

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: NA0261

Size: 100T/48S

Components:

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

Reagent I: Powder ×2. Storage at -20°C.

Reagent II: Liquid 5 mL×2. Storage at 4°C.

Reagent III: Liquid 12 mL×1. Storage at 4°C.

Reagent IV: Liquid 5 mL×1. Storage at 4°C.

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

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

Reagent VII: Liquid 4 mL×1. Storage at room temperature.

Standard solution: Powder×1. Storage at 4°C. 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°C.

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

Preparation of phosphorus determination reagent: prepare according to the proportion of H₂O: 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/Microplate reader, desktop centrifuge, cryogenic centrifuge, constant temperature water bath/constant temperature incubator, micro glass cuvette/96 well plate, 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 0.05 g and add 0.5 mL of Extracting solution), homogenize on ice, centrifuge at 4°C, 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 0.5 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°C, 15000g for 10 min, and the supernatant is put on ice for testing.

3. Liquid: direct detection.

II. Determination procedure:

1. Preheat the Spectrophotometer/Microplate reader 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 0.96、0.48、0.24、0.12、0.06、0.03、0.015 $\mu\text{mol/mL}$.

3. Add reagents with the following list: (Operate in 1.5 mL EP tube)

(1) Enzymatic reaction

Reagent (μL)	Test tube	Control tube
Sample	20	20
Working solution	80	
ortex mixing, 37°C (mammalian) or 25°C (plant and other) reaction for 30 min		
Reagent III	100	100
Working solution	-	80
Vortex mixing, 25°C, 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	80	80	-	-
Standard	-	-	80	-
Reagent IV	-	-	-	80
Phosphorus determination reagent	160	160	160	160

Vortex mixing, 40°C color for 10 min; take 200 μL of reaction solution in micro glass cuvette/96 well plate, 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 $x(\mu\text{mol/mL})$.

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.

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

(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.

$$5'\text{-NT activity (U/g mass)} = x \times V_{RT} \div (W \times V_S \div V_{ST}) \div T \times 10^3 = 166.67 \times x \div W$$

(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.

$$5'\text{-NT activity (U/10}^4 \text{ cell)} = x \times V_{RT} \div (\text{cell number} \times V_S \div V_{ST}) \div T \times 10^3 = 166.67 \times x \div \text{cell number}$$

(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.

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

V_S : sample volume added in enzymatic reaction, 0.02 mL; V_{RT} : total volume of enzymatic reaction, 0.2 mL; V_{ST} : volume added in Extracting solution, 0.5 mL; W : sample mass, g; C_{pr} : sample protein concentration, mg/mL; cell number: in tens of thousands; T : enzymatic reaction time, 30 min; 10^3 : unit conversion, $1 \mu\text{mol} = 10^3 \text{ nmol}$.

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 0.1g of mouse liver, and then take the sample for treatment. take the supernatant and operate according to the determination steps. Calculate with 96 well plate the $\Delta A_T = A_T - A_C = 0.449 - 0.334 = 0.115$, and bring the standard curve $y = 1.5514x + 0.0038$, calculate $x = 0.0717$, calculate the enzyme activity according to the sample quality:

$$5'\text{-NT activity (U/g mass)} = 333.3 \times x \div W = 333.3 \times 0.0717 \div 0.1 = 238.98 \text{ U/g mass.}$$

2. Take 0.1 g of barnyard grass for sample treatment. take the supernatant and operate according to the determination steps. Calculate with 96 well plate $\Delta A_T = A_T - A_C = 0.245 - 0.196 = 0.049$, and bring in the standard curve $y = 1.5514x + 0.0038$, calculate $x = 0.0291$, calculate the enzyme activity according to the sample quality:

$$5'\text{-NT activity (U/g mass)} = 333.3 \times x \div W = 333.3 \times 0.0291 \div 0.1 = 97.00 \text{ U/g mass.}$$

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