm or fluorescence at Ex/Em 535/587 nm in a microplate reader.

Data Analysis

Calculation of Results

Correct background by subtracting the value derived from the 0 PC control from all sample and standard readings (The background reading can be significant and must be subtracted from sample readings).

Plot PC standard curve. Apply sample readings to the standard curve. PC concentrations of the test samples can then be calculated:

C = Sa/Sv (nmol/µl, µmol/ml or mM)

Where:

Sa is the PC content of unknown samples (in nmol) from standard curve,

Sv is sample volume (µl) added into the assay wells.

Phosphatidylcholine avg molecular weight is 768 g/mol.