(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

NAD Kinase (NADK) Activity Assay Kit

Catalog No.: BC00154

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	NAD Kinase (NADK) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Bacteria, cells, tissues, serum, plasma
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (optimal detection wavelength 340 nm)

Product Introduction

NADK (EC2.7.1.23) is widely found in animals, plants, microorganisms, and cultured cells. It is currently the only known enzyme in living organisms capable of catalyzing the phosphorylation of NAD^+ to $NADP^+$. It catalyzes the phosphorylation of NAD(H) using ATP or inorganic polyphosphate [poly(P)] as a phosphoryl group donor to generate NADP(H). Therefore, NADK plays a crucial role in the synthesis of NADP(H) and in regulating the balance between NAD(H) and NADP(H).

Principle

NADK catalyzes the phosphorylation of NAD^+ to generate $NADP^+$, which can be reduced to NADPH by glucose-6-phosphate dehydrogenase; the level of NADK activity can be reflected by measuring the rate of increase of NADPH at 340 nm.

Components

Components	Size	Storage
Extract	50 mL × 1 bottle	2~8°C
Reagent 1	25 mL × 1 bottle	2~8°C
Reagent 2	50 mL × 1 bottle	2~8°C

Reagent 3	Powder × 1 bottle	-20℃
Reagent 4	Powder × 1 bottle	-20℃

Storage

The kit has a shelf life of 3 months

Preparation before the experiment

Sample processing

1. Preparation of bacterial or cell samples: First, collect bacteria or cells into centrifuge tubes, centrifuge, and discard the supernatant; according to the ratio of bacteria or cell number (10^4) : extraction liquid volume (mL) of 500-1000:1 (it is recommended to add 1 mL of extraction liquid for 5 million bacteria or cells), sonicate to disrupt the bacteria or cells (ice bath, power 20% or 200W, sonicate for 3 seconds, with 10-second intervals, repeat 30 times); centrifuge at 8000g, 4°C for 10 minutes, collect the supernatant, and place it on ice for testing.
2. Tissue sample preparation: Homogenize the tissue sample in an ice bath at a ratio of 1:5-10 (tissue mass (g): extraction liquid volume (mL) – it is recommended to weigh approximately 0.1g of tissue and add 1mL of extraction liquid). Centrifuge at 8000g, 4°C for 10min, collect the supernatant, and place it on ice for analysis.
3. Serum (plasma) samples: direct detection.

Operation process

1. Preheat the spectrophotometer for at least 30 minutes, adjust the wavelength to 340 nm, and zero it with distilled water.
2. Incubate solvent one and solvent two in a water bath at 37°C (mammals) or 25°C (other species) for at least 15 minutes.
3. Preparation of working solution I: Add 12 mL of solvent I to reagent 3, mix thoroughly and set

aside for use; prepare and use immediately.

4. Preparation of working solution II: Add 45 mL of solvent II to reagent 4 mix thoroughly and use immediately.

Reagents(μL)	Test tube	Control tube
Sample	100	100
Working solution I	400	--
Reagent 1	--	400
Mix thoroughly, incubate in a water bath at 37°C (mammals) or 25°C (other species) for 15 minutes, immediately boil for 2 minutes (cover tightly to prevent water loss), and then cool. Centrifuge at 1000g at room temperature for 10 minutes, and collect the supernatant.		
Supernatant	200	200
Working solution II	800	800
After adding the reagents and mixing well, let it stand at room temperature for 15 minutes and then measure the absorbance at 340 nm for 30 seconds. Calculate $\Delta A = A_{test} - A_{control}$.		

Result calculation

1. Calculated based on sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that produces 1 nmol of NADP per minute by catalyzing the production of 1 mg of tissue protein.

$$\text{NADK Activity (nmol/min/mgprot)} = \frac{\Delta A \times V_{\text{reaction total}} \times 10^9}{\varepsilon \times d \times V_{\text{sample}} \times Cpr \times T} = 53.59 \times \Delta A \div Cpr$$

2. Calculated by organizational quality

Unit definition: One unit of enzyme activity is defined as the amount of 1 nmol NADP produced per minute by the catalytic activity of 1 g of tissue.

$$\text{NADK Activity (nmol/min/g)} = \frac{\Delta A \times V_{\text{reaction total}} \times 10^9 \times V_{\text{sample total}}}{\varepsilon \times d \times V_{\text{sample}} \times W \times T} = 53.59 \times \Delta A \div W$$

3. Calculated by bacterial or cell density

Unit definition: One unit of enzyme activity is defined as the production of 1 nmol NADP per minute by 10,000 bacteria or cells.

$$\text{NADK Activity}\ (\text{nmol/min/}10^4\text{cell}) = \frac{\Delta A \times V_{\text{reaction total}} \times 10^9 \times V_{\text{sample total}}}{500 \times \varepsilon \times d \times V_{\text{sample}} \times T} = 0.107 \times \Delta A$$

4. Calculated based on sample volume (e.g., serum plasma)

Unit definition: One unit of enzyme activity is defined as the amount of NADP produced per ml of sample per minute by catalysis.

$$\text{NADK Activity}\ (\text{nmol/min/mL}) = \frac{\Delta A \times V_{\text{reaction total}} \times 10^9}{\varepsilon \times d \times V_{\text{sample}} \times T} = 53.59 \times \Delta A$$

Annotation

$V_{\text{reaction total}}$: Total volume of the reaction system, 5*10 L

V_{total} : 1 mL of the extracted solution added;

T: Reaction time, 15 min;

Cpr: Sample protein concentration, mg/mL (prot refers to protein);

W: Sample quality;

500: Total number of cells or bacteria, 5 million.

Precautions

This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.