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CheKine™ Micro Phosphatidylcholine (PC) Content Assay Kit

Cat #: KTB2261 Size: 96 T/48 S 192 T/96 S

	Micro Phosphatidylcholine (PC) Content Assay Kit		
REF	**Cat #:** KTB2261	LOT	**Lot #:** Refer to product label
	Detection range: 0.05-1 μmol/mL		**Sensitivity:** 0.05 μmol/mL
	Applicable samples: Animal and Plant Tissues, Cells, Plasma, Serum		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Phosphatidylcholine (PC) is a key structural component of eukaryotic cell membranes and a major precursor of important second messenger molecules such as diacylglycerol, phosphatidic acid, lysophosphatidic acid, and arachidonic acid, which can be further metabolized into a variety of intracellular signaling molecules. PC is widely distributed in animal and plant tissues and can be obtained from dietary sources such as egg yolk, soybean, and aquatic products, as well as through endogenous biosynthesis. PC not only maintains the structural integrity and fluidity of cell membranes but also plays a critical role in hepatic lipid synthesis and transport, as well as in the digestion and absorption of biliary lipids, and is thus closely associated with hepatic lipid metabolic homeostasis. Aberrant PC levels are correlated with pathological conditions including fatty liver disease and liver injury; therefore, the quantification of phosphatidylcholine serves as an important experimental indicator in studies of lipid metabolism and cell membrane physiology and pathology. CheKine™ Micro α-Ketoglutaric Acid (α-KG) Content Assay Kit can be used to detect biological samples such as animal and plant tissues, cells, plasma, serum or other liquid samples. In the kit, phospholipase D (PLD) catalyzes the hydrolysis of phosphatidylcholine to yield choline and phosphatidic acid (PA); choline is subsequently oxidized by choline oxidase (COD) to produce betaine and H_2O_2; the generated H_2O_2, under the catalysis of peroxidase (POD), couples with a chromogenic substrate to form a red quinone dye exhibiting maximum absorption at 550 nm, which is directly proportional to the phosphatidylcholine content in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	192 T	
Extraction Buffer	70 mL	70 mL×2	4℃
Reagent Ⅰ	40 μL	80 μL	-20℃
Reagent Ⅱ	6.5 mL	13 mL	4℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×2 vials	-20℃
Reagent Ⅳ	25 mL	50 mL	4℃, protected from light

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Standard	1 mL	1 mL	-20℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 550 nm
- 96-well plate or micro glass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water, PBS
- Homogenizer or mortar (for tissue samples), Ultrasonic disruptor (for cells)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅰ: Ready to use as supplied; Before opening, perform a low-speed centrifugation first; Place on ice during the experiment; The remaining reagent can also be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.

Reagent Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Working Reagent Ⅲ: Prepared before use;Take one and add 260 μL deionized water to it, then dissolve it fully for use. Place on ice during the experiment; The remaining reagent can also be stored at -20℃ for 4 weeks after aliquoting to avoid repeated freezing and thawing.

Reagent Ⅳ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Working Solution Ⅰ: Prepared before use; For each test/standard/blank well, prepare 50 μL Working Solution Ⅰ by mixing 0.5 μL Reagent Ⅰ and 49.5 μL Reagent Ⅱ. The volume ratio of preparation is 1: 99. Working Solution Ⅰ is freshly prepared.

Working Solution Ⅱ: Prepared before use; For each test/control/standard/blank well, prepare 180 μL Working Solution Ⅱ by mixing 2 μL Working Reagent Ⅲ and 178 μL Reagent Ⅳ. The volume ratio of preparation is 2: 178. Working Solution Ⅱ is freshly prepared.

Standard: Ready to use as supplied; Equilibrate to room temperature before use; 10 μmol/mL PC Standard; Store at -20℃. Using 10 μmol/mL PC Standard, prepare standard curve dilution as described in the table:

Num.	Standard (μL)	Extraction Buffer (μL)	Concentration (μmol/mL)
Std.1	50 μL of 10 μmol/mL PC	450	1
Std.2	40 μL of 10 μmol/mL	460	0.8
Std.3	30 μL of 10 μmol/mL	470	0.6
Std.4	40 μL of 10 μmol/mL	960	0.4
Std.5	500 μL of 0.4 μmol/mL	500	0.2
Std.6	500 μL of 0.2 μmol/mL	500	0.1
Std.7	500 μL of 0.1 μmol/mL	500	0.05
Blank	0	500	0

Notes: For each experiment, use freshly prepared diluted Standard solution. Diluted Standard solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, intact cells or aliquoted tissue blocks can be stored at -80℃ for 1 month. During measurement, the temperature and duration of thawing shall be controlled. When thawing

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at room temperature, sample thawing shall be completed within 4 h.

1. Animal or plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 12,000 g for 10 min at 4℃. Take supernatant for assay.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, centrifuge at 800 g for 2 min, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 10 min at 4℃. Take supernatant for assay.
3. Plasma, Serum: Tested directly..

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 550 nm. visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 96-well plate or micro glass cuvette)

Reagent	Test Well (μL)	Control Well (μL)	Blank Well (μL)	Standard Well (μL)
样本	20	20	0	0
Extraction Buffer	0	0	20	0
Standard	0	0	0	20
Working Solution Ⅰ	50	0	50	50
Reagent Ⅱ	0	50	0	0
Mix well, incubate at 37℃ for 10 min.				
Working Solution Ⅱ	180	180	180	180
Mix well, incubate at 37℃ for 20 min.				

3. Measure the absorbance value at 550 nm. The Test Well is marked as A_{Test}, the Contro Well is marked as $A_{Control}$, The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$,. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.05, increase the sample quantity appropriately. If ΔA_{Test} is greater than $\Delta A_{Standard}$ of 1 μmol/mL or A_{Test} is greater than 1.6, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve. The measured ΔA_{Test} is substituted into the equation to obtain the y-value (μmol/mL).

2. Calculation of the PC content

(1) Calculated by fresh weight of samples

$PC\ (\mu mol/g)=(V_{Sample}\times y)\div(W\times V_{Sample}\div V_{Total\ Sample})\times n$**$=y\div W\times n$**

(2) Calculated by number of cells

$PC\ (\mu mol/10^4)=(V_{Sample}\times y)\div(N\times V_{Sample}\div V_{Total\ Sample})\times n$**$=y\div N\times n$**

(3) Calculated by volume of liquid samples

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PC (μmol/mL)=(V_{Sample}×y)÷V_{Sample}×n=**y×n**

(4) Calculated by protein concentration

PC (μmol/mg prot)=(V_{Sample}×y)÷(V_{Sample}×Cpr)×n=**y÷Cpr×n**

V_{Sample}: Added the sample volume, 0.02 mL; $V_{Total\ Sample}$: Added Extraction Buffer volume, 1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; N: total number of cells, with the unit of 10^4 (e.g., if the cell count is $5×10^6$, N=500); n: the sample dilution factor.

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

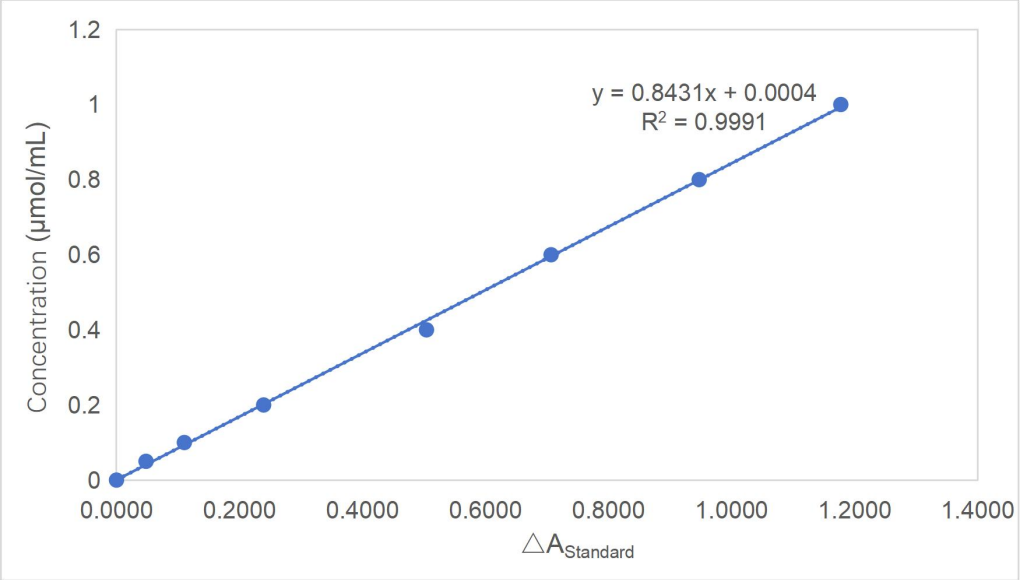


Figure 1. Standard Curve of PC assay.

Examples:

Test 0.106 g soaked soybean seed, following the above protocol and measured with the 96-well plate:

$\Delta A_{Test}=A_{Test}-A_{Control}$=1.0102-0.7306=0.2796, substituting into the standard curve, the calculated value of y=0.2361.

Calculated by fresh weight of samples: PC (μmol/g)=**y÷W×n**=2.227 μmol/g

Recommended Products

Catalog No.	Product Name
KTB2200	CheKine™ MicroTriglyceride (TG) Assay Kit
KTB2220	CheKine™ Micro Total Cholesterol (TC) Assay Kit
KTB2230	CheKine™ Micro Free Fat Acid (FFA) Content Assay Kit
KTB2231	CheKine™ Free Fatty Acid (FFA/NEFA) Content Assay Kit (Enzymatic Method)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

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