Coenzyme II NADP (H) Content Assay Kit (MTT Chromogenic Method)

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: BC1100

Size:50T/24S

Components:

Acid Extract solutiont : Liquid 25 mL×1. Storage at 2-8℃.

Alkaline Extract solutiont: Liquid 25 mL×1. Storage at 2-8℃.

Reagent I : Liquid 10 mL×1. Storage at 2-8℃.

Reagent II : Powder×1. Storage at -20℃. Add 4mL of distilled water before using to dissolve it. Reagents can be stored at 2-8°C for 4 weeks after dissolution.

Reagent III : Liquid 8 mL×1. Storage at 2-8℃..

Reagent IV A: Powder×1. Storage at -20℃.

Reagent IV B: Liquid 5mL×1. Storage at 2-8℃. Dissolve reagent IV A into reagent IV B just before use. Store the reagents in aliquots after dissolving, avoid repeated freezing and thawing, and store at -20°C for 4 weeks.

Reagent V : Liquid 30 mL×1. Storage at 2-8℃.

Reagent VI : Liquid 50 mL×1. Storage at 2-8℃.

NADP standard: Powder×1. Storage at -20℃.Add 1.27 mL of distilled water before use to obtain a standard of 5 μmol/mL, which can be stored at -20°C for 2 weeks.

NADPH standard: Powder×1. Storage at -20℃.Add 1.2 mL of distilled water before use to obtain a standard of 5 μmol/mL, which can be stored at -20°C for 2 weeks.

Product Description:

Coenzyme II NADP (H) is widely present in animals, plants, microorganisms and cultured cells. The determination of $NADP^+$ and NADPH content can calculate the content of NADP (NADPH + $NADP^+$) and the ratio of NADPH/$NADP^+$. The changes are related to the pentose phosphate pathway and biosynthesis and resistance The oxidation reaction is closely related. The ratio of NADPH/$NADP^+$ is not only one of the main signs of cell redox state, but also has an important regulatory role in PPP pathway, biosynthesis and antioxidant metabolism.

The $NADP^+$ and NADPH in the sample were extracted with acidic and alkaline extracts, respectively. NADPH reduces the oxidized thiazole blue (MTT) to formazan through the hydrogen transfer effect of PMS, and detects the absorbance at 570nm to determine the NADPH content. Use 6-phosphate glucose dehydrogenase to reduce $NADP^+$ to NADPH to detect $NADP^+$ content.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, centrifuge, water bath, mortar/homogenizer, sonicator, adjustable pipette, micro glass cuvettes/96 well plates , ice, and distilled water.

Sample Preparation：

1. Serum

Extract $NADP^+$: Take 0.1 mL of serum (slurry), add 0.5 mL of acidic extract, boil for 5 minutes

(cover tightly to prevent water loss), and after cooling in an ice bath, centrifuge at 10,000g at 4°C for 10 minutes; take 200 μL of supernatant, add an equal volume of alkaline extract ; Mix well, centrifuge at 10,000g at 4°C for 10 min, take the supernatant, and store on ice for testing.

Extract NADPH: Take 0.1 mL of serum (slurry), add 0.5 mL of alkaline extract, boil for 5 minutes (cover tightly to prevent water loss), after cooling in an ice bath, centrifuge at 10,000g at 4°C for 10 minutes, take 200 μL of supernatant, and add an equal volume of acidic extract ; Mix well, centrifuge at 10000g at 4°C for 10min, take the supernatant and store on ice for testing.

2. Tissue

Extract NADP$^+$: Weigh about 0.1g of tissue, add 0.5mL of acidic extract, grind in ice bath, boil for 5min (cover tightly to prevent water loss), after cooling in ice bath, centrifuge at 10,000g at 4°C for 10min, take 200μL of supernatant, add an equal volume of alkaline extract ; Mix wel, centrifuged at 10,000 g at 4°C for 10 min, and the supernatant was taken and stored on ice for testing.

Extract NADPH: Weigh about 0.1 g of tissue, add 0.5 mL of alkaline extraction solution, grind in an ice bath, boil for 5 min (cover tightly to prevent water loss), after cooling in an ice bath, centrifuge at 10,000 g at 4°C for 10 min, take 200 μL of supernatant, and add an equal volume of acidic extract ; Mix well, centrifuged at 10,000 g at 4°C for 10 min, and the supernatant was taken and stored on ice for testing.

3. Bacteria or cells

Extract NADP$^+$: Collect 5 million cells or bacteria, add 0.5mL of acidic extract, ultrasonically disrupt for 1min (intensity 20% or 200W, ultrasonic for 2s, stop for 1s), boil for 5min (cover tightly to prevent water loss), cool in ice bath, 10000g centrifuge at 4°C for 10min, take 200uL of the supernatant into another new centrifuge tube, add an equal volume of alkaline extract to neutralize, mix well, centrifuge at 10,000g at 4°C for 10min, take the supernatant and store it on ice for testing .

Extract NADPH: Collect 5 million cells or bacteria, add 0.5mL alkaline extract, ultrasonically disrupt for 1min (intensity 20% or 200W, ultrasonic for 2s, stop for 1s), boil for 5min (cover tightly to prevent water loss), cool in an ice bath, Centrifuge at 10,000g at 4°C for 10min, take 200uL of the supernatant into another new centrifuge tube, add an equal volume of acidic extract to neutralize, mix well, centrifuge at 10,000g at 4°C for 10min, take the supernatant, and store it on ice for testing .

Determination procedure:

1. Preheat the spectrophotometer/ microplate reader more than 30 minutes, adjust the wavelength to 570 nm, the spectrophotometer set zero with distilled water.
2. NADP+ standard: diluted with distilled water to a standard solution of 2.5,1.25, 0.625, 0.3125, 0.15625, 0.078, 0.039, 0nmol/mL (0nmol/mL is a blank tube).
3. NADPH standard: diluted with distilled water to a standard solution of 1.25, 0.625, 0.3125, 0.15625, 0.078, 0nmol/mL (0nmol/mL is a blank tube).
4. Adding sample table (add samples in the 1.5mL brown EP tube in sequence according to the following table):

Reagent name (μL)	Control tube (A_1)	Test tube (A_2)	Standard tube
Sample	20	20	-
Standard	-	-	20
Reagent V	200	-	-

Reagent I	80	80	80
Reagent II	30	30	30
Reagent III	60	60	60
Reagent IV	30	30	30
		Mix well and let stand for 20min at room temperature in the dark	
Reagent V		200	200
Mix thoroughly, and after standing for 5 minutes, centrifuge at 20,000g at 25°C for 5 minutes, discard the supernatant, and add it to the pellet.			
Reagent VI	400	400	400

Mix well, take 200μL into a micro glass cuvettes/96 well plates, measure at 570nm, read the absorbance value, $NADP^+$ is marked as: ΔA $NADP^+$= A_2- A_1, NADPH is marked as ΔA NADPH= A_2'- A_1', NADP standard tube is marked as ΔAs= As-Ab. The NADPH standard tube is marked as ΔAs ' = As '-Ab. (The standard curve only needs to be done 1-2 times).

Calculation:

1. Standard curve drawing:

(1) Drawing of $NADP^+$ standard curve: According to the concentration of the standard tube (x_1, nmol/mL) and the absorbance ΔAs (y_1, ΔAs), establish a standard curve. From the standard curve, plug ΔA into the equation to get x_1 (nmol/mL).

(2) Drawing of the NADPH standard curve: According to the concentration of the standard tube (x_2, nmol/mL) and the absorbance ΔAs ' (y_2, ΔAs '), establish a standard curve. From the standard curve, plug ΔA into the equation to get x_2 (nmol/mL).

2. Calculation of $NADP^+$ and NADPH content：

①Calculation of $NADP^+$ content

(1) Calculated by liquid volume:

$NADP^+$content (nmol/mL) = $x_1 \times (Ve + Vse) \div V\ serum = 11 \times x_1$

(2) Calculated by sample protein concentration

$NADP^+$ (nmol/mg prot) = $x_1 \times Ve \div (Ve \times Cpr) = x_1 \div Cpr$

(3) Calculate content according to the fresh weight of the sample

$NADP^+$ (nmol/g fresh weight) = $x_1 \times Ve \div W = x_1 \div W$

(4) Calculated by the number of cells:

$NADP^+$ content (nmol/10^4 cell) = $x_1 \times Ve \div 500 = 0.002 \times x_1$

②Calculation of NADPH content

(1) Calculated by liquid volume:

NADPH content (nmol/mL) = $x_2 \times (Ve + Vs) \div Vs = 11 \times x_2$

(2) Calculated by sample protein concentration

NADPH (nmol/mg prot) = $x_2 \times Ve \div (Ve \times Cpr) = x_2 \div Cpr$

(3) Calculate the content according to the fresh weight of the sample

NADPH (nmol/g fresh weight) = $x_2 \times Ve \div W = x_2 \div W$

(4) Calculated the content by the number of cells:

NADPH (nmol/104 cell) = $x_2 \times Ve \div 500 = 0.002 \times x_2$

Ve: volume of added extract, 1 mL;

Vse: volume of serum (plasma), 0.1 mL;
Cpr: sample protein concentration, mg/mL;
W: sample mass, g;
500: the total number of bacteria or cells, 5 million.

Experimental example:

1. Determination of $NADP^+$: Weigh 0.1g of green radish leaves, extract according to the extraction steps, and then operate according to the determination steps. After measuring the absorbance value in a glass cuvette, calculate $\Delta A = A_2 - A_1 = 0.095 - 0.066 = 0.029$, standard curve $y_1 = 0.5586x + 0.0295$, according to the standard curve, $x_1 = 0.050$, $NADP^+$ content: $NADP^+$ (nmol/g mass) = $x_1 \div W = 0.503$ nmol/g mass.
   Determination of NADPH: Weigh 0.1g of green radish leaves, extract according to the extraction steps, and then operate according to the determination steps. After measuring the absorbance value in the glass cuvette, calculate $\Delta A = A_2' - A_1' = 0.101 - 0.065 = 0.036$, the standard curve $y_2 = 0.518x + 0.0113$, according to the standard curve, $x_2 = 0.048$, NADPH content: NADPH (nmol/g mass) = $x_2 \div W = 0.477$ nmol/g mass.
2. Determination of $NADP^+$: Weigh 0.1g of rabbit liver, extract it according to the extraction steps, and then operate according to the determination steps. After measuring the absorbance value in a glass cuvette, calculate $\Delta A = A_2 - A_1 = 0.307 - 0.137 = 0.170$, standard curve $y_1 = 0.5586x + 0.0295$, according to the standard curve, $x_1 = 0.295$, NADP+ content: $NADP^+$ (nmol/g mass) = $x_1 \div W = 2.950$nmol/g mass.
   Determination of NADPH: Weigh 0.1g of rabbit liver, extract it according to the extraction steps, and then operate according to the determination steps. After measuring the absorbance value in a glass cuvette, calculate $\Delta A = A_2' - A_1' = 0.116 - 0.076 = 0.040$, standard curve $y_2 = 0.518x + 0.0113$, according to the standard curve, $x_2 = 0.055$, NADPH content: NADPH (nmol/g mass) = $x_2 \div W = 0.554$ nmol/g mass.
3. Determination of $NADP^+$: Take 0.1mL of horse serum, extract it according to the extraction steps, and then operate according to the determination steps. After measuring the absorbance value in the glass cuvette, calculate $\Delta A = A_2 - A_1 = 0.068 - 0.057 = 0.011$, standard curve $y_1 = 0.5586x + 0.0295$, according to the standard curve, $x_1 = 0.019$, $NADP^+$ content: $NADP^+$ (nmol/mL) = 11 $x_1 = 0.210$ nmol/mL .
   Determination of NADPH: Take 0.1mL of horse serum, extract it according to the extraction steps, and then operate according to the determination steps. After measuring the absorbance value in a glass cuvette, calculate $\Delta A = A_2' - A_1' = 0.080 - 0.060 = 0.020$, the standard curve $y_2 = 0.518x + 0.0113$, according to the standard curve, $x_2 = 0.017$, NADPH content: NADPH (nmol/mL)=11 $x_2 = 0.185$ nmol/mL .

Note:

1. If the number of samples for one-time determination is large, reagents I, II and III can be prepared into a mixed solution in proportion.
2. Avoid light during the reaction.
3. Since each measuring tube needs to set up a control tube, 50 tubes of this kit can measure 24 $NADP^+$ or NADPH.
4. If the measured absorbance value exceeds the linear range absorbance value, you can increase the sample volume or dilute the sample before measuring.