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

Catalog No：E-BC-K781-M

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

Measuring instrument：Microplate reader (405-415 nm)

Detection range：0.383-16.588 U/L

Elabscience® Phospholipase C (PLC) Activity 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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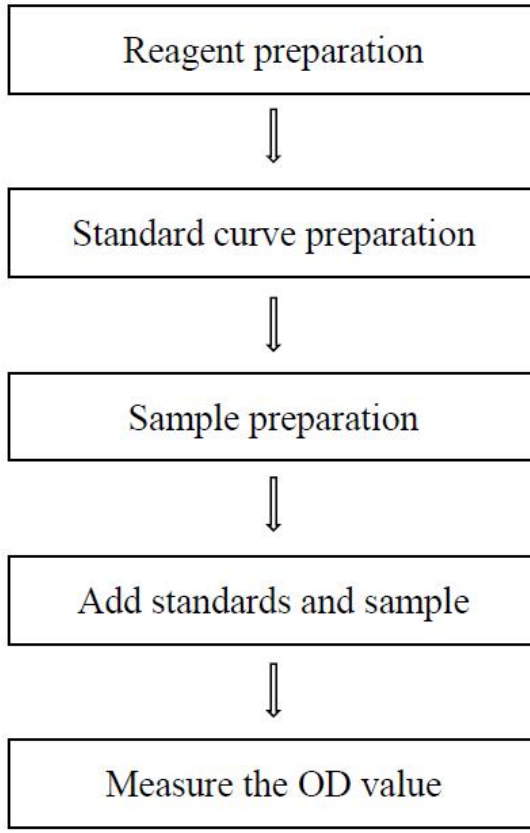
Assay summary



Intended use

This kit can be used to detect the phospholipase C (PLC) activity in serum, plasma, cell, animal and plant tissue samples.

Detection principle

Phospholipase C (PLC) is a family of enzymes that are widely distributed in animals, plants, and microorganisms. These enzymes catalyze the hydrolysis of phosphodiester bonds in membrane phospholipids, generating important second messenger molecules. PLC also plays a crucial role in neural signal transmission and neuroprotection, contributing to the understanding and potential treatment of neurodegenerative diseases. Furthermore, PLC is involved in insulin signal transduction and lipid metabolism, making it a significant focus in diabetes and obesity research. In recent years, phosphatidylcholine-specific phospholipase C (PC-PLC) has garnered increasing attention for its role in cell signal transduction. It has been confirmed that PC-PLC participates in the transduction of various apoptotic signaling pathways..

PLC catalyzes the substrate to produce chromogenic substance has maximum absorption at 410 nm, and the PLC enzyme activity is calculated by measuring OD value at 410 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL × 1 vial	20 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate	0.75 mL × 1 vial	1.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	5 mmol/L Standard Solution	1 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	48 wells	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 (405-415 nm, optimum wavelength: 410 nm), Incubator (37℃)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② 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 double distilled water and 100 μL of 5 mmol/L standard solution). Keep it on ice during use protected from light and used up within same day.

③ The preparation of working solution:

For each well, prepare 120 μL of working solution (mix well 108 μL of buffer solution and 12 μL of substrate). Keep it on ice during use protected from light and used up within same day.

④ The preparation of standard curve:

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

Dilute 0.5 mmol/L standard solution with double distilled water diluent 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	⑥	⑦	⑧
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
Double distilled water (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation:

Serum (plasma) samples: detect directly.

Tissue samples:

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

② Wash tissue in normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer 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.

⑤ Meanwhile, determine the protein concentration of supernatant (Animal tissue: E-BC-K318-M. Plant tissue: E-BC-K168-M).

Cell (adherent or suspension) samples:

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

② Wash cells with normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) 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.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
Mouse serum	2-5
Rat serum	2-5
Human serum	2-5
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	2-5
10% Mouse heart tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mung bean sprout tissue homogenate	2-5
10% Edamame tissue homogenate	2-5
10% Peanut tissue homogenate	2-5
10% Broccoli tissue homogenate	2-5
2×10^6 HL-60 cells	1
1.8×10^6 Hela cells	1
2×10^6 CHO cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

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

② Add 120 μL of working solution to each well.

③ Mix fully with microplate reader for 5 s and measure the OD value of sample well at 410 nm, as A_1. Incubate at 37°C for 30 min and measure the OD value of each well at 410 nm, as A_2. (The standard curve is fitted to the standard well in A_2 value.)

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean A_2 value of the blank (Standard #①) from all standard readings. This is the absoluted A_2 value.

3. Plot the standard curve by using A_2 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:

Definition: The amount of enzyme in 1 L serum or plasma per 1 min that produce 1 μmol product at 37 °C is defined as 1 unit.

$$\underset{(U/L)}{\text{PLC activity}} = (\Delta A_{410} - b) \div a \div T \times f \times 1000$$

2. Tissue and cell samples:

Definition: The amount of enzyme in 1 g tissue or cell protein per 1 min that produce 1 μmol product at 37 °C is defined as 1 unit.

$$\underset{(U/gprot)}{\text{PLC activity}} = (\Delta A_{410} - b) \div a \div T \times f \div C_{pr} \times 1000$$

[Note]

ΔA_{410}: $\Delta A_{410} = A_2 - A_1$.

T: Reaction time, 30 min.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

1000: Unit conversion, 1 mmol/L= 1000 μmol/L.

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 (U/L)	5.00	10.00	15.00
%CV	1.8	2.0	3.0

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 (U/L)	5.00	10.00	15.00
%CV	6.7	7.8	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 100.3%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/L)	5	10	15
Observed Conc. (U/L)	5.0	10.0	15.2
Recovery rate (%)	100	100	101

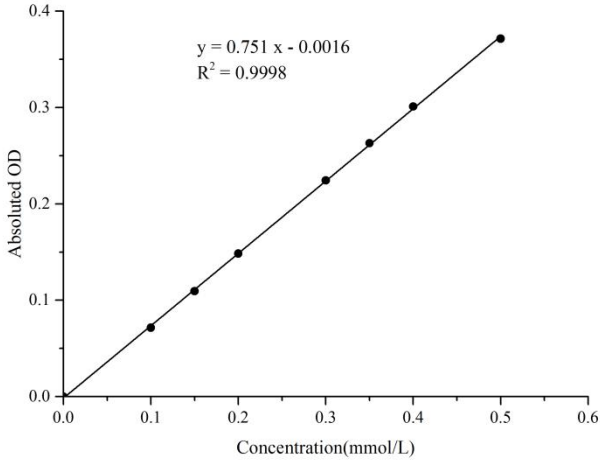
Sensitivity

The analytical sensitivity of the assay is 0.383 U/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
OD value	0.101	0.175	0.216	0.255	0.330	0.372	0.406	0.479
	0.109	0.178	0.213	0.252	0.329	0.364	0.406	0.474
Average OD value	0.105	0.177	0.215	0.254	0.330	0.368	0.406	0.477
Absoluted OD value	0	0.072	0.110	0.149	0.225	0.263	0.301	0.372



Appendix II Example Analysis

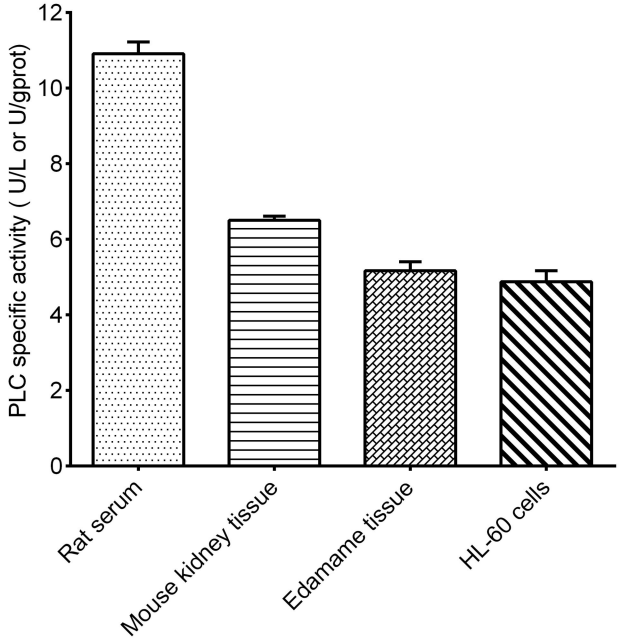
Example analysis:

Take 20 μL of rat serum which dilute for 2 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.751 x - 0.0016, the A_1 of the sample well is 0.417, the A_2 of the sample well is 0.585, $\Delta A_{410} = A_2 - A_1 = 0.585 - 0.471 = 0.114$, and the calculation result is:

$$\text{PLC activity (U/L)} = (0.114 + 0.0016) \div 0.751 \div 30 \times 2 \times 1000 = 10.26 \text{ U/L}$$

Detect rat serum (dilute for 2 times), 10% mouse kidney tissue homogenate (the concentration of protein is 9.635 gprot/L, dilute for 2 times), 10% edamame tissue homogenate (the concentration of protein is 3.201 gprot/L, dilute for 2 times) and 1.8×10^6 HL-60 cells (the concentration of protein is 1.444 gprot/L) 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.