（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K796-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader(540-560 nm)

Detection range：0.003-0.500 mmol/L

Elabscience® Phosphatidylcholine (PC) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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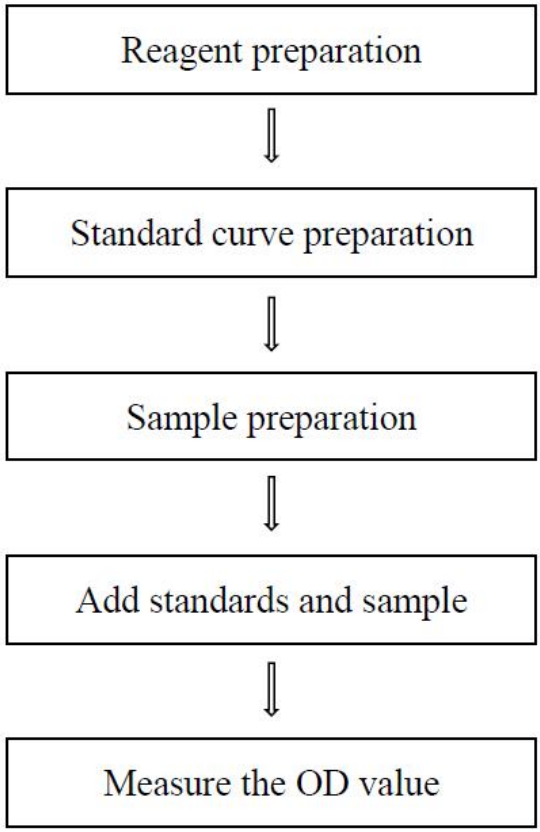
Assay summary



Intended use

This kit can be used to measure phosphatidylcholine content in serum, plasma, plant (animal) tissue and cell samples.

Detection principle

Phosphatidylcholine (PC) is an important component of eukaryotic cell membranes and a major source of second messenger diacylglycerol, phosphatidic acid, lysophosphatidic acid, and arachidonic acid, which can be further metabolized into other signaling molecules. It is widely found in a variety of foods, especially egg yolks, soy, fish, meat, and some seeds, and it can also be consumed through dietary supplements. PC provides structural stability and fluidity of cell membranes. In the liver, PC is involved in lipid synthesis and transport, helping to maintain lipid balance. As an important component of bile, PC promotes the digestion and absorption of lipid. PC supplements are used to intervene and treat liver diseases such as fatty liver disease and cirrhosis, and are used by athletes to improve physical fitness and endurance. Therefore, the detection of PC has important application value in medical diagnosis, disease management and health evaluation.

The detection principle of this kit: Enzyme catalyze the PC and chromogenic agent to produce, with maximum absorption at 550 nm, and the content of PC in the sample can be calculated by measuring the OD value at 550 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Buffer Solution	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months
Reagent 3	Catalyst	0.1 mL × 1 vial	0.2 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months shading light
Reagent 5	Chromogenic Agent	2.5 mL × 1 vial	5 mL × 1 vial	-20°C, 12 months shading light
Reagent 6	5 mmol/L Standard Solution	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months shading light
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (540-560 nm, optimum wavelength: 550 nm), Incubator (37℃)

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of catalyst working solution:

Before testing, please prepare sufficient catalyst working solution according to the test wells. For example, prepare 500 μL of catalyst working solution (mix well 495 μL of extraction solution and 5 μL of catalyst). The catalyst working solution should be prepared on spot. Keep the prepared solution on ice for detection, and use it up within 1 day.

③ The preparation of enzyme working solution:

Dissolve one vial of enzyme reagent with 250 μL of double distilled water, mix well to dissolve. Store at -20℃ for 2 days protected from light.

④ The preparation of measuring working solution:

For each well, prepare 40 μL of measuring working solution (mix well 36 μL of buffer solution, and 4 μL of enzyme working solution). The measuring working solution should be prepared on spot, keep measuring working solution on ice protected from light for detection and used it up within 1 day.

⑤ The preparation of 0.5 mmol/L standard solution:

Before testing, please prepare sufficient 0.5 mmol/L standard solution. For example, prepare 1000 μL of 0.5 mmol/L standard solution (mix well 900 μL of extraction solution and 100 μL of 5 mmol/L standard solution). The 0.5 mmol/L standard solution should be prepared on spot, keep the prepared solution on ice protected from light for detection and used it up within 1 day.

⑥ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 0.5 mmol/L standard with extraction solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.10, 0.15, 0.20, 0.30, 0.35, 0.40, 0.50 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑦	⑧	⑨
Concentration (mmol/L)	**0**	**0.10**	**0.15**	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**
0.5 mmol/L standard (μL)	0	40	60	80	120	140	160	200
Extraction solution (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

Cell sample:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse heart tissue homogenate	1
Mouse serum	1-3
Mouse plasma	1-3
Rat serum	1-3
Rat plasma	1-3
Human serum	1-3
10% Rose petal tissue homogenate	1
10% Corn tissue homogenate	1
10% Ligustrum leaf tissue homogenate	1
10% Garlic tissue homogenate	1
1×10^6 293T cell	1
1×10^6 Hela cell	1
1×10^6 Jurkat cell	1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Add 20 μL of sample to the corresponding wells.

② Add 50 μL of catalyst working solution to each well.

③ Mix fully with microplate reader for 5 s, then incubate at 37°C for 10 min.

④ Add 100 μL of buffer solution into each well.

⑤ Add 40 μL of measuring working solution into each well.

⑥ Add 40 μL of chromogenic agent into each well.

⑦ Mix fully with microplate reader for 5 s , measure the OD value of each well at 550 nm with microplate reader, as A_1.Incubate at 37°C for 30 min, measure the OD value of each well with microplate reader, as A_2.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean ΔA value of the blank (Standard #①) from all standard readings. This is the absoluted ΔA value.

3. Plot the standard curve by using absoluted ΔA value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) samples:

$$\text{PC content (mmol/L)} = (\Delta A_{550} - b) \div a \times f$$

2. Tissue sample:

$$\text{PC content (mmol/kg wet weight)} = (\Delta A_{550} - b) \div a \div \frac{m}{V} \times f$$

3. Cell sample:

$$\text{PC content (μmol/10^6)} = (\Delta A_{550} - b) \div a \div \frac{n}{V} \times f$$

[Note]

ΔA_{550}: The change OD value of the sample, $\Delta A_{550} = A_2 - A_1$

m: The tissue wet weight, g

n: The number of cell sample, 10^6

V: The volume of sample homogenate, Ml

f: Dilution factor of sample before tested

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.15	0.25	0.40
%CV	2.0	2.0	2.1

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.15	0.25	0.40
%CV	2.7	5.3	9.9

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 94.3%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.25	0.4
Observed Conc. (mmol/L)	0.14	0.24	0.38
Recovery rate (%)	94	94	95

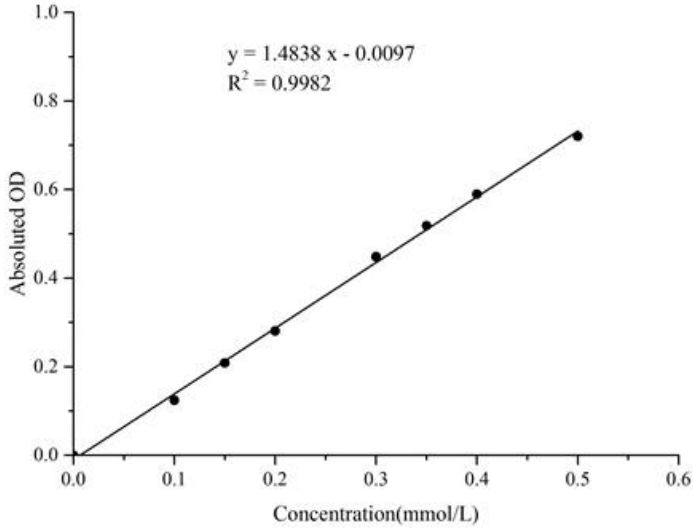
Sensitivity

The analytical sensitivity of the assay is 0.003 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**0.10**	**0.15**	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**
A_1	0.041	0.049	0.059	0.071	0.090	0.099	0.103	0.118
	0.041	0.048	0.057	0.069	0.088	0.095	0.104	0.117
A_2	0.041	0.172	0.267	0.351	0.546	0.621	0.692	0.841
	0.041	0.173	0.266	0.350	0.529	0.610	0.694	0.835
ΔA	0.000	0.123	0.208	0.280	0.456	0.522	0.589	0.723
	0.000	0.125	0.209	0.281	0.441	0.515	0.590	0.718
Average ΔA	0.000	0.124	0.209	0.281	0.449	0.519	0.590	0.721
Absolute ΔA	0	0.124	0.209	0.281	0.449	0.519	0.590	0.721



Appendix II Example Analysis

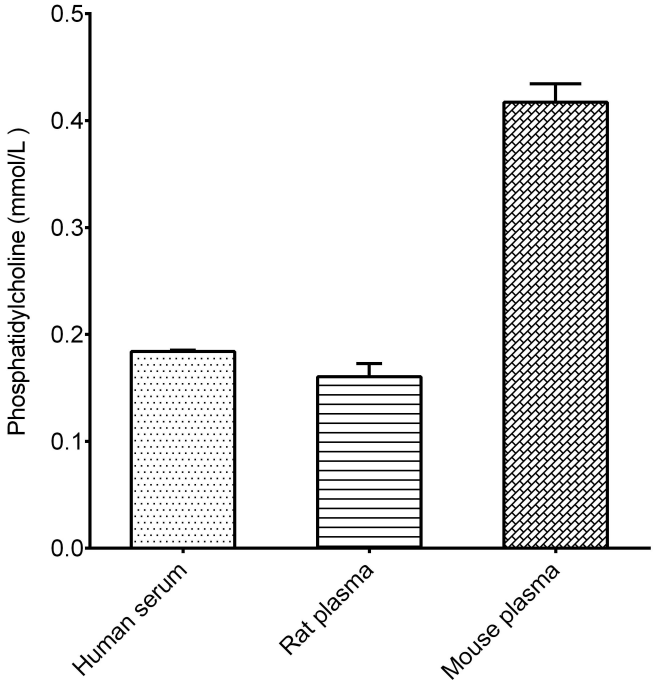
Example analysis：

Take 20 μL of human serum sample (diluent for 2 times) and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 1.4838 x - 0.0097, the A_1 value of the sample well is 0.060, the A_2 value of the sample well is 0.195, $\Delta A_{550} = A_2 - A_1 = 0.195 - 0.060 = 0.135$, and the calculation result is:

$$\text{PC content (mmol/L)} = (0.135 + 0.0097) \div 1.4838 \times 2 = 0.195 \text{ mmol/L}$$

Detect human serum (diluent for 2 times), rat plasma (diluent for 2 times) and mouse plasma (diluent for 2 times), according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.