NADKinase (NADK) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No:BC1030

Size:50T/24S

Components:

Extract solution: 30mL×1, store at 4℃.

Reagent I:10mL×1, store at 4℃.

Reagent II: 25mL×1, store at 4℃.

Reagent III:Powder×1, store at-20℃. Add 3.75 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 2.5 mL ofdistilled waterwhen the solution will be used, fully dissolve it, and store at 4℃.

Reagent V:Powder×2, store at -20℃, Add 3 mL ofdistilled waterwhen the solution will be used, fully dissolve it, and store at 4℃.

Reagent VI:Powder×1, store at -20℃. Add 6 mL ofdistilled waterwhen the solution will be used, fully dissolve it, and store at 4℃.

Reagent VII:Powder×1, store at 4℃.Add 6 mL ofdistilled waterwhen the solution will be used, fully dissolve it, store at 4℃ and protect from light.

Reagent VIII: 42 μL×1, store at 4℃. Add 3 mLof distilled water andfully dissolve it, which can be stored at - 20℃ after sub-package.Repeated freeze-thaw is prohibited.

Reagent IX:50mL×1, store at 4℃.

Reagent X: Provide for oneself, 95% ethanol.

Standard: Powder×1, storage at 4℃. Add 1.9mLof distilled water to obtain 2 μmol/mL NADP standard. Dilute the 2 μmol/mL NADP standard100 times before use to obtain20 nmol/mL NADP standard solution for use.

Description:

NAD Kinase(NADK, EC 2.7.1.23) is widely found in animals, plants, microorganisms and cultured cells. It is the only enzyme that can catalyzes the phosphorylation of NAD^+ to $NADP^+$ in organisms. It can catalyzethe 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, water bath, 1 mL glass cuvette, 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 1mL of extract solution to 5 million bacteria or cells, break the bacteria or cells by ultrasonic (power 200W, ultrasonic 3s, interval 10s, repeat for 15imes). Centrifugate 8000×g for 10minutes at 4℃, take thesupernatant and keep it on ice for test.

b. Tissue:

Weigh about 0.1g of tissue, add 1mL 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. Preheat ultraviolet spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set zero with 95% ethanol.

2. 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

3. Preheat Spectrophotometer/Microplate reader for 30minuters, adjust wavelength to 570nm, set zero with 95% ethanol.

4. Operation table: (operation in EP tube)

Reagent Name (μL)	Test tube (T)	Contrast tube (C)	Standard tube(S)	Black tube (B)
Sample	50	50	-	-
Standard	-	-	50	-
Distilled water	-	-	-	50

Reagent I	70	120	120	120
Reagent III	50	-	-	-
Reagent IV	30	30	30	30
Mix well, place it in water bath at37 ℃ (mammal) or 25℃ (other species) for 15minutes, and boil immediately for 2minutes (cover tightly to prevent water loss). After centrifugation at 10000rpm for 5minutes, take 100 μL of supernatant and put it into 1.5mL EP tubes, and continue to add the following reagents.				
Reagent II	250	250	250	250
Reagent V	75	75	75	75
Reagent VI	75	75	75	75
Reagent VII	75	75	75	75
Reagent VIII	35	35	35	35
Stand for 20minutes at room temperature in dark.				
Reagent IX	500	500	500	500
Mix well and let stand for 5min, centrifuge 15000g for 5minutesat room temperature. Discard the supernatant and leave the sediment.				
Reagent X	1000	1000	1000	1000

After fully dissolving the precipitate, measure the absorbance value at 570nm, 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 $\Delta A_S$is taken as the y-axis. The linear equation y=kx+b is obtained. Take$\Delta A_T$to the equation to acquire x.

2.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 systemevery 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 systemevery gram tissue.

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

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 systemevery milligram protein.

NADK(U/mg prot)＝x×V_S÷(Cpr×V_S)÷T =0.067× x÷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 systemevery 10 thousand germ or cells protein.

NADK(U/10^4 cell)＝x×V_S÷(500×V_S÷V_E)÷T =1.33×10^{-4}× 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÷T=0.067×x

V_S: Add the volume ofsample, 0.05 mL;

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

Cpr: Sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

500:5 million cells;

T: Reaction time, 15minutes.

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 experimentbefore 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 0.6, 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, then detect according to the measured steps. Measure A_T=0.455, A_C=0.213, calculate ΔA_T= A_T-A_C =0.455-0.213=0.242, Take ΔA_T to the standard curve y=0.1662x-0.0343 , calculate x=（0.242+0.0343）/0.1662=1.66, calculate the enzyme activity according to sample weight:

NADK(U/g weight)＝0.067×x÷W× dilution ratio =0.067×1.66÷0.1×2=2.22 U/g weight.

2. Take serum of mouse to detect directly, measure A_T=0.131, A_C=0.094, calculate ΔA_T= A_T-A_C =0.131-0.094=0.037, Take ΔA_T to the standard curve y=0.1662x-0.0343, calculate x =（0.037+0.0343）/0.1662=0.429, calculate the enzyme activity according to volume of serum:

NADK（U/mL）=0.067×x =0.067×0.429=0.028 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	Coenzyme I NAD(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