Phosphatidylcholine Assay Kit (Fluorometric)

Phosphatidylcholine Assay Kit (Fluorometric) can be used to measure Phosphatidylcholine levels in plasma or serum, tissue homogenates, or cell culture supernatants.

Catalog number: ARG82860

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Phosphatidylcholines (PC) are a class of phospholipids that incorporate choline as a headgroup. They are a major component of biological membranes and can be easily obtained from a variety of readily available sources, such as egg yolk or soybeans, from which they are mechanically or chemically extracted using hexane. They are also a member of the lecithin group of yellow-brownish fatty substances occurring in animal and plant tissues. Dipalmitoyl phosphatidylcholine (a.k.a. lecithin) is a major component of pulmonary surfactant and is often used in the L / S ratio to calculate fetal lung maturity. While phosphatidylcholines are found in all plant and animal cells, they are absent in the membranes of most bacteria, including Escherichia coli. Purified phosphatidylcholine is produced commercially. [Provide by Wikipedia: Phosphatidylcholine]

PRINCIPLE OF THE ASSAY

This Phosphatidylcholine Assay Kit (Fluorometric) is a simple fluorometric assay that measures the amount of Phosphatidylcholine (PC) present in serum, plasma, and tissue samples. The assay is based on the enzyme driven reaction that will detect phosphatidylcholine via phosphatidylcholine-specific phospholipase D enzyme and choline oxidase. First, phospholipase D hydrolyzes phosphatidylcholine into choline and phosphatidic acid. Choline is then oxidized by choline oxidase to produce hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific fluorescence probe. Horseradish peroxidase catalyzes the reaction between the probe and

hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of phosphatidylcholine standard within the 96-well microtiter plate format.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Upon receipt, store the Standard, 100X Fluorescence Probe, HRP, and Choline Oxidase at -20°C. Avoid multiple freeze/thaw cycles. Store the Phospholipase D at -80°C. Store the remaining kit components at 4°C.

Component	Quantity	Storage information
Black 96 well Microplate	1 plate	4°C
350X Phospholipase D	30 µL	-80°C
Choline Oxidase	50 µL	-20°C
HRP (100 U/mL solution in glycerol)	100 µL	-20°C
100X Fluorescence Probe, one tube in DMSO	100 µL	-20°C (Protect from light)
10X Assay Buffer	25 mL	4°C
Standard (100 mg/mL)	25 µL	-20°C
10X Standard Diluent Buffer	1 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation at 530-570 nm and emission at 590-600 nm
- 1X PBS
- Deionized or distilled water
- Pipettes, pipette tips and multichannel micropipette reservoir
- (optional) Chloroform
- (optional) Superoxide dismutase

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.
- Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL.
- Avoid samples containing DTT or β-mercaptoethanol since the

Fluorescence Probe is not stable in the presence of thiols (above 10 µM).

- Choline can generate high background if present in samples. If choline may be present, run a background control without Phospholipase D. Subtract this value from sample reading values.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2000 x g for 10 minutes at 4°C. Collect the serum and store on ice. Serum sample must be diluted at least 1:50 to 1:400 with 1X Assay Buffer.

Plasma: Collect blood with heparin or citrate and centrifuge at 1000 x g for 10 minutes at 4°C. Collect the plasma layer and store on ice. Take care to avoid disturbing the white buffy layer. Plasma sample must be diluted at least 1:50 to 1:400 with 1X Assay Buffer.

Tissues or Cell suspensions: Homogenize 250 mg of sample (wet tissue or cell pellet) in 4.5 mL of chloroform/methanol (2:1, v/v). Centrifuge to remove debris. After centrifugation, incubate the homogenate at room temperature for 1 hour on an orbital shaker. Induce phase separation by adding 1.25 mL distilled water. Incubate 10 minutes at room temperature and centrifuge at 1000 x g for 10 minutes. Collect the lower (chloroform) organic phase and re-extract the upper phase with 2 mL of solvent mixture whose composition is $CHCl_3$/MeOH/water (86:14:1, v/v/v). Combine organic phases and dry in a vacuum centrifuge. Dissolve in 200 µL of $CHCl_3$/MeOH/water (60:30:4.5, v/v/v) for storage. Before phosphatidylcholine assay, samples must be diluted at least 1:50 to 1:400 with 1X Assay Buffer.

Note:

- Samples should be assayed immediately or stored at -80°C prior to performing the assay.
- Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL.
- Avoid samples containing DTT or β-mercaptoethanol since the Fluorescence Probe is not stable in the presence of thiols (above 10 µM).
- Choline can generate high background if present in samples. If choline may be present, run a background control without Phospholipase D. Subtract this value from sample reading values.

REAGENT PREPARATION

- **1X Assay Buffer:** equilibrate the 10X Assay Buffer to room temperature prior to using. Dilute the 10X Assay Buffer to 1X with distilled water. (E.g., mix 25 mL of 10X Assay Buffer with 225 mL of distilled water for 250 mL total) Mix to homogeneity. Store the 1X Assay Buffer at 4°C up to six months.
- **1X Standard Diluent Buffer:** equilibrate the 10X Standard Diluent Buffer to room temperature prior to using. Dilute the 10X Standard Diluent Buffer to 1X with distilled water. (E.g., mix 25 mL of 10X Assay Buffer with 225 mL of distilled water for 250 mL total) Mix to homogeneity. Store the 1X Assay Buffer at 4°C up to six months.
- **Reaction Reagent:** prepare a Reaction Reagent by diluting the Choline Oxidase 1:200, HRP 1:500, Fluorescence Probe 1:100, and Phospholipase D 1:350 in 1X Assay Buffer.
- **Standards:** Prepare fresh phosphatidylcholine standards by first diluting a portion of the 100 mg/mL Phosphatidylcholine Standard stock solution 1:100 in 1X Standard Diluent. (E.g., Add 5 µL of Phosphatidylcholine Standard in 495 µL of 1X Standard Diluent). Vortex thoroughly. This provides a 100 mg/dL concentration. Dilute standard as follows.

Standard tube	Phosphatidylcholine (mg/dL)	1X Assay Buffer (µL)	Standard, 100 mg/dL (µL)
S1	10	450	50
S2	5	250	250 of S1
S3	2.5	250	250 of S2
S4	1.25	250	250 of S3
S5	0.625	250	250 of S4
S6	0.313	250	250 of S5
S7	0.156	250	250 of S6
S0	0	500	0

Note: Do not store diluted phosphatidylcholine standard solution.

ASSAY PROCEDURE

Each phosphatidylcholine standard and samples should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

	Standard well	Sample well
Each diluted Standard	10 µL	
Each Sample		10 µL
Reaction Reagent	100 µL	100 µL
Tap plate to mix briefly and thoroughly. Incubate for **60 minutes** at **37°C** in the dark.		
Read the fluorescence intensity at **λex/em** = **530-570/590-600 nm**.		

CALCULATION OF RESULTS

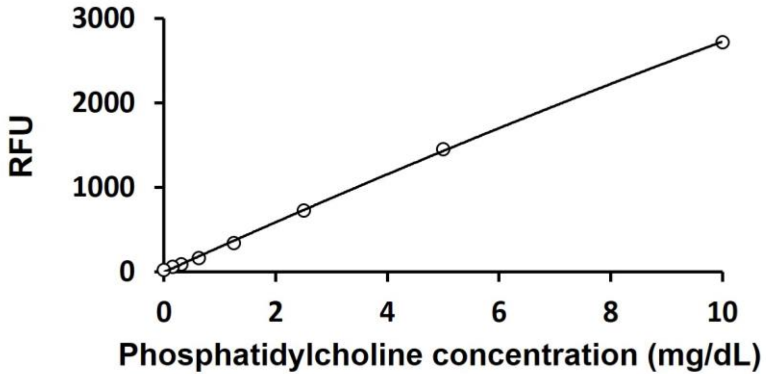
1. Calculate the average fluorescence values for every standard, control, and sample. Subtract the average zero standard (S0) value from itself and all standard and sample values. This is the corrected fluorescence.
2. Plot the corrected fluorescence for the standards against the final concentration of the phosphatidylcholine standards curve.
3. Determine the phosphatidylcholine concentration of the samples with the equation obtained from the linear regression analysis of the standard curve. Substitute the corrected fluorescence values for each sample. Only use values within the range of the standard curve. Remember to account for dilution factors.

   Total phosphatidylcholine (mg/dL)

   = (Sample corrected fluorescence / Slope) x Sample dilution factor

EXAMPLE OF RESULT

The following figures demonstrate typical results with the Phosphatidylcholine Assay Kit (Fluorometric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.5 mg/dL