Product Information

Phosphatidylcholine Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2086-100 100 assays	Cap Color	Part Number
PC Assay Buffer	25 ml	WM	K2086-C-1
OxiRed Probe	0.2 ml	Red	K2086-C-2
PC Hydrolysis Enzyme	lyophilized	Purple	K2086-C-3
PC Development Mix	lyophilized	Green	K2086-C-4
PC Standard (10 µmol)	lyophilized	Yellow	K2086-C-5

II. Introduction:

Phosphatidylcholine (PC) is a class of phospholipid that incorporates choline as the lipid headgroup. PC is a major component of pulmonary surfactant and biological membranes. PC can transport by phosphatidylcholine transfer protein (PCTP) between membranes within the cell. PC is also involved in membrane-mediated cell signaling through the second messenger phosphatidic acid.

The Phosphatidylcholine Colorimetric/Fluorometric Assay Kit provides a sensitive, simple and convenient way for detection of phosphatidylcholine in various biological fluids based on colorimetric and fluorometric method. The assay is based on an enzyme-coupled assay in which PC is hydrolyzed to release choline, which is then oxidized resulting in development of the OxiRed probe to yield fluorescence (Ex/Em 535 nm 587 nm) and absorbance (570 nm). The kit can measure PC in the range of 0.1 to 10 nmol per sample. PC in serum is about 0.2 - 2.5 mM (~ 50 - 200 mg/dL).

III. Storage and Handling:

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.

IV. Reagent Reconstitution and General Consideration:

PC Probe: Ready to use as supplied. Warm to > 18℃ to melt frozen DMSO prior to use. Store at -20℃; protect from light and moisture. Stable for 2 months.

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.

V. Phosphatidylcholine Assay Protocol:

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, 1, 2, 3, 4, 5 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, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well of the standard.

2. Sample Preparation:

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

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 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 significant background. If choline is present in your samples, perform a background control without the PC Hydrolysis Enzyme and subtract this value 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.

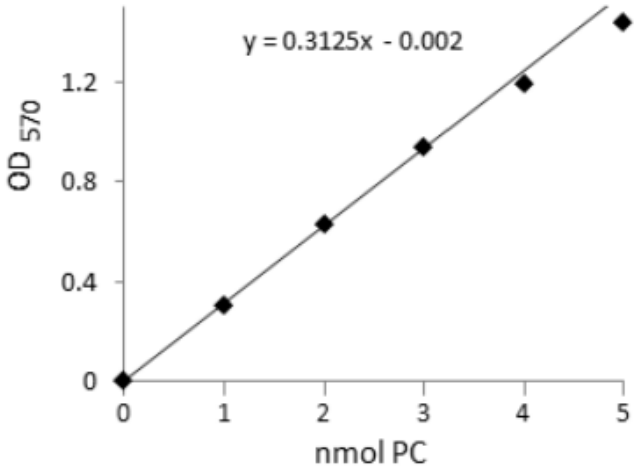
5. Calculation: Correct background by subtracting the value derived from the 0 PC control from all sample and standard readings (The background reading can be significant and must be subtracted from sample readings). Plot PC standard curve. Apply sample readings to the standard curve. PC concentrations of the test samples can then be calculated:

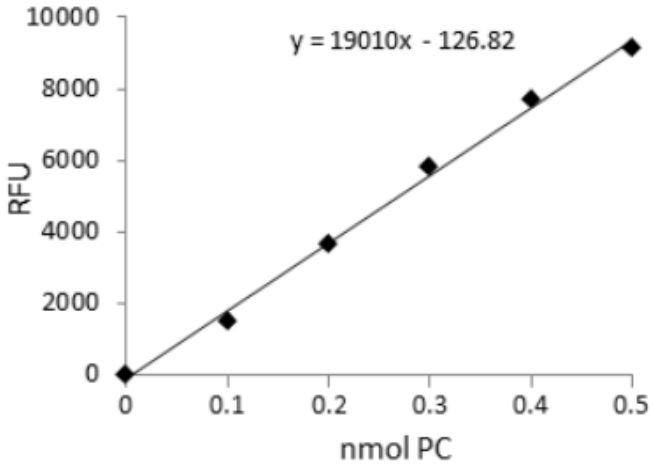
C = Sa/Sv (nmol/µl, or mM)

Where: Sa is the PC content of unknown samples (in nmol) fro standard curve,

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

Phosphatidylcholine avg molecular weight is 768 g/mol.





General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely

	• Plate read at incorrect wavelength • Use of a different 96-well plate	• Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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