NADP Phosphatase (NADPase) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC1110

Size:50T/24S

Components

Extractsolution: 30mL×1. Storage at 4℃.

Reagent I: 20mL×1. Storage at 4℃.

Reagent II: Powder ×2. Storage at 4℃.Add 1.5mL of Reagent I to fully dissolve for later use and prepare it fresh.

ReagentIII:Powder ×1. Storage at 4℃. Add 20 mL of distilled water for use, dissolves and save at 4 ℃ for a week.

ReagentIV: Powder ×1. Storage at 4℃. Add 20 mL of distilled water for use, dissolves and storage at 4 ℃ for a week.

Reagent V：20mL×1, Storage at room temperature.

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

Preparation of 0.5 μmol/mL standard phosphorus application solution: dilute the standard for 20 times, namely take 0.5 mL standard solution add 9.5mL distilled water, fully mix.

Preparation of phosphorus detect reagent: it is prepared that the ratio of distilled water :Reagent III: Reagent IV: ReagentV=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.

Reagents and Equipment Required but Not Provided

Low temperature centrifuge, water-bath, adjustable pipette, spectrophotometer, 1 mL glasscuvette, 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

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 660 nm and set zero with distilled water.

2. Operate table:

a. Enzymatic reaction:

Reagent Name (μL)	Test Tube (A_T)	Contrast Tube (A_C)
Reagent I	300	300
Reagent II	100	-
Distilled water	-	100
37℃ (mammals) or 25℃(other species)preheat for 5 minutes.		
Sample	100	100

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.

b. Detect phosphorus content:

Reagent Name (μL)	Standard Tube (A_S)	Blank Tube (A_B)	Test Tube (A_T)	Contrast Tube (A_C)
0.5 mol/mL standard phosphorus application solution	100	-	-	-
Distilled water	-	100	-	-
Supernatant	-	-	100	100
detected phosphorus reagent	1000	1000	1000	1000

Mix thoroughly, at 37 ℃ (mammals) or 25 ℃ (other species) water bath for 30 minutes, cool to room temperature.Record the absorbance value, note as A_S, A_B, A_T, A_C, and calculate $\Delta A_S=A_S-A_B$, $\Delta A_T= A_T-A_C$.

III. Calculation of Enzyme activity of NADPase.

1. Calculate by protein concentration:

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

NADPase (U/mg prot) $=\Delta A_T\div\Delta A_S\div C_S\times V_{SU}\div(Cpr\times V_S\times V_{SU}\div V_{EN})\div T=0.125\times\Delta A_T\div\Delta A_S\div Cpr$。

2. Calculate by sample fresh weight:

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

NADPase (U/g fresh weight) $=\Delta A_T\div\Delta A_S\div C_S\times V_{SU}\div(W\times V_S\div V_{EX}\times V_{SU}\div V_{EN})\div T=0.125\times\Delta A_T\div\Delta A_S\div W$.

C_S: 0.5 μmol/mL phosphorus standard application solution;

V_{SU}: Supernatant liquid volume in constant phosphorus test, 0.1 mL;

Cpr: Sample protein concentration, mg/mL;

V_S: The sample volume in the enzymatic reaction, 0.1 mL;

V_{EN}: Total volume of enzymatic reaction, 0.5 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. 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 tube 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.
3. Because the Extract solution contains a certain concentration of protein (about 1 mg/mL), the protein content of the Extract solution itself needs to be subtracted when determining the protein concentration of the sample.

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 $\Delta A_T=A_T-A_C=0.410-0.385=0.025$， $\Delta A_S=A_S-A_B=0.365-0.005=0.360$, calculate the enzyme activity according to sample weight:

NADPase (U/g weight)=$0.125\times\Delta A_T\div\Delta A_S\div W=0.125\times0.025\div0.360\div0.1=0.087$ 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$\Delta A_T= A_T-A_C=0.510-0.107=0.403$， $\Delta A_S=A_S-A_B=0.365-0.005=0.360$, calculate the enzyme activity according to sample weight:

NADPase (U/g weight) =$0.125\times\Delta A_T\div\Delta A_S\div W=0.125\times0.403\div0.360\div0.1=1.399$ 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：

BC0260/BC0265	6-Phosphate Dehydrogenase(G6PDH)Activity Assay Kit
BC0400/BC0405	Isocitrate Dehydrogenase Cytoplasmic(ICDHc) Assay Kit
BC2100/BC2105	6-phosphogluconate Dehydrogenase(6-PGDH)Activity Assay Kit