Coenzyme I NAD (H) Content Assay Kit (WST-1 Chromogenic Method)

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: NA0172

Size:100T/48S

Components:

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

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

Reagent I : Liquid 6 mL×1. Storage at 2-8℃.

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

Reagent III : Liquid 4 mL×1. Storage at 2-8℃..

Reagent IV: Liquid 0.9 mL×1. Storage at -20℃.

Reagent V : Liquid 15 mL×1. Storage at 2-8℃.

NAD standard: Powder×1. Storage at -20℃.Add 1.5 mL of distilled water before use to obtain a standard of 2 μmol/mL, which can be stored at -20°C for 2 weeks.

NADH standard: Powder×1. Storage at -20℃.Add 1.4 mL of distilled water before use to obtain a standard of 2 μmol/mL, which can be stored at -20°C for 2 weeks.

Product Description:

Coenzyme I, including reduced and oxidized forms, plays a role in transferring hydrogen in biological oxidation. Oxidized coenzyme I, also known as nicotinamide adenine dinucleotide (NAD^+), is a coenzyme of dehydrogenase, which plays an irreplaceable role in glycolysis, gluconeogenesis, tricarboxylic acid cycle and respiratory chain. The intermediate product will pass off the hydrogen to NAD, making it NADH (reduced coenzyme I). NADH acts as a hydrogen carrier to synthesize ATP by chemical osmotic coupling in the respiratory chain. NAD(H) has important physiological significance in the body, and is closely related to material metabolism, energy metabolism, anti-aging, anti-oxidation and the occurrence of some diseases. Decreased levels of coenzyme I in the body can lead to cell damage or decline.

The NAD^+ and NADH in the samples were extracted with acidic and alkaline extracting solutions, respectively. Under the action of 1-mPMS, WST-1 can react with NADH to produce water-soluble formazan, which has a characteristic absorption peak at 450 nm, while NAD^+ can be dehydrogenated by ethanol The enzyme was reduced to NADH, which was further detected by WST-1.

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 NAD^+: 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 NADH: 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 NAD^+: 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 well, centrifuged at 10,000 g at 4°C for 10 min, and the supernatant was taken and stored on ice for testing.

Extract NADH: 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 NAD^+: 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 NADH: 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 450 nm, the spectrophotometer set zero with distilled water.
2. NAD+ standard: diluted with distilled water to a standard solution of 1.25、0.625、0.3125、0.15625、0.078、0.039、0.0195、0nmol/mL (0nmol/mL is a blank tube).
3. NADH standard: diluted with distilled water to a standard solution of 10、5、2.5、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 (μL)	Control tube (A_1)	Test tube (A_2)	Standard tube
Sample	10	10	-
Standard	-	-	10
Reagent V	100	-	-
Reagent I	50	50	50

Reagent II	15	15	15
Reagent III	30	30	30
Reagent IV	7	7	7
Mix well and let stand for 1h at room temperature in the dark			
Reagent V		100	100

Mix well, measure at 450nm, read the absorbance value, NAD^+ is marked as: $\Delta A\ NAD^+ = A_2 - A_1$, NADH is marked as $\Delta A\ NADH = A_2' - A_1'$, NAD standard tube is marked as ΔAs= As-Ab. The NADH standard tube is marked as ΔAs ' = As '-Ab. (The standard curve only needs to be done 1-2 times, Each test tube needs to correspond to a control tube).

Calculation:

1. Standard curve drawing:

(1) Drawing of NAD^+ 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 NADH 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 NAD^+ and NADH content:

①Calculation of NAD^+ content

(1) Calculated by liquid volume:

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

(2) Calculated by sample protein concentration

NAD^+ (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

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

(4) Calculated by the number of cells:

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

②Calculation of NADH content

(1) Calculated by liquid volume:

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

(2) Calculated by sample protein concentration

NADH (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

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

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

NADH (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. Avoid light during the reaction.

2. If the measured absorbance value exceeds the absorbance value in the linear range, the sample volume can be increased or the sample can be diluted before the measurement.

Experimental example:

1. Determination of NAD^+: 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~~=~~0.113~~0.119-~~0.089~~0.106=~~0.024~~0.013, standard curve y1=~~0.5982~~0.373x+~~0.0038~~0.0012, according to the standard curve ~~song~~, x_1=0.~~034~~032, and the NAD+ content is:

   NAD^+ (nmol/g mass) = $x_1 \div W$=0.~~34~~ 32 nmol/g mass.

   Determination of NADH: 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~~=~~0.250~~0.172-~~0.168~~0.128=~~0.082~~0.044, standard curve y2= ~~0.4452~~0.1479x~~-0.0008~~, according to the standard curve ~~song~~, x_2=0.~~186~~297, and the NADH content is:

   NADH (nmol/g mass) = $x_2 \div W$=~~1.86~~2.97 nmol/g mass.

2. Determination of NAD^+: 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 the glass cuvette, calculate $\Delta A = A_2 - A_1$~~ΔAt=At-Ac~~=~~0.068~~0.119-~~0.045~~0.105=~~0.019~~0.014, standard ~~The~~ curve y1=0.373x+0.0012~~0.5982x+0.0038~~, according to the standard curve, x_1=0.~~025~~034, and the NAD^+ content is:

   NAD^+ (nmol/g mass) = $x_1 \div W$=~~0.25~~0.34nmol/g mass.

   Determination of NADH: 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~~=~~0.217~~0.145-~~0.118~~0.112=~~0.09~~0.0339, standard curve y2= 0.1479x ~~y2 =0.4452x-0.0008~~, according to the standard curve, x_2=0.~~224~~223, the NADH content is:

   NADH (nmol/g mass) = $x_2 \div W$=~~2.24~~2.23 nmol/g mass.

3. Determination of NAD^+: Take 0.1 mL 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 $\Delta A = A_2 - A_1$~~ΔAt=At-Ac~~=0.~~113~~114-0.~~074~~097=~~0.039~~0.017, standard curve y1=0.373x+0.0012~~y1 =0.5982x+0.0038~~, according to the standard curve, x_1=0.~~059~~042, and the NAD^+ content is:

   NAD^+ (nmol/mL ~~nmol/g mass~~) = $x_1 \div W$=~~0.59~~ 0.462 nmol/mL ~~nmol/g mass~~.

   Determination of NADH: Take 0.1 mL of horse 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 $\Delta A = A_2' - A_1$~~ΔAt= At- Ac~~=~~0.120~~0.142-~~0.097~~0.135=~~0.023~~0.007, standard curve y2= 0.1479x ~~y2 = 0.4452 x-0.0008~~, according to the standard curve, x_2=0.~~053~~047, the NADH content is:

NADH (nmol/mL ~~nmol/g mass~~) = $x_2 \div W$=~~0.53~~0.517 nmol/mL ~~nmol/g mass~~.