Phosphatidylcholine Colorimetric Assay Kit

Item No. 10009926

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity
10010066	PC Buffer (10X)	1 vial
10010067	PC Color Detector	2 vials
10010068	PC Enzyme Mixture	2 vials
10010069	PC-Specific PLD	1 vial
10010070	Phosphatidylcholine Standard	1 vial
10010071	PC Detergent Solution	1 vial
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate
400012	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure absorbance between 585-600 nm.
2. Adjustable pipettes and a multichannel pipette.
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable.

INTRODUCTION

Background

Phosphatidylcholine (PC) is the major membrane phospholipid in mammalian cells, and regulation of its biosynthesis and turnover is critical to maintaining membrane structure and function. PC consists of two fatty acids and one molecule of phosphocholine bound to a glycerol molecule. The fatty acids on the *sn*-1 or *sn*-2 position can be saturated, unsaturated, or *trans*; the double bonds in the unsaturated are naturally fatty acids in the *cis* configuration. PC is also the major source of choline in the body. Choline and the compounds derived from it serve many vital functions including the synthesis of cell signaling molecules (*i.e.*, Platelet Activating Factor and sphingophosphorylcholine) and the neurotransmitter, acetylcholine, which is involved in muscle control and memory.

PC exerts an important influence on the regulation of lipid homeostasis by being one of the major phospholipids in plasma lipoproteins, comprising ~70% of the total phospholipids in the circulation.[1] An enzyme present in circulation, Lecithin:cholesterol acyltransferase (LCAT), removes one fatty acid from PC and attaches it to cholesterol, converting cholesterol into a cholesteryl ester (see Figure 1, on page 6).[2] The cholesteryl ester is taken up by a high-density lipoprotein and transported back to the liver where the cholesterol is excreted from the body in the form of a bile salt. Elevated levels of cholesterol and cholesteryl esters have been linked to atherosclerosis and heart disease.[3,4] It has recently been shown that administration of myriocin (an inhibitor of sphingomyelin synthesis) into apolipoprotein E knockout mice dramatically decreased sphingomyelin (SM) levels, increased PC levels, thus decreasing the SM/PC ratio in the plasma, and significantly decreased the atherosclerotic lesion area.[5,6] The data suggests that SM might play a promoting role, whereas PC might play a preventative role in the development of atherosclerosis. Sensitive and reliable techniques for the quantification of PC will aid in the elucidation of the role that PC plays in various diseases.

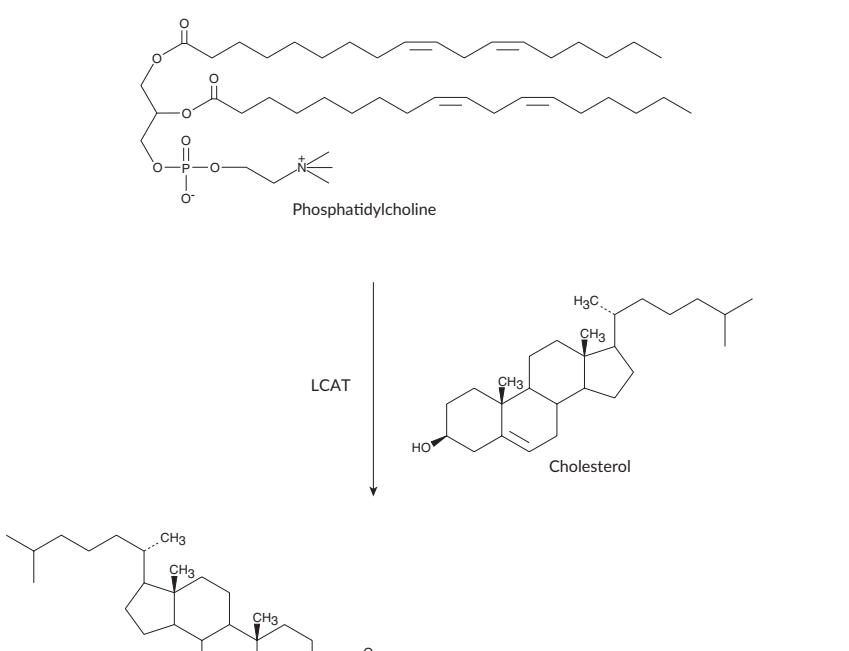


Figure 1.

About This Assay

Cayman's Phosphatidylcholine Colorimetric Assay Kit provides a specific, sensitive, and convenient method for quantifying phosphatidylcholine in plasma or serum. In this assay, phosphatidylcholine-specific phospholipase D (PC-Specific PLD) is first used to hydrolyze phosphatidylcholine to choline and phosphatidic acid. The newly formed choline is then used to generate hydrogen peroxide in a reaction catalyzed by choline oxidase. Finally, with peroxidase as a catalyst, hydrogen peroxide reacts with DAOS and 4-aminoantipyrine to generate a blue dye with an optimal absorption at 595 nm (see Figure 2).[7]

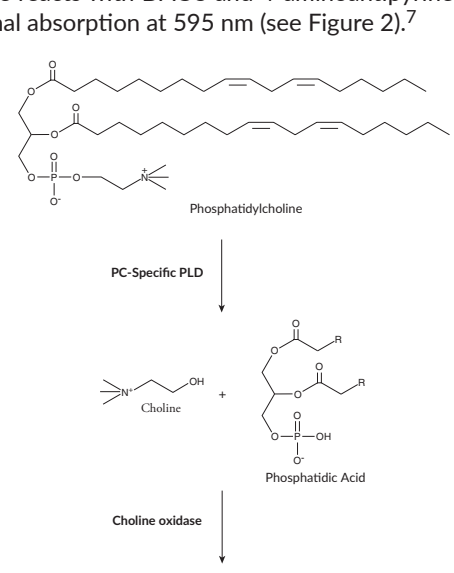


Figure 2. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **PC Buffer (10X) - (Item No. 10010066)**

   Mix 3 ml of PC Buffer concentrate with 27 ml of HPLC-grade water. This final Buffer (50 mM Tris-HCl, pH 8.0, containing 0.66 mM $CaCl_2$) should be used in the assay and for diluting reagents. When stored at 4°C, this 1X Buffer is stable for at least six months.

2. **PC Color Detector - (Item No. 10010067)**

   Each vial contains a lyophilized powder of DAOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline) and 4-aminoantipyrine. Reconstitute the Color Detector in 3 ml of 1X PC Buffer. The reconstituted Color Detector is stable for eight hours at room temperature.

3. **PC Enzyme Mixture - (Item No. 10010068)**

   Each vial contains a lyophilized powder of choline oxidase and horseradish peroxidase. Prior to use in the assay, reconstitute the vial with 1 ml of 1X PC Buffer. Store the reconstituted Enzyme Mixture on ice until ready to use. The reconstituted Enzyme Mixture is stable for 24 hours at 4°C.

4. **PC-Specific PLD - (Item No. 10010069)**

   This vial contains a solution of phosphatidylcholine-specific phospholipase D. It is ready to use as supplied.

5. **Phosphatidylcholine Standard - (Item No. 10010070)**

   This vial contains a standard of phosphatidylcholine (see **Standard Preparation** on page 10).

6. **PC Detergent Solution - (Item No. 10010071)**

   This vial contains a Triton X-100 solution. It is ready to use as supplied.

Sample Preparation

Plasma

The typical concentration of phosphatidylcholine in human plasma is 50-200 mg/dl.[7]

1. Collect blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
3. Plasma does not need to be diluted before assaying.

Serum

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.
4. Serum does not need to be diluted before assaying.

ASSAY PROTOCOL

Standard Preparation

Add 1 ml of PC Detergent Solution (Item No. 10010071) to the Phosphatidylcholine Standard (Item No. 10010070) and vortex briefly. Transfer 200 µl of the standard to another glass vial and add 1.3 ml of PC Detergent Solution to obtain a 200 mg/dl PC Stock Solution. The reconstituted PC Stock is stable for one month at -20°C. Take seven clean glass test tubes and mark them A-G. Add the amount of PC Stock and PC Detergent Solution to each tube as described in Table 1, below. *Note: Bubbles may form in some of the standards, they will disperse in a few minutes and not affect the assay in any way. Diluted standards are stable for four hours.*

Tube	200 mg/dl PC Stock (µl)	PC Detergent Solution (µl)	PC Concentration (mg/dl)
A	0	500	0
B	50	450	20
C	100	400	40
D	150	350	60
E	200	300	80
F	250	250	100
G	375	125	150

Table 1. Preparation of phosphatidylcholine standards

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of phosphatidylcholine standards and samples to be measured in duplicate is shown below in Figure 3. We suggest you record the contents of each well on the template sheet provided on page 18.

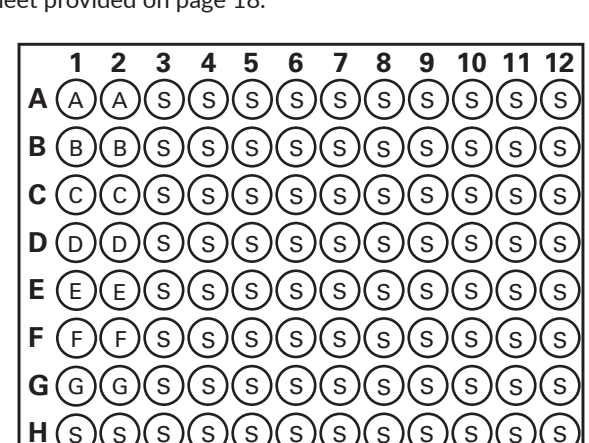


Figure 3. Sample plate format

Pipetting Hints

- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 110 µl in all wells.
- The incubation temperature is 37°C.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the standards and samples be assayed at least in duplicate.
- Twenty-seven samples can be assayed in triplicate, or forty-one samples in duplicate.
- Monitor the absorbance at 585-600 nm using a plate reader.

Performing the Assay

1. **Preparation of Reaction Mixture -** In a suitable tube, prepare the Reaction Mixture according to the table below. The Reaction Mixture is stable for 24 hours at 4°C.

Reagent	50 wells	100 wells
PC Color Detector	3 ml	6 ml
PC Enzyme Mixture	1 ml	2 ml
PC-Specific PLD	30 µl	60 µl
1X PC Buffer	970 µl	1.94 ml

Table 2. Reaction Mixture preparation

2. **Phosphatidylcholine Standard Wells -** add 10 µl of standard (tubes A-G) per well in the designated wells on the plate (see suggested plate configuration, Figure 3, page 11).
3. **Sample Wells -** add 10 µl of sample (either undiluted plasma or serum) to two or three wells. *NOTE: The amount of sample added to the well should always be 10 µl.*
4. Initiate the reactions by adding 100 µl of Reaction Mixture to each well.
5. Carefully shake the microwell plate for a few seconds to mix.
6. Cover with plate cover and incubate at 37°C for 60 minutes.
7. Read the absorbance at a wavelength between 585-600 nm using a plate reader.

ANALYSIS

Calculations

1. Determine the average absorbance of each standard and sample.
2. Subtract the absorbance of standard A (0 mg/dl) from itself and all other standards and samples to yield the corrected absorbance value (CAV).
3. Graph the CAV of the standards as a function of the final phosphatidylcholine concentration (mg/dl) from Table 1. See Figure 4 (on page 15) for a typical standard curve.
4. Calculate the phosphatidylcholine concentration of the original samples using the equation obtained from the linear regression of the standard curve by substituting the CAV for each sample into the equation. *NOTE: The phosphatidylcholine concentration is calculated back to the original sample and not what is in the well.*

$$\text{Phosphatidylcholine (mg/dl)} = \left[\frac{\text{(CAV) - (y-intercept)}}{\text{Slope}} \right]$$

Performance Characteristics

Precision:

When a series of 16 human plasma and serum measurements were performed on the same day, the intra-assay coefficient of variation was 1.6 and 1.3%, respectively. When a series of human plasma and serum measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 2.1 and 1.2%, respectively.

Sensitivity:

The lower limit of detection (LLOD) is 0.212 mg/dl.

The lower limit of quantification (LLOQ) is 2.5 mg/dl.

Representative Phosphatidylcholine Standard Curve

The standard curve presented here is an example of the data typically provided with this kit; however, your results will not be identical to these. You must run a new standard curve - do not use Figure 4 to determine the values of your samples.

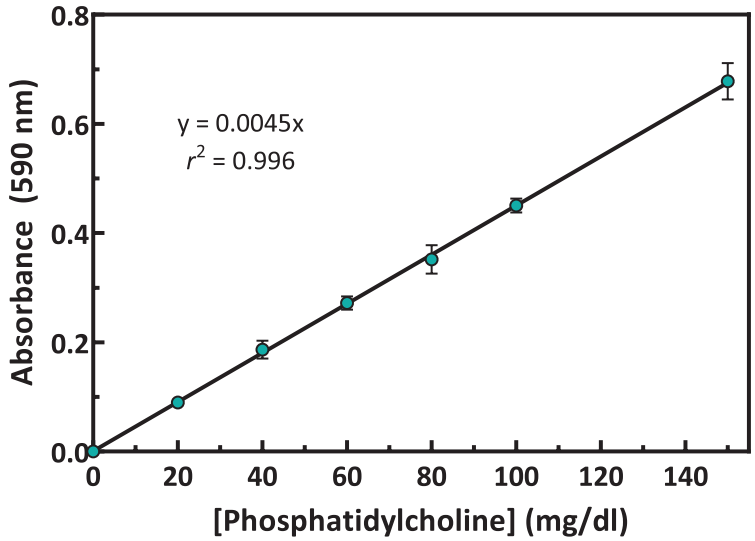


Figure 4. Phosphatidylcholine standard curve

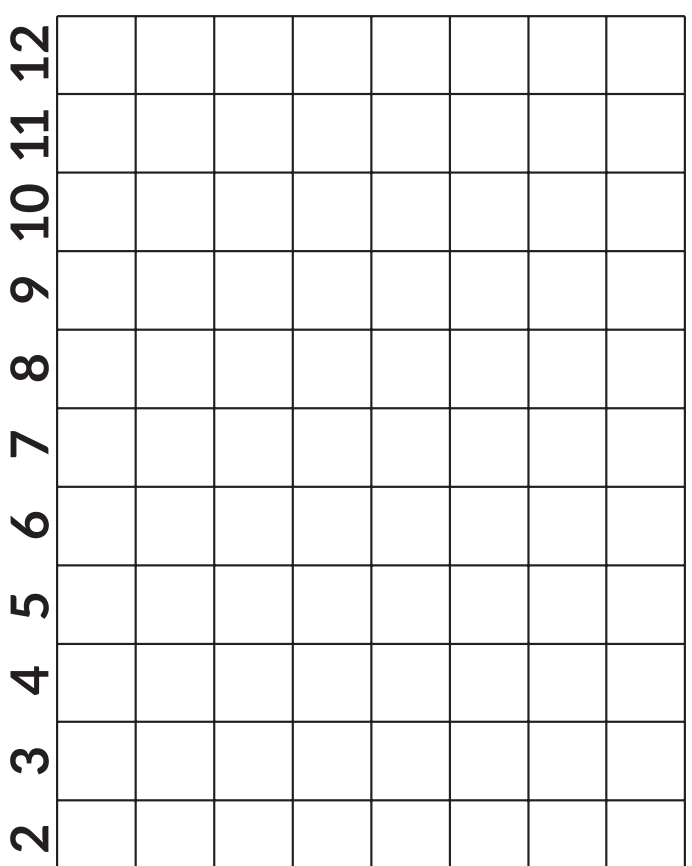
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No color development in any of the wells	The reaction mixture was not prepared correctly	Make sure to follow the directions when preparing the reaction mixture and re-assay
No color development in the standard wells	The standards were not diluted correctly	Make sure to follow the directions when preparing the standards and re-assay
Sample absorbance values are above the highest point in the standard curve	Phosphatidylcholine concentration was too high in the sample	Dilute sample 1:2 with 1X PC buffer and re-assay; *NOTE: Remember to account for the dilution factor when calculating phosphatidylcholine concentration*

References

1. Phillips, G.B. and Dodge, J.T. Composition of phospholipids and of phospholipid fatty acids of human plasma. *J. Lipid Res.* **8**, 676-681 (1967).
2. Jauhiainen, M. and Dolphin, P.J. Human plasma lecithin-cholesterol acyltransferase. An elucidation of the catalytic mechanism. *J. Biol. Chem.* **261(15)**, 7032-7043 (1986).
3. Matsuura, E. and Lopez, L.R. Are oxidized LDL/β2-glycoprotein I complexes pathogenic antigens in autoimmune-mediated atherosclerosis? *Clinical & Developmental Immunology* **11(2)**, 103-111 (2004).
4. Linsel-Nitschke, P. and Tall, A.R. HDL as a target in the treatment of atherosclerotic cardiovascular disease. *Nature Reviews Drug Discovery* **4**, 193-205 (2005).
5. Hojjati, M.R., Li, Z., Zhou, H., *et al.* Effect of myriocin on plasma sphingolipid metabolism and atherosclerosis in apoE-deficient mice. *J. Biol. Chem.* **280(11)**, 10284-10289 (2005).
6. Park, T.-S., Panek, R.L., Mueller, S.B., *et al.* Inhibition of sphingomyelin synthesis reduces atherogenesis in apolipoprotein E-knockout mice. *Circulation* **110**, 3465-3471 (2004).
7. Hojjati, M.R. and Jiang, X.-C. Rapid, specific, and sensitive measurements of plasma sphingomyelin and phosphatidylcholine. *J. Lipid Res.* **47(3)**, 673-676 (2006).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.