NAD Kinase (NADK) Activity Assay Kit

Note: Take two or three different samples for prediction before the formal determination.

Detection equipment: Spectrophotometer/Microplate

Cat No: BC1035

Size:100T/48S

Components:

Extract solution: 60 mL×1, store at 4℃.

Reagent I:5 mL×1, store at 4℃.

Reagent II: 10 mL×1, store at 4℃.

Reagent III:Powder×1, store at -20℃. Add 3 mL of distilled water when the solution will be used, fully dissolve it, and it can be separately packed for preservation. It can be preserved for two weeks at -20℃, and repeated freeze-thaw is forbidden. Dilute the reagent III 100 times according to the number of samples before use.

Reagent IV: Powder×1, store at -20℃. Add 1 mL of distilled water when the solution will be used, fully dissolve it, and store at 4℃.

Reagent V: Powder×1, store at -20℃, Add 2 mL of distilled water when the solution will be used, fully dissolve it, and store at 4℃.

Reagent VI: Powder×1, store at -20℃, Add 2 mL of distilled water when the solution will be used, fully dissolve it, and store at 4℃.

Reagent VII: Powder×1, store at 4℃, Add 2 mL of distilled water when the solution will be used, fully dissolve it, store at 4℃ and protect from light.

Reagent VIII: 14 μL×1, store at 4℃, Add 0.986 mL of distilled water and fully dissolve it, which can be stored at - 20℃ after subpackage, and repeated freeze-thaw is prohibited.

Reagent IX: 20 mL×1, store at 4℃.

Reagent X: Provide for oneself, 95% ethanol.

Standard: Powder×1, storage at 4℃. Add 1.9 mL of distilled water to obtain 2 μmol/mL NADP standard. Dilute the 2 μmol/mL NADP standard 100 times before use to obtain 20 nmol/mL NADP standard solution for use.

Description:

NADK (EC 2.7.1.23) is widely found in animals, plants, microorganisms and cultured cells. It is the only enzyme that can catalyze the phosphorylation of NAD^+ to $NADP^+$ in organisms. It can catalyze the phosphorylation reaction of NAD(H) with ATP or inorganic polyphosphate [poly(P)] as a phosphoryl donor to generate NADP(H). Therefore, NAD kinase plays an important role in the synthesis of NADP(H) and the regulation of the balance between NAD(H) and NADP(H).

NADK catalyzes the phosphorylation of NAD^+ to produce $NADP^+$, which can be reduced to NADPH by

glucose-6-phosphate dehydrogenase. NADPH could reduce oxidized thiazole blue (MTT) through the dehydrogenation of PMS. The absorbance value of MTT at 570 nm can be reflect the activity of NADK.

Required but not provided

Table centrifuge, spectrophotometer/microplate reader, water bath, micro glass cuvette/96 well flat – bottom plate (UV plate), adjustable pipette, mortar/homogenizer, anhydrous ethanol, ice and distilled water.

Protocol:

I. Crude enzyme extraction:

1. Preparation of bacterial, cell or tissue samples:

a. Bacteria or cultured cells:

Collect the bacteria or cells into the centrifuge tube, discard the supernatant, add 1 mL of Extract solution to 5 million bacteria or cells, break the bacteria or cells by ultrasonic (power 20%, ultrasonic 3s, interval 10s, repeat for 30 times). Centrifugate 8000 ×g for 10 minutes at 4℃, take the supernatant and keep it on ice for test.

b. Tissue:

Weigh about 0.1 g of tissue, add 1 mL of Extract solution, homogenate on ice bath. Centrifuge at 8000×g for 10 minutes at 4℃, take the supernatant and keep it on ice for test.

2. Serum sample: direct detection.

II. Procedure

1. Preparation of standard: mix Reagent III and standard to prepare standard according to the following table.

Standard (μL)	Reagent III (μL)	Standard tube concentration (nmol/mL)
0	50	0
5	45	2
10	40	4
15	35	6
20	30	8
25	25	10
30	20	12
35	15	14

2. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 570 nm, set zero with 95% ethanol.

3. Operation table: (operation in EP tube)

Reagent Name (μL)	Test tube (T)	Control tube (C)	Standard tube (S)	Black tube (B)
Sample	10	10	-	-
Standard	-	-	10	-
Distilled water	-	-	-	10

Reagent I	14	24	24	24
Reagent III	10	-	-	-
Reagent IV	6	6	6	6
Mix well, place it in water bath at 37 ℃ (mammal) or 25℃ (other species) for 15 minutes, and boil immediately for 2 minutes (cover tightly to prevent water loss). After centrifuge at 10000 rpm for 5 minutes, take 20 μL of supernatant and put it into 1.5 mL EP tubes, and continue to add the following reagents.				
Reagent II	50	50	50	50
Reagent V	15	15	15	15
Reagent VI	15	15	15	15
Reagent VII	15	15	15	15
Reagent VIII	7	7	7	7
Stand for 20 minutes at room temperature in dark.				
Reagent IX	100	100	100	100
Mix well and let stand for 5 minutes, centrifuge at 20000 ×g for 5 minutes at room temperature. Discard the supernatant and leave the sediment.				
Reagent X	200	200	200	200

After fully dissolving the precipitate, put it in a micro cuvette/96 well flat-bottom plate to measure the absorbance value at 570 nm, record as A_T, A_C, A_S, A_B, calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$.

III. Calculate the activity of NADK

1. Making of standard curve.

When making the standard curve, the concentration of the NADP standard solution is taken as the x-axis, and the ΔA_S is taken as the y-axis. The linear equation y=kx+b is obtained. Take ΔA_T to the equation to acquire x.

A. Calculation of NADK activity

1.Tissue

(1). Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce 1 nmol of NADP per minute in the reaction system every milligram tissue protein.

NADK(U/mg prot)= $x \times V_S \div (Cpr \times V_S) \div T = 0.067 \times x \div Cpr$

(2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce 1 nmol of NADP per minute in the reaction system every gram tissue.

NADK(U/g fresh weight)= $x \times V_S \div (W \times V_S \div V_S) \div T = 0.067 \times x \div W$

2.Germ or cells

(1) Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce 1 nmol of NADP per minute in the reaction system every milligram protein.

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NADK(U/mg prot)＝$x \times V_S \div (Cpr \times V_S) \div T = 0.067 \times x \div Cpr$

(2) Germ or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce 1 nmol of NADP per minute in the reaction systemevery10 thousand germ or cells protein.

NADK(U/10^4 cell)＝ $x \times V_S \div (500 \times V_S \div V_E) \div T = 1.33 \times 10^{-4} \times x$

3.Serum

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce 1 nmol of NADP per minute in the reaction system every milliliter serum.

NADK(U/mL)＝$x \div T = 0.067 \times x$

V_S: Add the volume of sample, 0.01 mL;

Cpr: Sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

V_E: Added the volume of extract solution, 1 mL;

500:5 million of cells;

T: Reaction time, 15 minutes.

Note:

1. The extraction of crude enzyme solution must be completed at 0℃-4℃ to prevent enzyme denaturation and deactivation. It is suggested that two samples with large differences should be selected for pre-experiment before the formal experiment.
2. Reagents III, IV, V, VI, VII and VIII must be placed on ice during the determination.
3. When the initial absorption value is greater than 0.4, it is recommended to dilute the sample with PBS and then measure it.
4. If the number of measured samples is too large, Reagents II, V, VI and VII can be proportioned into working solution for use.

Experimental instances：

1. Take 0.1g of rat kidney, add 1mL of extract solution, homogenate and grind. Take the supernatant and dilute it twice, according to the measured steps, put it in 96 well flat-bottom plate to measure A_T=0.260, $A_{C=}$0.125, calculate $\Delta A_T = A_T - A_C$ =0.260-0.125=0.135, Take ΔA_T to the standard curve y=0.0994x-0.0429, calculate x=0.135+0.0429）/0.0994=1.7897, calculate the enzyme activity according to sample weight:

NADK(U/g weight)＝0.067×x÷W×Dilution Ratio = 0.067×1.7897÷0.1×2=2.3982 U/g weight.

2. Dilute serum of mouse twice and detect directly, measure A_T=0.093, $A_{C=}$0.076, calculate $\Delta A_T = A_T - A_C$ =0.093-0.076=0.017, Take ΔA_T to the standard curve y=0.0994x-0.0429, calculate x =（0.017+0.0429）/0.0994=0.6026, calculate the enzyme activity:

NADK（U/mL）=0.067×x×Dilution Ratio =0.067×0.6026×2=0.0808 U/mL.

References：

[1] Pollak N, Niere M, Ziegler M. NAD kinase levels control the NADPH concentration in human cells[J]. Journal of Biological Chemistry, 2007, 282(46): 33562-33571.

Related products：

BC0310/BC0315	CoenzymeINAD(H) Content Assay Kit
BC1040/BC1045	NAD-Malate Dehydrogenase（NAD-MDH）Assay Kit
BC1050/BC1055	NADP-Malate Dehydrogenase（NADP-MDH）Assay Kit
BC0630/BC0635	NADH oxidase(NOX) Activity Assay Kit