Phospholipase D (PLD) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Cat No: BC2410

Size: 50T/48S

Components:

Extract solution I: Liquid 60 mL×1. Storage at 2-8℃.

Extract solution II: Liquid 0.6 mL×1. Storage at -20℃. The reagent is easy to volatilize. After use, it needs to be quickly sealed and wrapped with a sealing film.

Reagent I: Liquid 70 mL×1. Storage at 2-8℃.

Reagent II: Liquid 10 mL×1. Storage at 2-8℃.

Reagent III: Powder×2, Before use, add 3 mL of absolute ethanol to dissolve completely; after the reagent is packed, store it at 2-8℃, and avoid freeze and thaw repeatedly.

Reagent IV: liquid 45 mL×1, stored at 2-8℃.

Standard: liquid 1 mL×1, Storage at 2-8℃. Choline solution with a concentration of 50μmol/mL, Before use, dilute the standard solution 100 times to 500nmol/mL standard solution with Reagent 1 (you can take 10μL of 50μmol/mL choline solution and add 990μL of distilled water to mix).

Product Description:

Phospholipase D catalyzes the hydrolysis of phosphatidyl diester bond at the end of phosphatidylcholine to form phosphatidylic acid and choline. Choline is catalyzed by choline oxidase to form betaine and hydrogen peroxide. Hydrogen peroxide oxidizes 4-aminoantipyrine and bisphenol to pink substance under the action of catalase. There is a characteristic absorption peak at 500 nm.

Reagents and Equipment Required but Not Provided:

Balance, mortar/cell ultrasonic crusher, ultra freezing centrifuge, Visible spectrophotometer, 1 mL glass cuvette, Constant temperature water bath, Anhydrous ethanol, Ice.

Procedure:

I. Sample preparation:

1. Tissue: add the extract solution according to the ratio of mass(g): volume of extract solution(mL):

1:5~10 (it is recommended to weigh about 0.1g and add 0.99 mL of extract solution I and 0.01 mL of extract solution II), homogenize in ice bath and centrifuge at 4℃, 10000g for 5 min, then centrifuge all the supernatant at 4℃,100 000g for 30 min, discard the supernatant and dissolve the precipitate in 1 mL of Reagent I.

2. Cells: according to the number of cells (10^4): the volume of extract solution (mL) is

500-1000:1 (it

is recommended to add 0.99 mL extract solution I and 0.01 mL of extract solution II to 5 million cells), ice bath ultrasonic wave is used to crush cells (power 300W, ultrasonic 3s, interval 7s, total time 3 min); then centrifugation at 4℃ and 10000 g for 5 min, then centrifuge all the supernatant at 4℃ and 100 000 g for 30 min, discard the supernatant, take the precipitate and dissolve in 1 mL of Reagent I.

3. Serum: direct determination.

II. Determination procedure:

1.Preheat spectrophotometer for more than 30 minutes, adjust the wavelength to 500 nm, set the counter to zero with distilled water.

2.Sample determination (adding the following reagents to the EP tube):

Reagent name	Blank tube(A_B)	Standard tube(A_S)	Test tube(A_T)
Reagent I（μL）	100	-	-
Reagent II（μL）	150	150	150
Standard（μL）	-	100	-
Sample（μL）	-	-	100
Reagent III（μL）	100	100	100
Mix well and react at 30℃ for 30 min, boiling water bath for 10 minutes, open the lid, natural cooling for 5 minutes.			
Reagent IV（μL）	700	700	700
After reacting at 30℃ for 30 min, measure the absorbance at 500 nm, and record it as A_B、A_S and A_T respectively. $\Delta A_T=A_T-A_B$, $\Delta A_S=A_S-A_B$, The standard curve and blank tube only need to be measured 1-2 times.			

III. Calculation:

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme activity catalyzes the hydrolysis of phosphatidylcholine to produce 1 nmol choline per minutes every milligram protein.

PLD activity (U/mg prot) = $\Delta A_T \div \Delta A_S \times C_S \times Vr \div (Cpr \times Vr) \div T = 16.7 \times \Delta A_T \div \Delta A_S \div Cpr$.

2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme activity catalyzes the hydrolysis of phosphatidylcholine to produce 1 nmol choline per minutes every gram tissue.

PLD activity (U/g weight) = $\Delta A_T \div \Delta A_S \times C_S \times Vr \div W \div T = 16.7 \times \Delta A_T \div \Delta A_S \div W$.

3) Cells number:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme activity catalyzes the hydrolysis of phosphatidylcholine to produce 1 nmol choline per minutes every the cells number of 10^4.

PLD activity (nmol/min/10^4 cell) = $\Delta A_T \div \Delta A_S \times C_S \times Vr \div N \div T = 16.7 \times \Delta A_T \div \Delta A_S \div N$.

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4) Liquid volume:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme activity catalyzes the hydrolysis of phosphatidylcholine to produce 1 nmol choline per minutes every milliliter serum.

PLD activity (nmol/min/mL) = $\Delta A_T \div \Delta A_S \times C_S \times V_B \div V_B \div T = 16.7 \times \Delta A_T \div \Delta A_S$.

C_S: standard concentration, 500 nmol/mL; Vr: Total volume of extract (Extract solution I + Extract solution II),1mL; V_B: Serum (plasma) volume, 0.1mL; Cpr: sample protein concentration, mg/mL; W: sample mass, g; T: reaction time, 30 min; N : number of cells, 10^4.

Note:

1. After color development, if there is precipitation, centrifuge at 8000g and 25℃ for 5 min, and then take the supernatant for determination.
2. The absorbance value should not exceed 1, otherwise the enzyme solution should be diluted with reagent I and multiplied by the dilution multiple in the calculation formula.

Related Products：

BC2420/BC2425	Phospholipase C(PLC) Activity Assay Kit
BC2430/BC2435	Phospholipase A2(PLA2) Activity Assay Kit

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