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

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

Operation Equipment: Spectrophotometer

Cat No: NA0171

Size:50T/24S

Components:

Acid Extract solutiont : Liquid 15 mL×1. Storage at 2-8℃.

Alkaline Extract solutiont: Liquid 15 mL×1. Storage at 2-8℃.

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

Reagent II : Powder×1. Storage at -20℃. Add 12mL of distilled water before using to dissolve it. Reagents can be stored at 2-8°C for 4 weeks after dissolution.

Reagent III : Liquid 12 mL×1. Storage at 2-8℃.

Reagent IV: Liquid 12 mL×1. Storage at 2-8℃.

Reagent V : Liquid 70 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/$NADP^+$, and its changes are related to the pentose phosphate pathway and biosynthesis and Antioxidative responses are closely related. The NADPH/$NADP^+$ ratio is not only one of the main markers of cellular redox state, but also plays an important regulatory role in the PPP pathway, biosynthesis and antioxidant metabolism.

The $NADP^+$ and NADPH in the samples were extracted with acidic and basic extraction solutions, respectively. Under the action of 1-mPMS, WST-1 can react with NADPH to produce water-soluble formazan, which has a characteristic absorption peak at 450nm, while $NADP^+$ can be reduced to NADPH by 6-phosphate glucose dehydrogenase, which is further detected by WST-1.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, centrifuge, water bath, mortar/homogenizer, sonicator, adjustable pipette, 1 mL glass cuvette, 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 more than 30 minutes, adjust the wavelength to 450 nm, 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, 0.0195, 0.01, 0nmol/mL (0nmol/mL is a blank tube).
3. NADPH 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).
4. Adding sample table (add samples in the 1.5mL brown EP tube in sequence according to the following table):

Reagent (μL)	Control tube (A_1)	Test tube (A_2)	Standard tube
Sample/ Standard	100	100	100
Reagent V	1000	-	-

Reagent I	400	400	400
Reagent II	150	150	150
Reagent III	150	150	150
Reagent IV	150	150	150
Mix well and let stand for 1h at room temperature in the dark			
Reagent V		1000	1000

Mix well, measure at 450nm, read the absorbance value, $NADP^+$ is marked as: $\Delta A\ NADP^+ = A_2 - A_1$, NADPH is marked as $\Delta A\ NADPH = A_2' - A_1'$, NADP standard tube is marked as $\Delta As = As - Ab$. The NADPH standard tube is marked as $\Delta As' = As' - Ab$. (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 $\Delta As'$ (y_2, $\Delta As'$), 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.

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. Simultaneously modify the calculation formula.

Experimental example:

1. Determination of $NADP^+$: Weigh 0.1g, extract according to the extraction steps and then operate according to the determination steps. After measuring the absorbance value in the glass cuvette, calculate ΔA~~t~~=~~At~~A2-~~Ac~~A1=0.052-0.036=0.016, standard curve y1= 0.333x-0.0063, according to the standard curve, x1=0.067, and the $NADP^+$ content is:

   $NADP^+$ (nmol/g mass) = x1÷W=0.67 nmol/g mass.

   Determination of NADPH: Weigh 0.1g of holly 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 $\Delta A = A_2' - A_1'$~~ΔAt=At-Ac~~=0.153-0.096=0.057, standard curve y2= 0.0914x-0.0065, according to the standard curve, x2=0.695, NADPH content: NADPH (nmol/g mass) = x2÷W=6.947 nmol/g mass.

2. Determination of $NADP^+$: Weigh 0.1g of mouse 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 ΔA=A2-A1~~ΔAt=At-Ac~~=0.042-0.028=0.014, standard The curve y1=0.333x-0.0063, according to the standard curve, x1=0.061, $NADP^+$ content: $NADP^+$ (nmol/g mass) = x1÷W=0.61nmol/g mass.

   Determination of NADPH: Weigh 0.1g of mouse 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 $\Delta A = A_2' - A_1'$~~ΔAt=At-Ac~~=0.19-0.063=0.127, standard curve y2 =0.0914x-0.0065, according to the standard curve x2=1.461, NADPH content: NADPH (nmol/g mass) = x2÷W=14.61 nmol/g mass.

3. Determination of $NADP^+$: Take 0.1 mL of bovine serum, extract it according to the extraction steps, and operate according to the determination steps. After measuring the absorbance value in the glass cuvette, calculate ΔA=A2-A1~~ΔAt=At-Ac~~=0.048-0.030=0.018, standard curve y1 =0.333x-0.0063, according to the standard curve x1=0.073, $NADP^+$ content:

   $NADP^+$ (nmol/mL) = 11×x1=0.803 nmol/mL.

   Determination of NADPH: Take 0.1 mL of bovine 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 $\Delta A = A_2' - A_1'$~~ΔAt=At-Ac~~=0.056-0.032=0.024, standard curve y2=0.0914 x-

0.0065, according to the standard curve x2=0.334, NADPH content: NADPH (nmol/mL) = 11×x2=3.671 nmol/mL.