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NADP Phosphatase (NADPase) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: AK0487

Size:100T/48S

Components:

Extract solution: 50 mL ×1. Storage at 4℃ .

Reagent I: 15 mL×1. Storage at 4℃ .

Reagent II: Powder×2. Storage at 4℃ . Add 1.1 mL of Reagent I to fully dissolve for later use and prepare when the solution will be used.

Reagent III: Powder×1. Storage at 4℃ . Add 8 mL ofdistilled water for use, dissolves and storage at 4 ℃ for a week.

ReagentIV: Powder×1. Storage at 4℃ . Add 8 mL ofdistilled water for use, dissolves and storage at 4 ℃ for a week.

Reagent V: 8 mL×1. Storage at room temperature.

Standard: 1 mL×1. 10 mmol/L standard phosphorus stock solution, Storage at 4℃ .

Preparation of 1 μmol/mL standard phosphorus application solution: dilute the standard for 10 times, namely 9 mL of distilled water is added into 1 mL of standard solution plus, fully mix.

Preparation of phosphorus detect reagent: it is prepared that the ratio of distilled water: Reagent III : Reagent IV : Reagent V =2:1:1:1. The prepared phosphorus detect reagent should be light yellow. If it is colorless, the reagent is invalid. If it is blue, it is polluted by phosphorus. Phosphorus detect reagent is prepared when the solution will be used.

Note: it is better to use the new beaker, glass rod and glass pipette to prepare reagent, or to use disposable plastic utensils to avoid phosphorus pollution.

Product description：

NADPase mainly exists in plant tissues and is the only enzyme in organisms that catalyzes the degradation of $NADP^+$ to NAD^+. NADPase and NADK regulate the balance between NAD and NADP.

NADPase can catalyze the hydrolysis of $NADP^+$ to NAD^+ and inorganic phosphorus, the activity of NADPaseis determined by determining the content of inorganic phosphorus.

Reagents and Equipment Required but Not Provided

Refrigerated centrifuge, water-bath, adjustable pipette, spectrophotometer/microplate reader, micro glasscuvette/96 well plate, homogenizer/mortar and distilled water.

Procedure

I. Extraction of crude enzyme solution:

Tissue samples: Take about 0.1 g of sample, and add 1.0 mL of Extract solution for full grinding. Centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and place it on ice under test.

II. Test procedure

a. Preheat the spectrophotometer/microplate reader, for more than 30 minutes, adjust the wavelength to 660 nm and adjust zero with distilled water.

b. Operate table:

Enzymatic reaction:

Reagent Name (μL)	Test Tube (A_T)	Contrast Tube (A_C)
Reagent I	120	120
Reagent II	40	-
Distilled water	-	40
37℃ (mammals) or 25℃ preheat for 5 minutes (other species)		
Sample	40	40

37℃ (mammals) or 25℃ (other species) accurately react for 20 minutes, boiling water bath for 5 minutes (cover tightly, in order to prevent moisture loss). After cooling, centrifuge at 10000 ×g for 10 minutes at room temperature, take the supernatant.

Detect phosphorus content:

Reagent Name (μL)	Standard Tube (A_S)	Blank Tube (A_B)	Test Tube (A_T)	Contrast Tube (A_C)
1 μmol/mL standard phosphorus application solution	20	-	-	-
Distilled water	-	20	-	-
Supernatant	-	-	20	20
Fixed phosphorus reagent	200	200	200	200

Mix thoroughly, 37℃ (mammals) or 25℃ (other species) water bath 30 minutes, cool to room temperature. Then draw 200 μL to micro glass cuvette or 96 well plate, and record the absorbance value at 660 nm, note as A_S, A_B, A_T, A_C, and calculate the $\Delta A_S = A_S - A_B$, $\Delta A_T = A_T - A_C$.

III. Calculation of Enzyme activity of NADPase:

1. Calculate by protein concentration:

Definition: One unit of enzyme activity is defined as the amount of NADPasecatalyzesthe decompose NADP to produce 1 μmol of inorganic phosphorus per minuteevery milligram of tissue protein.

$$NADPase\ (U/mg\ prot) = \Delta A_T \div \Delta A_S \div C_S \times V_{SU} \div (Cpr \times V_S \times V_{SU} \div V_{EN}) \div T$$
$$= 0.25 \times \Delta A_T \div \Delta A_S \div Cpr$$

2. Calculate by sample fresh weight:

Definition: One unit of enzyme activity is defined as the amount of NADPasecatalyzesthe decompose NADP to produce 1 μmol of inorganic phosphorus per minute every gram tissue.

$$NADPase\ (U/g\ fresh\ weight) = \Delta A_T \div \Delta A_S \div C_S \times V_{SU} \div (W \times V_S \div V_{EX} \times V_{SU} \div V_{EN}) \div T$$
$$= 0.25 \times \Delta A_T \div \Delta A_S \div W$$

C_S : 1 μmol/mL phosphorus standard application solution;
V_{SU} : The supernatant liquid volume in constant phosphorus test, 0.02 mL;
Cpr: Sample protein concentration, mg/mL;
V_S : The sample volume in the enzymatic reaction, 0.04 mL;
V_{EN} : Total volume of enzymatic reaction, 0.2 mL;
T: Reaction time, 20 minutes;
$V_{EX:}$ Extract solution volume, 1 mL;
W: Sample fresh weight, g.

Note:

1. This method has the characteristics of trace, sensitive and rapid. So the test tube has strict requirements that must have not phosphorus, if the test tube pass phosphate or phosphate buffer, must be washed very clean, first boiled with detergent and water, then with tap water, finally rinse with distilled water. Using disposable plastic or new glass pipe is the best, avoiding phosphorus pollution is the key to detect success or failure.
2. The blank tube and the standard tube only need to be done once.

Experimental instances:

1. Take 0. 1g of liver, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. Calculate $\Delta A_T=A_T-A_C=0.265-0.254=0.011$, $\Delta A_S=A_S-A_B=0.533-0.047=0.486$, calculate the enzyme activity according to sample weight:
NADPase (U/gweight) $=0.25\times\Delta A_T\div\Delta A_S\div W=0.25\times0.011\div0.486\div0.1=0.057$ U/g weight.
2. Take 0. 1g Setaria (root crop), add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. Calculate $\Delta A_T=A_T-A_S=0.336-0.107=0.229$, $\Delta A_S=A_S-A_B=0.533-0.047=0.486$, calculate the enzyme activity according to sample weight:
NADPase (U/g weight) $=0.25\times\Delta A_T\div\Delta A_S\div W=0.25\times0.229\div0.486\div0.1=1.18$ U/g weight.

References:

[1] Kawai S, Mori S, Mukai T, et al. Cytosolic NADP phosphatases I and II from Arthrobacter sp. strain KM: implication in regulation of NAD+/NADP+ balance[J]. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms, 2004, 44(3): 185- 196.

Related products:

AK0570/AK0569	6-Phosphate Dehydrogenase(G6PDH)Activity Assay Kit
AK0550/AK0549	Isocitrate Dehydrogenase Cytoplasmic(ICDHc) Assay Kit
AK0408/AK0407	6-phosphogluconate Dehydrogenase(6-PGDH)Activity Assay Kit