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

Coenzyme I NAD(H) Content Assay Kit

Catalog No.: BC00141

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	Coenzyme I NAD(H) Content Assay Kit
Detection Method	Colorimetric
Sample Type	Serum, plasma, tissue, bacteria, cells
Assay Type	Quantitative
Detection Instrument	Visible spectrophotometer (optimal detection wavelength 570 nm)

Product Introduction

Coenzyme I (NAD(H)) is widely found in animals, plants, microorganisms, and cultured cells. NAD^+ is the main hydrogen acceptor in glycolysis (EMP) and the tricarboxylic acid cycle (TCA). The generated NADH transfers electrons to oxygen via the electron transport chain (also known as the respiratory chain, ETC), synthesizing ATP while simultaneously generating a large amount of reactive oxygen species (ROS). At the same time, NADH is regenerated back into NAD^+ . The majority of oxidative reactions in the breakdown of carbohydrates, lipids, and proteins are completed through this system. The NAD(H) content and the NADH/NAD^+ ratio can be used to evaluate the intensity of glycolysis and the TCA cycle. Higher NAD(H) and NADH/NAD^+ ratios indicate higher cellular oxygen consumption and a state of peroxidation. Furthermore, an elevated NADH/NAD^+ ratio can inhibit glycolysis and the TCA cycle. In addition, NAD^+ degradation products play important regulatory roles in cell signaling, metabolism, and gene expression.

Principle

NAD^+ and NADH were extracted from the samples using acidic and alkaline extraction solutions, respectively. NADH reduced oxidized thiazolyl blue (MTT) to formazan via hydrogen transfer through PMS, and the absorbance was measured at 570 nm. NAD^+ was reduced to NADH by alcohol dehydrogenase, and the MTT reduction method was used for further detection.

Components (If both NAD^+ and NADH are measured, the number of samples that can be measured by the kit is halved)

Components	Size	Storage
Acidic extract	50 mL	2~8℃
Alkaline extract	50 mL	2~8℃
Reagent 1	15 mL	2~8℃
Reagent 2	4 mL	2~8℃
Reagent 3	1 bottle of powder	-20℃
	Add 4 mL of distilled water when in use, mix well, and store unused reagent at 4℃ for up to one week.	
Reagent 4	1 bottle of powder	2~8℃
	Add 4 mL of distilled water when in use, mix well, and store unused reagent at 4℃ for up to one week.	
Reagent 5	1.8 mL	2~8℃
Reagent 6	30 mL	2~8℃
Reagent 7	50 mL	2~8℃

Storage

The kit has a shelf life of 3 months

Preparation before the experiment

Sample processing

Extraction of NAD^+ and NADH from serum (plasma)

1. NAD^+ extraction: The ratio of serum (plasma) volume (mL) to acidic extraction solution volume (mL) is 1:5-10 (it is recommended to take about 0.1mL of serum (plasma) and add 1mL of acidic extraction solution). Boil for 5 minutes (cover tightly to prevent water loss). After cooling in an ice bath, centrifuge at 10000g at 4℃ for 10 minutes. Transfer the

supernatant to another new centrifuge tube, add an equal volume of alkaline extraction solution to neutralize, mix well, centrifuge at 10000g at 4℃ for 10 minutes, collect the supernatant, and place on ice for testing.

2. NADH extraction: The ratio of serum (plasma) volume (mL) to alkaline extraction solution volume (mL) is 1:5-10 (it is recommended to take about 0.1 mL of serum (plasma) and add 1 mL of alkaline extraction solution). Boil for 5 min (cover tightly to prevent water loss). After cooling in an ice bath, centrifuge at 10000 g at 4℃ for 10 min. Transfer the supernatant to another new centrifuge tube, add an equal volume of acidic extraction solution to neutralize, mix well, centrifuge at 10000 g at 4℃ for 10 min, collect the supernatant, and place on ice for testing.

Extraction of NAD^+ and NADH from tissues

1. NAD^+ extraction: The ratio of tissue mass (g) to acidic extract volume (mL) is 1:5-10 (it is recommended to take about 0.1g of tissue and add 1mL of acidic extract). Grind in an ice bath and boil for 5min (cover tightly to prevent moisture loss). After cooling in an ice bath, centrifuge at 10000g at 4℃ for 10min. Transfer the supernatant to another new centrifuge tube, add an equal volume of alkaline extract to neutralize, mix well, centrifuge at 10000g at 4℃ for 10min, collect the supernatant, and place on ice for analysis.
2. NADH extraction: The ratio of tissue mass (g) to alkaline extraction solution volume (mL) is 1:5-10 (it is recommended to take about 0.1g of tissue and add 1mL of alkaline extraction solution). Grind in an ice bath and boil for 5 minutes (cover tightly to prevent moisture loss). After cooling in an ice bath, centrifuge at 10000g at 4℃ for 10 minutes. Transfer the supernatant to another new centrifuge tube, add an equal volume of acidic extraction solution to neutralize, mix well, centrifuge at 10000g at 4℃ for 10 minutes, collect the supernatant, and place on ice for analysis.

Extraction of NAD^+ and NADH from cells or bacteria

1. NAD^+ extraction: First, collect cells or bacteria into centrifuge tubes, discard the supernatant, and add acidic extract at a ratio of 500-1000: 1 (for every 10^4 bacteria or cells, add 1 mL of

acidic extract). Sonicate for 1 min (ice bath, intensity 20% or 200W, sonicate for 2 seconds, pause for 1 second), then boil for 5 min (tightly capped to prevent moisture loss). After cooling in an ice bath, centrifuge at 10000g at 4℃ for 10 min. Transfer the supernatant to a new centrifuge tube, add an equal volume of alkaline extract to neutralize, mix well, centrifuge at 10000g at 4℃ for 10 min, collect the supernatant, and place on ice for analysis.

2. NADH extraction: First, collect cells or bacteria into centrifuge tubes, discard the supernatant, and add alkaline extract at a ratio of 500-1000:1 (bacteria or cells: alkaline extract volume (mL) for 10^4 cells/bacteria). Sonicate for 1 min (ice bath, intensity 20% or 200W, sonicate for 2 seconds, pause for 1 second), then boil for 5 min (tightly capped to prevent moisture loss). After cooling in an ice bath, centrifuge at 10000g at 4℃ for 10 min. Transfer the supernatant to a new centrifuge tube, add an equal volume of acidic extract to neutralize, mix well, centrifuge at 10000g at 4℃ for 10 min, collect the supernatant, and place on ice for analysis.

Operation process

Reagents	Test tube	Control tube
Sample (μL)	50	50
Reagent 1 (μL)	250	250
Reagent 2 (μL)	75	75
Reagent 3 (μL)	75	75
Reagent 4 (μL)	75	75
Reagent 5 (μL)	35	35
Reagent 6 (μL)	--	500
Mix well and let stand at room temperature in the dark for 20 minutes.		
Reagent 6 (μL)	500	--
Mix thoroughly, let stand for 5 minutes, then centrifuge at 20,000 g at room temperature for 5 minutes. Discard the supernatant and collect the precipitate (this precipitate is purplish-black).		
Reagent 7 (μL)	1000	1000

Mix thoroughly (the purplish-black precipitate will dissolve after mixing; if a white or near-white precipitate remains, centrifuge at 20,000 g for 5 minutes at room temperature and then take the supernatant reading). Measure the absorbance of each tube at a wavelength of 570 nm using a 1 cm path length cuvette and zeroing with distilled water.

Note: Preheat the spectrophotometer for at least 30 minutes in advance; if a large number of samples are to be measured at once, reagents one, two, three and four can be prepared into a working solution in proportion; avoid light during the reaction; for multiple samples, each test tube should correspond to a control tube.

Result calculation

Calculation of NAD^+ content

1. Calculation of NAD^+ content in serum (plasma) :

$$\begin{aligned} \text{NAD}^+\ \text{Content}\ (\text{nmol/mL}) &= (A_{test} - A_{control} - 0.099) \times 36.1 \div (V_2 \div V_1) \\ &= (A_{test} - A_{control} - 0.099) \times 722 \end{aligned}$$

2. Calculation of NAD^+ content in tissues, bacteria, or cells :

(1) Calculate based on sample fresh weight

$$\begin{aligned} \text{NAD}^+\ \text{Content}\ (\text{nmol/g tissues}) &= (A_{test} - A_{control} - 0.099) \times 36.1 \div (W \div V_1) \\ &= (A_{test} - A_{control} - 0.099) \times 722 \div W \end{aligned}$$

(2) Calculated based on bacterial or cell density

$$\begin{aligned} \text{NAD}^+\ \text{Content}\ (\text{nmol}/10^4\text{Cells}) &= (A_{test} - A_{control} - 0.099) \times 36.1 \div (500 \div V_1) \\ &= (A_{test} - A_{control} - 0.099) \times 0.1444 \end{aligned}$$

Calculation of NADH content

1. Calculation of NADH content in serum (plasma) :

$$\begin{aligned} \text{NADH Content}\ (\text{nmol/mL}) &= (A_{test} - A_{control} - 0.065) \times 24.7 \div (V_2 \div V_1) \\ &= (A_{test} - A_{control} - 0.065) \times 494 \end{aligned}$$

2. Calculation of NADH content in tissues, bacteria, or cells :

(1) Calculate based on sample fresh weight

$$\begin{aligned} \text{NADH Content}\ (\text{nmol/g tissues}) &= (A_{test} - A_{control} - 0.065) \times 24.7 \div (W \div V_1) \\ &= (A_{test} - A_{control} - 0.065) \times 19.4 \div W \end{aligned}$$

(2) Calculated based on bacterial or cell density

$$\text{NADH Content}\ (\text{nmol}/10^4\text{Cells}) = (A_{test} - A_{control} - 0.065) \times 24.7 \div (500 \div V_1)$$
$$= (A_{test} - A_{control} - 0.065) \times 0.0988$$

Annotation

V_1 : Volume of extract added, 2 mL;

V_2 : Volume of serum (plasma) added: 0.1 mL;

W: Sample mass, g;

500: Total number of cells or bacteria, 5 million

Precautions

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