CREATIVE BIOMART®
Assay Kit

Phosphatidylcholine Assay Kit

Product Information

Cat.No. Kit-0662

Product Overview

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.

Storage

at -20°C. Stability ≥ 1 year

Shipping

Wet ice

Size

96 wells

Materials Required 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.

Technical Notes

• 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.
• The final volume of the assay is 110 µl in all wells.
• The incubation temperature is 37°C.

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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- 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.

Preparation

Reagent Preparation

1. PC Buffer (10X) -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 CaCl2) 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 -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 -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 -This vial contains a solution of phosphatidylcholine-specific phospholipase D. It is ready to use as supplied.
5. Phosphatidylcholine Standard -This vial contains a standard of phosphatidylcholine.
6. PC Detergent Solution -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.

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.

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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.

Standard Preparation
Add 1 ml of PC Detergent Solution to the Phosphatidylcholine Standard 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.
Table 1. Preparation of phosphatidylcholine standards:
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

Assay Protocol

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.
Table 2. Reaction Mixture preparation:
Reagent 50 wells 100 wells

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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
2. Phosphatidylcholine Standard Wells - add 10 µl of standard (tubes A-G) per well in the designated wells on the plate.
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

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.
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.
Phosphatidylcholine (mg/dl) = (CAV) - (y-intercept)/ Slope

Sensitivity

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 20-150 mg/dl phosphatidylcholine.

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