Phospholipase C (PLC) Activity Assay Kit

Note: The reagents in this product are subject to change, please note and follow these instructions.
Operation Equipment: Spectrophotometer/microplate reader
Cat No: BC2425
Size: 100T/96S

Components:
Extract solution I: Liquid 110 mL×1. Storage at 2-8℃.
Extract solution II: Liquid 0.6 mL×2. Storage at -20℃. Reagent is volatile, screw the cap quickly after use and wrap the sealing film.
Reagent I: Liquid 110 mL×1. Storage at 2-8℃.
Reagent II: Liquid 15 mL×1. Storage at 2-8℃.
Reagent III: Liquid 10 mL×1. Storage at 2-8℃.
Standard: Liquid 1 mL×1×1. Store at 2-8℃. 5 μmol/mL p-nitrophenol solution.
Preparation of extraction solution: Before use, mix extraction solution I and extraction solution II according to the volume 1:1, do not mix all at once, and match as much as needed for the experiment.

Product Description:
Phospholipase C (EC 3.1.4.3) is a kind of lipid hydrolase that hydrolyzes glycerophosphate bond at C3 site of glycerophosphate. It is widely existed in the tissues and cells of microorganisms, animals and plants, and plays an important role in cell metabolism, cell transmission, growth and development.
Phospholipase C catalyzes the hydrolysis of NPPC to produce p-nitrophenol with a characteristic absorption peak at 410 nm.

Reagents and Equipment Required but Not Provided:
Visible spectrophotometer/ microplate reader, water bath, ultra fast freezing centrifuge, adjustable pipette, balance, micro glass cuvette/96-well plate, mortar/homogenizer/cell ultrasonicator, distilled water, ice.

Procedure:
I. Sample preparation:
1. Tissue: add the extract 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 1 ml extract solution), homogenize in ice bath and centrifuge at 4℃ and 10000g for 5 min, then centrifuge all the supernatant at 4℃ and 100000g 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 1 ml extract solution 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, centrifugation at 4℃ and 100000 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/ microplate reader for 30 minutes, adjust wavelength to 410 nm, spectrophotometer set zero with distilled water.

2.Standard solution dilution: 5μmol/mL p-nitrophenol solution is diluted to 1.25、0.625、0.3125、0.15625、0.078、0.039、0.02、0.01μmol/mL with distilled water..

3. Operation table

Reagent name (μL)	Blank tube(B)	Test tube(T)	Standard tube(S)
Standard	-	-	20
Sample	-	20	-
Reagent I	20	-	-
Reagent II	100	100	100
Mix well and react at 37℃ for 30 min			-
Reagent III（μL）	80	80	80
Mix well, measure the absorbance at 410 nm, and record it as A_B and A_T respectively, $\Delta A = A_T - A_B$, $\Delta As = A_S - A_B$. Standard curves and blank tubes only need to be done 1-2 times。			

III. Calculation:

1. Standard curve

According to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔAs (y, ΔAs), establish a standard curve. According to the standard curve, bring ΔA (y, ΔA) into the formula to calculate the sample concentration (x, μmol/mL).

2. **Calculation**

1) Tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that hydrolysis of NPPC produces 1 nmol of p-nitrophenol every milligram of protein per minute.

PLC Activity (U/mg prot) = x×Vs÷(Vs×Cpr) ÷T = 0.033× x÷ Cpr

2) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount enzyme that hydrolysis of NPPC produces 1 nmol of p-nitrophenol every gram of tissue per minute.

PLC Activity (U/g weight) = x×Ve÷W÷T = 0.033× x÷W

3) Liquid

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that hydrolysis of NPPC produces 1 nmol of p-nitrophenol every milliliter of liquid sample per minute.

PLC Activity (U/mL) = x÷T = 0.033× x

4) Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that

hydrolysis of NPPC produces 1 nmol of p-nitrophenol every 10^4 cells or bacteria per minute at 40℃.

PLC Activity (U/10^4 cell) = x×Ve÷N÷T= 0.033× x÷N

Vs: Sample volume (mL), 0.1 mL;
Ve: Volume of Reagent I added as extract, 1 mL;
Cpr: Supernatant sample protein concentration (mg/mL);
T: Reaction time (min), 30 minutes;
W: Sample weight, g;
N: Number of cells 10 thousand as unit.

Related Products:

BC2410/BC2415	Phospholipase D (PLD) Activity Assay Kit
BC2430/BC2435	Phospholipase A2(PLA2) Activity Assay Kit