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

Catalog No：E-BC-K783-M

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

Measuring instrument：Microplate reader（540-560 nm）

Detection range：0.68-16.73 U/L

Elabscience® Phospholipase D (PLD) 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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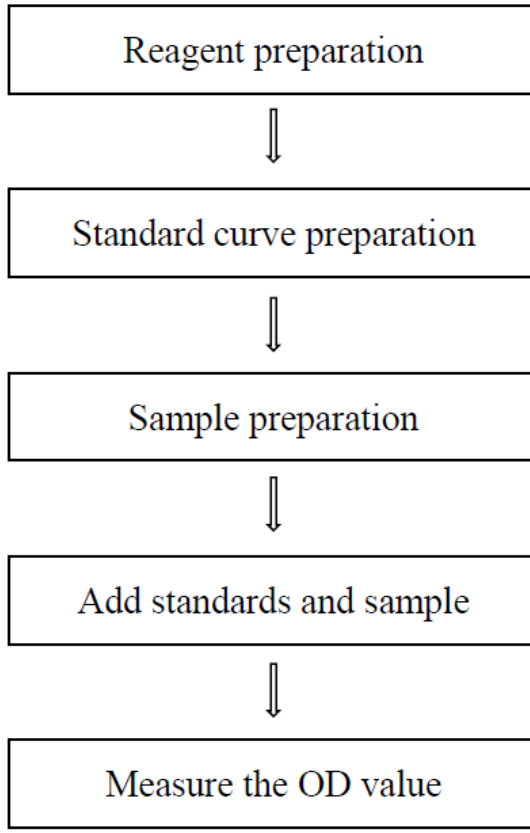
Assay summary



Intended use

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

Detection principle

Phospholipase D (PLD) exists widely in natural plants and animals, and PLD plays a key role in phospholipase metabolism. PLD hydrolyzes phosphodiester bonds of glycerol phospholipids to produce phosphatidylic acid and nitrogenous base. Abnormal PLD activity and expression are associated with Alzheimer's disease, stroke, cancer, and other brain diseases.

The detection principle of this kit is that PLD catalyze the substrate and the chromogenic agent to product colored substance, which has the maximum absorption at the wavelength of 550 nm. By measuring the OD value at 550 nm, PLD activity in the sample is calculated according to the standard curve.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	50 mL ×1 vial	50 mL ×2 vials	-20 ℃, 12 months
Reagent 2	Substrate	1.5 mL ×1 vial	1.5 mL ×2 vials	-20 ℃, 12 months, shading light
Reagent 3	Catalyst	Powder ×1 vial	Powder ×2 vials	-20 ℃, 12 months, shading light
Reagent 4	Chromogenic Agent	2.5 mL ×1 vial	5 mL ×1 vial	-20 ℃, 12 months, shading light
Reagent 5	10 mmol/L Standard Solution	0.75 mL ×1 vial	1.5 mL ×1 vial	-20 ℃, 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：

Dissolve one vial of catalyst with 450 μL of double distilled water, mix well to dissolve. Store at -20 ℃ for 7 days protected from light.

③ The preparation of measuring working solution:

For each well, prepare 180 μL of measuring working solution (mix well 150 μL of buffer solution, 24 μL of substrate and 6 μL of catalyst working solution). Keep it on ice during use and used up within same day.

④ 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 950 μL of buffer solution and 50 μL of 10 mmol/L standard solution). Keep it on ice during use and used up within same day.

⑤ 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 buffer solution 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
Buffer solution (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 cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL buffer solution 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).

② Dilution of sample

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

Sample type	Dilution factor
Mouse serum	1
Mouse plasma	1
Human serum	1
Rabbit plasma	1
10% Mouse liver tissue homogenate	2-4
10% Mouse kidney tissue homogenate	2-4
10% Mouse heart tissue homogenate	2-4
10% Rat liver tissue homogenate	2-4
10% Peanut tissue homogenate	1-3
10% Edamame tissue homogenate	1-3
10% Corn seed tissue homogenate	1-3

Note: The diluent is buffer solution. 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 180 μL of measuring working solution to each well.

③ Add 40 μL of chromogenic agent to each well.

④ Mix fully with microplate reader for 5 s and measure the OD value of each well at 550 nm, as A_1.

④ Incubate at 37 ℃ for 30 min and measure the OD value of each well at 550 nm, as A_2. $\Delta A = A_2 - A_1$.

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:

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.

$$\text{PLD specific activity (U/L)} = (\Delta A_{550} - b) \div a \div T \times f \times 1000$$

Tissue sample:

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

$$\text{PLD specific activity (U/gprot)} = (\Delta A_{550} - b) \div a \div T \times f \div C_{pr} \times 1000$$

[Note]

ΔA_{550}: $\Delta A_{550} = \Delta A_{sanple} - \Delta A_{blank}$.

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

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

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	1.7	2.2	2.8

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	1.8	6.1	9.5

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 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/L)	5	10	15
Observed Conc. (U/L)	4.9	9.9	15.0
Recovery rate (%)	98	99	100

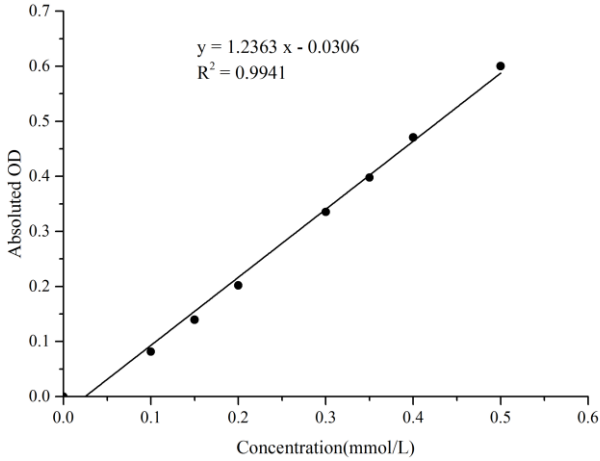
Sensitivity

The analytical sensitivity of the assay is 0.68 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**
A_1	0.066	0.164	0.200	0.240	0.293	0.318	0.341	0.390
	0.068	0.159	0.200	0.238	0.286	0.316	0.340	0.381
A_2	0.077	0.257	0.349	0.454	0.642	0.731	0.830	1.005
	0.078	0.250	0.351	0.449	0.629	0.720	0.814	0.988
ΔA	0.011	0.093	0.149	0.214	0.349	0.413	0.489	0.615
	0.010	0.091	0.151	0.211	0.343	0.404	0.474	0.607
Average ΔA	0.011	0.092	0.150	0.213	0.346	0.409	0.482	0.611
Absoluted ΔA	0	0.082	0.140	0.202	0.336	0.398	0.471	0.601



Appendix II Example Analysis

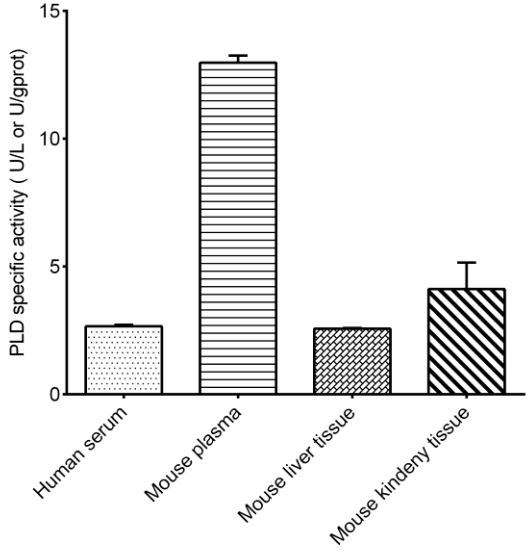
Example analysis:

Take 20 μL of 10% mouse liver tissue homogenate which dilute for 2 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 1.2363 x - 0.0306, the A_1 of the sample well is 0.096, the A_2 of the sample well is 0.679, $\Delta A_{sample} = A_2 - A_1 = 0.679 - 0.096 = 0.583$, the A_1 of the blank well is 0.066, the A_2 of the blank well is 0.077, $\Delta A_{blank} = A_2 - A_1 = 0.077 - 0.066 = 0.011$, $\Delta A_{550} = \Delta A_{sample} - \Delta A_{blank} = 0.583 - 0.011 = 0.572$, the concentration of protein is 12.54 gprot/L, and the calculation result is:

$$\text{PLD activity (U/gprot)} = (0.572 + 0.0306) \div 1.2363 \div 30 \times 2 \div 12.54 \times 1000 = 2.59 \text{ U/gprot}$$

Detect human serum, mouse plasma, 10% mouse liver tissue homogenate (the concentration of protein is 12.54 gprot/L, dilute for 2 times) and 10% mouse kindey tissue homogenate (the concentration of protein is 10.47 gprot/L, dilute 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.