Phosphatidylcholine Assay Kit

(Catalog #K576-100; 100 Reactions; Store kit at –20°C)

I. Introduction:

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. BioVision's 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 nm 587 nm) and absorbance (570 nm). BioVision's Phosphatidylcholine 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)

II. Kit Contents:

Components	K576-100	Cap Code	Part No.
PC Assay Buffer	25 ml	WM	K576-100-1
OxiRed Probe	0.2 ml	Red	K576-100-2A
PC Hydrolysis Enzyme	lyophilized	Purple	K576-100-4
PC Development Mix	lyophilized	Green	K576-100-5
PC Standard (10 µmol)	lyophilized	Yellow	K576-100-6

III. Storage and Handling:

Store the kit at -20°C, 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°C to melt frozen DMSO prior to use. Store at -20°C; 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°C if they will not all be used at once. Avoid repeated freeze/thaw cycles. Use within two months.

PC Standard: Dissolve in 200 µl dH2O to generate 50 mM (50 nmol/µl) PC Standard solution. Keep on ice while in use. Store at -20°C.

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 dH2O 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 *
44 µl Assay Buffer	46 µl Assay Buffer
2 µl PC Hydrolysis Enzyme	----------
2 µl Development Mix	2 µl Development Mix
2 µl PC Probe**	2 µl PC Probe

***** 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.

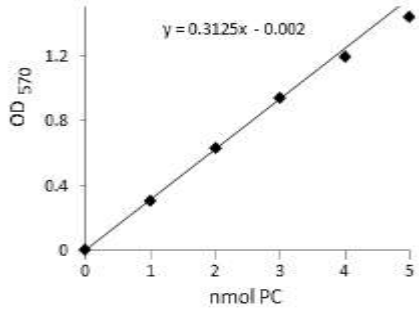
Add 50 µl of the **Reaction Mix** to each well containing the PC standard and test samples. Mix well. Incubate the reaction for 30 min at room temperature, protect from light.

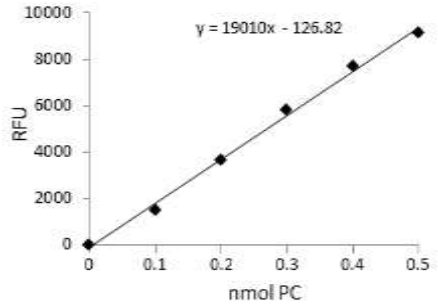
4. Measure O.D. at 570 nm, or fluorescence at Ex/Em 535/587 nm in a microplate reader

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:

$C = S_a/S_v$ (nmol/µl, or mM)

Where: S_a is the PC content of unknown samples (in nmol) from standard curve,
S_v is sample volume (µl) added into the assay wells.
Phosphatidylcholine avg molecular weight is 768 g/mol.





RELATED PRODUCTS:

NAD(P)/NAD(P)H Quantification Kit	ADP/ATP Ratio Assay Kit
Ascorbic Acid Quantification Kit	Glutathione Detection Kit
Total Antioxidant Capacity (TAC) Assay Kit	Fatty Acid Assay Kit
Ethanol Assay Kit	Uric Acid Assay Kit
Pyruvate Assay Kit	Lactate Assay Kit I & II
Creatinine Assay Kit	Nitric Oxide Assay Kit
Ammonia Assay Kit	Free Glycerol Assay Kit
Triglyceride Assay Kit	Hemin Assay Kit
Choline/Acetylcholine Quantification Kit	Glucose Assay Kit
Sarcosine Assay Kit	L-Amino Acid Assay Kit
Glycogen Assay Kit	Cholesterol Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till 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	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• 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 an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		