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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:** AK0042

**Size:** 100T/48S

**Components:**

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

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

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

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

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

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

Reagent Ⅶ: Liquid 4 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/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℃, 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 4C , 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 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 | 20 | 20 |
| Working solution | 80 | |
| ortex mixing, 37℃ (mammalian) or 25℃ (plant and other) reaction for 30 min | | |
| Reagent Ⅲ | 100 | 100 |
| Working solution | - | 80 |
| 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 | 80 | 80 | - | - |
| Standard | - | - | 80 | - |
| Reagent Ⅳ | - | - | - | 80 |
| Phosphorus determination reagent | 160 | 160 | 160 | 160 |
| Vortex mixing, 40℃ 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 $\Delta A_S$ 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) = $x \times V_{RT} \div (W \times V_S \div V_{ST}) \div T \times 10^3 = 166.67 \times x \div W$

(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'-NT activity (U/$10^4$ cell) = $x \times V_{RT} \div (\text{cell number} \times V_S \div V_{ST}) \div T \times 10^3 = 166.67 \times x \div$ 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, 0.02 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; 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. 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'-NT activity (U/g mass) =333.3×x ÷ W=333.3×0.0717 ÷ 0. 1=238.98 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'-NT activity (U/g mass) =333.3 × x ÷W=333.3×0.0291÷0. 1=97.00 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