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5'- Nucleotidase (5'-NT) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: AK0043

Size: 50T/24S

Components:

Extracting solution：Liquid 30 mL×1. Storage at -20℃ .

Reagent I ： Powder ×2. Storage at -20℃ .

Reagent Ⅱ: Liquid 12 mL×2. Storage at 4℃ .

Reagent Ⅲ: Liquid 30 mL×1. Storage at 4℃ .

Reagent Ⅳ: Liquid 25 mL×1. Storage at 4℃ .

Reagent Ⅴ: Powder ×1. Storage at 4℃ . Before use, add 12 mL of distilled water, fully dissolve, and store the unused reagent at 4℃ for two weeks.

Reagent Ⅵ: Powder×1. Storage at 4℃ . Before use, add 12 mL of distilled water, fully dissolve, and store the unused reagent at 4℃ for two weeks.

Reagent Ⅶ: Liquid 12 mL×1. Storage at room temperature.

Standard solution: Powder×1. Storage at 4℃ . 8 mg of phosphorus standard. Before use, 4.6 mL of Reagent IV is added to prepare a standard solution of 10 μmol/mL. After dissolution, the solution is stored at 4℃ .

Working solution： Reagent Ⅰ are added into a bottle of Reagent Ⅱ to dissolve completely; the unused reagents ar e packed and stored at - 20℃ for one week, and prepare when the solution will be used.

Preparation of phosphorus determination reagent: prepare according to the proportion of H_2O: Reagent Ⅴ: Reagent Ⅵ: Reagent Ⅶ = 2:1:1:1, and the prepared phosphorus determination reagent shall be light yellow. If colorless, reagent fails; if blue, it is phosphorus pollution (please use how much to match as required).

Product Description:

5'-nucleotidase (5'-NT) is a kind of hydrolase with low substrate specificity, which can act on a variety of nucleotides. It widely exists in various plant, animal tissues, serum and plasma. 5'-NT is a special phosphate hydrolase, which acts on nucleoside-5'-phosphate such as AMP (adenosine-5'-phosphate or adenosine monophosphate) to produce inorganic phosphate and nucleoside. The activity of 5'-NT can be calculated by determining the content of inorganic phosphorus.

Reagents and Equipment Required but Not Provided:

Balance, Spectrophotometer, desktop centrifuge, cryogenic centrifuge, constant temperature water bath/constant temperature incubator, 1 mL glass cuvette, transferpettor, mortar/homogenizer, ice, distilled water.

Procedure:

I. Sample preparation (the sample size can be adjusted appropriately, and the specific proportion can be referred to the literature):

1. Tissue: The ratio of mass (g): volume of Extracting solution (mL) is 1:5- 10 (it is recommended to weigh about 0.1 g and add 1 mL of Extracting solution), homogenize on ice, centrifuge at 4℃, 15000 g for 10 min, and place the supernatant on ice for testing.

2. Cells: The ratio of the number of cells (10^4): the volume of distilled water (mL) is 500- 1000:1 (it is recommended to add 1 mL distilled water to 5 million cells), the cells are broken by ice bath ultrasonic wave (power 300W, ultrasonic 3s, interval 7s, total time 3 min); then the cells are centrifuged at 4℃, 15000g for 10 min, and the supernatant is put on ice for testing.

3. Liquid: direct detection.

II. Determination procedure:

1. Preheat the Spectrophotometer for 30 minutes, adjust the wavelength to 660 nm, set zero with distilled water.
2. The starch standard solution is diluted with Reagent Ⅳ to 0.48、0.24、0.12、0.06、0.03、0.015 μmol/mL.
3. Add reagents with the following list: (Operate in 1.5 mL EP tube)

(1) Enzymatic reaction

Reagent (μL)	Test tube	Control tube
Sample	100	100
Working solution	400	
ortex mixing, 37℃ (mammalian) or 25℃ (plant and other) reaction for 30 min		
Reagent Ⅲ	500	500
Working solution	-	400
Vortex mixing, 25℃, 8000 rpm centrifugation for 10 min, take the supernatant for color reaction		

(2) Color reaction

Reagent (μL)	Test tube	Control tube	Standard tube	Blank tube
Supernatant	400	400	-	-
Standard	-	-	400	-
Reagent Ⅳ	-	-	-	400
Phosphorus determination reagent	800	800	800	800
Vortex mixing, 40℃ color for 10 min; take 1 mL of reaction solution in 1 mL glass cuvette, measure the absorbance value A at 660 nm, respectively record as A_T, A_C, A_S, A_B, calculate $\Delta A_S = A_S - A_B$, $\Delta A_T = A_T - A_C$(blank tube only needs to measure 1-2 times).				

III. Calculation:

1. Drawing of standard curve: draw the standard curve with ΔA_S as y axis, and the standard solution concentration as x axis, and get the standard equation y=kx+b, and bring the ΔA into the equation to get

x(μmol/mL).

2. Calculation of 5'-NT activity

(1) Calculated according to protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol inorganic phosphorus per minute every milligram tissue protein in the reaction system.

5'-NT activity (U/mg prot) = $x \times V_{RT} \div (V_S \times Cpr) \div T \times 10^3 = 333.3 \times x \div Cpr$

(2) Calculated by sample mass

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol inorganic phosphorus per minute every milligram tissue in the reaction system.

5'-NT activity (U/g mass) = $x \times V_{RT} \div (W \times V_S \div V_{ST}) \div T \times 10^3 = 333.3 \times x \div W$

(3) Calculated by cell number

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol inorganic phosphorus per minute every 10^4 cells in the reaction system.

5'-NT activity (U/10^4 cell) = $x \times V_{RT} \div (\text{cell number} \times V_S \div V_{ST}) \div T \times 10^3 = 333.3 \times x \div$ cell number (4) Calculated according to volume of liquid:

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol inorganic phosphorus per minute every milliliter liquid in the reaction system.

5'-NT activity (U/mL) = $x \times V_{RT} \div V_S \div T \times 10^3 = 333.3 \times x$

V_S : sample volume added in enzymatic reaction, 0.1 mL; V_{RT} : total volume of enzymatic reaction, 1 mL; V_{ST} : volume added in Extracting solution, 1 mL; W: sample mass, g; Cpr: sample protein concentration, mg/mL; cell number: in tens of thousands; T: enzymatic reaction time, 30 min; 10^3 : unit conversion, 1 μmol = 10^3 nmol.

Note:

When the absorbance value is greater than 1 or ΔA is greater than 1, it is suggested that the sample be diluted with Reagent Ⅳ before determination.

Experimental example:

1. Take 0.1 g of mouse liver, and then take the sample for treatment. take the supernatant and operate according to the determination steps. Calculate: $\Delta A_T = A_T - A_C = 0.723 - 0.534 = 0.189$, and bring the standard curve y=2.3928x+0.0165, calculate x=0.0721, calculate the enzyme activity according to the sample quality:

5'-NT activity (U/g mass) =333.3×x÷W=333.3×0.0721÷0. 1=240.31 U/g mass.

2. Take 0.1 g of barnyard grass for sample treatment. take the supernatant and operate according to the determination steps. Calculate: $\Delta A_T = A_T - A_C = 0.367 - 0.281 = 0.086$, and bring in the standard curve y=2.3928x+0.0165, calculate x=0.0290, calculate the enzyme activity according to the sample quality: 5'-NT activity (U/g mass) =333.3×x÷W=333.3×0.0290÷0. 1=96.657 U/g mass.

Related products:

AK0337/AK0336 Creatine Kinase (CK) Activity Assay Kit

AK0065/AK0064 Pyrroline-5-carboxylic Acid Synthase (P5CS) Activity Assay Kit

AK0319/AK0318 Laccase Activity Assay Kit

AK0246/AK0245 Isocitrate Lyase (ICL) Activity Assay Kit

AK0149/AK0148 Acetate Kinase (ACK) Activity Assay Kit