CheKine™ Micro NAD+ Kinase (NADK) Activity Assay Kit

Cat #: KTB1022 Size: 48 T/96 T

	Micro NAD+ Kinase (NADK) Activity Assay Kit		
REF	**Cat #:** KTB1022	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

NAD+ Kinase (NADK, EC 2.7.1.23) is widely found in animals, plants, microorganisms and 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, NADK plays an important role in the synthesis of NADP(H) and the regulation of the balance between NAD(H) and NADP(H). CheKine™ Micro NAD+ Kinase (NADK) Activity Assay Kit provides a simple method for detecting NADK activity in a variety of biological samples such as serum, plasma, animal and plant tissues, cells, bacteria. In the assay, NADK catalyzes the phosphorylation of NAD+ to produce NADP+; NADP+ can be reduced to NADPH by Glucose-6-Phosphate dehydrogenase. NADPH has a characteristic absorption peak at 340 nm. The rate of NADPH increase at 340 nm can reflect NADK activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Assay Buffer Ⅰ	5 mL	10 mL	4℃
Assay Buffer Ⅱ	12.5 mL	25 mL	4℃
Substrate Mix	1	1	-20℃, protected from light
Enzyme Mix	1	1	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, incubate, water bath
- Deionized water

• Dounce homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Assay Buffer Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Assay Buffer Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent Ⅰ Preparation: Prepare Substrate Mix by mixing 2.5 mL (48 T)/5 mL (96 T) Assay Buffer Ⅰ before use. Aliquot the unused reagents and store them at -20℃, protected from light. Avoid freezing and thawing.

Working Reagent Ⅱ Preparation: Prepare Enzyme Mix by mixing 9 mL (48 T)/18 mL (96 T) Assay Buffer Ⅱ before use. Aliquot the unused reagents and store them at -20℃, protected from light. Avoid freezing and thawing.

Note: Substrate Mix and Enzyme Mix must be placed on ice during the measurement process. The reaction temperature has an influence on the results, so please keep it at 25℃ (general species) or 37℃ (mammal).

Sample Preparation

1. Serum or plasma samples: Directly test.
2. Animal tissue samples: Wash tissues with cold PBS to remove blood as much as possible. Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells or bacteria samples: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Plant tissue samples: Wash plant with cold PBS to remove impurities as much as possible. Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Preheat Assay Buffer Ⅰ and Assay Buffer Ⅱ in a 25℃ (general species) or 37℃ (mammal) water bath for more than 15 min.
3. Set the control tube and the test tube, and operate according to the sample addition and reaction process in the following table (operate using EP Tubes):

Reagent	Control Tube (μL)	Test Tube (μL)
Sample	20	20
Working Reagent Ⅰ	0	80
Assay Buffer Ⅰ	80	0
Mix well, incubate at 25℃ (general species) or 37℃ (mammal) for 15 min, boil immediately for 2 min (cover tightly to prevent water loss) and cool in an ice bath, room temperature, 10,000 g, centrifuge for 10 min, discard precipitation and keep the supernatant.		

4. Take 40 μL of the supernatant from each tube and add it to the 96-well UV plate or microquartz cuvette, then add 160 μL Working Reagent Ⅱ per well, and mix quickly.

5. Incubate for 15 min, read optical density at 340 nm. $\Delta A=A_{Test}-A_{Control}$.

Note: Every sample needs to set a control tube. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. Calculation formulae based on 96-well UV plates are as below

1. Calculation of NADK activity in serum (plasma)

Active unit definition: 1 nmol NADP was produced per min in 1 mL serum (plasma) reaction system is defined as a unit of enzyme activity.

NADK (nmol/min/mL)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div V_{Sample}\div T$**=107.18×ΔA**

2. Calculation of NADK activity in tissues, bacteria or cells

(1) Calculated by protein concentration

Active unit definition: 1 nmol NADP was produced per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

NADK (nmol/min/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(Cpr\times V_{Sample})\div T$**=107.18×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: 1 nmol NADP was produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

NADK (nmol/min/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\times V_{Sample}\div V_{Sample\ Total})\div T$**=107.18×ΔA÷W**

(3) Calculated by bacteria or cell numbers

Active unit definition: 1 nmol NADP was produced per min in 10^4 bacteria or cells reaction system is defined as a unit of enzyme activity.

NADK (nmol/min/10^4)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(500\times V_{Sample}\div V_{Sample\ Total})\div T$**=0.214×ΔA**

Where: V_{Total}: Total reaction volume, 1×10^{-4} L; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plates diameter, 0.5 cm; 10^9: 1 mol=10^9 nmol; V_{Sample}: sample volume added, 0.02 mL; $V_{Sample\ Total}$: Extraction Buffer added to samples, 1 mL; T: reaction time, 15 min; Cpr: sample protein concentration, mg/mL; W: sample weight, g; 500: Total number of bacteria or cells, 5×10^6.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Recommended Products

Catalog No.	Product Name
KTB1020	CheKine™ Micro Coenzyme Ⅰ NAD(H) Assay Kit
KTB1021	CheKine™ Micro NADH Oxidase (NOX) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.