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: NA0262

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 are 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) = $x \times V_{RT} \div (\text{cell number} \times V_S \div V_{ST}) \div T \times 10^3 = 333.3 \times x \div \text{cell number}$

(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) =$333.3 \times x \div W = 333.3 \times 0.0721 \div 0.1 = 240.31$ 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) =$333.3 \times x \div W = 333.3 \times 0.0290 \div 0.1 = 96.657$ U/g mass.

Related products:

NA0784/NA0543 Creatine Kinase (CK) Activity Assay Kit

NA0288/NA0287 Pyrroline-5-carboxylic Acid Synthase (P5CS) Activity Assay Kit

NA0738/NA0495 Laccase Activity Assay Kit
NA0724/NA0482 Isocitrate Lyase (ICL) Activity Assay Kit
NA0640/NA0377 Acetate Kinase (ACK) Activity Assay Kit