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

Coenzyme II NADP(H) Content Assay Kit

Catalog No.: BC00142

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 II NADP(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 II NADP(H) is widely present in animals, plants, microorganisms, and cultured cells. The determination of $NADP^+$ and NADPH content allows for the calculation of total NADP (NADPH + $NADP^+$) levels and the NADPH/$NADP^+$ ratio, which is closely associated with the pentose phosphate pathway, biosynthesis, and antioxidant responses. The NADPH/$NADP^+$ ratio serves not only as one of the primary indicators of cellular redox status but also plays a crucial regulatory role in the PPP pathway, biosynthesis, and antioxidant metabolism.

Principle

$NADP^+$ and NADPH are extracted from samples using acidic and alkaline extraction buffers, respectively. NADPH reduces oxidized thiazolyl blue (MTT) to formazan via the hydrogen transfer action of phenazine methosulfate (PMS). The absorbance is measured at 570 nm to determine NADPH content. $NADP^+$ content is detected by reducing $NADP^+$ to NADPH using glucose-6-phosphate dehydrogenase.

Components (If both $NADP^+$ and NADPH 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	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 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 $NADP^+$ and NADPH from serum (plasma)

1. $NADP^+$ 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. NADPH 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 $NADP^+$ and NADPH from tissues

1. $NADP^+$ 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. NADPH 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 $NADP^+$ and NADPH from cells or bacteria

1. $NADP^+$ 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. NADPH 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

Add samples sequentially into a 1.5 mL brown EP tube according to the table below, and the reaction process should be protected from light.

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)	Mix well and let stand at room temperature in the dark for 20 minutes.	500
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		
Reagent 7 (μL)	1000	1000

Mix thoroughly. Set the spectrophotometer to 570 nm wavelength using a 1 cm path length cuvette, zero with distilled water, and measure the absorbance of the control (A1) and the sample tube (A2). Calculate ΔA = A2 - A1.

Note: After adding Reagent 5 to the control tube, Reagent 6 must be added immediately; whereas for the sample tube, Reagent 6 should be added only after a 20-minute reaction following the addition of Reagent 5.

Result calculation

Calculation of $NADP^+$ content

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

$$\underset{(\text{nmol/mL})}{\text{NADP}^+\ \text{Content}} = \frac{4.57 \times (\triangle \text{A} - 0.062) \times \text{V1}}{\text{V3} \times \text{V1} \div \text{V2}} = 91.4 \times (\triangle \text{A} - 0.062)$$

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

(1) Calculate according to the sample protein concentration.

$$\underset{(\text{nmol/mgprot})}{\text{NADP}^+\text{Content}} = \frac{4.57 \times (\triangle \text{A} - 0.062) \times \text{V1}}{\text{V1} \times \text{Cpr}} = 4.57 \times (\triangle \text{A} - 0.062) \div \text{Cpr}$$

(2) Calculate based on sample fresh weight

$$\underset{(\text{nmol/g tissues})}{\text{NADP}^+\ \text{Content}} = \frac{4.57 \times (\triangle \text{A} - 0.062) \times \text{V1}}{\text{W} \times \text{V1} \div \text{V2}} = 9.14 \times (\triangle \text{A} - 0.062)) \div \text{W}$$

(3) Calculated based on bacterial or cell density

$$\underset{(\text{nmol}/10^4\text{Cells})}{\text{NADP}^+\ \text{Content}} = \frac{4.57 \times (\triangle \text{A} - 0.062) \times \text{V1}}{500 \times \text{V1} \div \text{V2}} = 0.0183 \times (\triangle \text{A} - 0.062)$$

Calculation of NADPH content

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

$$\underset{(\text{nmol/mL})}{\text{NADPH Content}} = \frac{7.2 \times (\triangle \text{A} - 0.072) \times \text{V1}}{\text{V3} \times \text{V1} \div \text{V2}} = 144 \times (\triangle \text{A} - 0.072)$$

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

(1) Calculate according to the sample protein concentration.

$$\underset{(\text{nmol/mgprot})}{\text{NADPH Content}} = \frac{7.2 \times (\triangle \text{A} - 0.072) \times \text{V1}}{\text{V1} \times \text{Cpr}} = 7.2 \times (\triangle \text{A} - 0.072) \div \text{Cpr}$$

(2) Calculate based on sample fresh weight

$$\text{NADPH Content}\ (\text{nmol/g tissues}) = \frac{7.2 \times (\triangle A - 0.072) \times V1}{W \times V1 \div V2} = 14.4 \times (\triangle A - 0.072) \div W$$

(3) Calculated based on bacterial or cell density

$$\text{NADPH Content}\ (\text{nmol}/10^4\text{Cells}) = \frac{7.2 \times (\triangle A - 0.072) \times V1}{500 \times V1 \div V2} = 0.0288 \times (\triangle A - 0.072)$$

Annotation

V1: Volume of sample added to the reaction system, 0.05 mL;

V2: Volume of extraction buffer added, 2 mL;

V3: Volume of serum (plasma) added, 0.1 mL;

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

W: Sample mass, g;

500: Total number of cells or bacteria, 5 million

Precautions

1. If a large number of samples are to be assayed at one time, Reagents 1, 2, 3, and 4 can be proportionally mixed to prepare a working solution, which should be prepared immediately before use.
2. Protect from light during the reaction process.
3. Since each tube requires a corresponding control tube, this kit (50 tubes) can be used to assay 24 samples for either $NADP^+$ or NADPH; if both are to be measured, 12 samples can be assayed.
4. The minimum detection limit is 1 nmol/mL or 1 nmol/g fresh tissue weight or 0.01 nmol/mgprot.
5. This product is for research use only and must not be used for clinical diagnosis. Do not take it.