Ver.260801

Coenzyme I NAD (H) Assay Kit

BC6601-01

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Coenzyme I include both reduced and oxidized forms and plays a role in hydrogen transfer in biological oxidation. Oxidized coenzyme I also called nicotinamide adenine dinucleotide (NAD^+) which is the coenzyme of dehydrogenase. It is an important role in glycolysis, gluconeogenesis, tricarboxylic acid cycle and respiratory chain. Intermediate product will transfer hydrogen to NAD make it become NADH (reduced coenzyme I). NADH acts as a carrier for hydrogen and synthesizes ATP by chemosmosis coupling in the respiratory chain. NADH has important physiological significance in the body. It is closely related to substance metabolism, energy metabolism, anti-cell aging, anti-oxidation and the occurrence of some diseases. A decrease in coenzyme I levels in the body can lead to cell damage or death.

Extract the sample of NAD^+ and NADH with acidic and alkaline extract solution respectively. NADH reduces the oxidized Thiazolyl Blue Tetrazolium Blue (MTT) to form formazan by hydrogen transfer from PMS, formazan has characteristic absorption at 570nm. NAD could be reduced to NADH by alcohol dehydrogenase. Further, MTT reduction method was used to detect NADH.

Kit components

Component	100T	Storage
Acid Extract solution	30mL	2-8℃
Alkaline Extract solution	30mL	2-8℃
Reagent I	15mL	2-8℃
Reagent II	5mL	2-8℃
Reagent III	9 mL	2-8℃
Reagent IV	2.5mL	-20℃
Reagent V	30mL	2-8℃
NAD Standard	Powder	-20℃
NADH Standard	Powder	-20℃

Required but Not Provided:

Spectrophotometer, water bath, desk centrifuge, transferpettor, 1mL glass cuvette, mortar/ homogenizer, ice and distilled water.

Solution Preparation:

1. **Reagent III:** Bright yellow solution, need to strictly avoid light.
2. **Reagent VI:** About 75mL liquid (Required but not provided). Mix 28.8mL of alcohol and 1.2mL of distilled water (Total 30mL,30T), store at RT for 4 weeks. A 30mL brown reagent bottle is provided in the kit. Please label the reagent name yourself.
3. **NAD standard:** Add 1.5mL of distilled water before use to prepare as 2μmol/mL, then dilute it to 1.25nmol/mL NAD standard solution. Before use, 25μL 2μmol/mL NAD standard solution was taken and 975μL distilled water was added to prepare 50nmol/mL NAD standard solution. Then 25μL 50nmol/mL NAD standard solution was mixed with 975μL distilled water to prepare 1.25nmol/mL NAD standard solution.
4. **NADH standard:** Add 1.4mL of distilled water before use to prepare as 2μmol/mL, then dilute it to 1.25nmol/mL NADH standard solution. Before use, 25μL 2μmol/mL NADH standard solution was taken and 975μL distilled water was added to prepare 50nmol/mL NADH standard solution. Then 25μL 50nmol/mL NADH standard solution was mixed with 975μL distilled water to prepare 1.25nmol/mL NADH standard solution.

Protocol

I. Sample preparation

1. Serum

   Extraction of NAD^+: Add 0.5mL of acid extract solution to 0.1mL of serum, boiling 5 minutes (Wrap the sealing film to prevent bursting), ice bath after cooling. Centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant 200μL and add the alkaline extract to about pH 7, then dilute with distilled water to 400μL. Mix thoroughly, centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant, preserve on ice for test. (Equivalent to 0.1mL serum (plasma) + 1.1mL extract solution= 1.2mL liquid volume)

   Extraction of NADH: Add 0.5mL of alkaline extract solution to 0.1mL of serum, boiling 5 minutes (Wrap the sealing film to prevent bursting), ice bath after cooling. Centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant 200μL and add the acidic extract to about pH 7, then dilute with distilled water to 400μL. Mix thoroughly, centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant, preserve on ice for test. (Equivalent to 0.1mL serum (plasma) + 1.1mL extract solution = 1.2mL liquid volume)

2. Tissue

   Extraction of NAD^+: Add 0.5mL of acid extract solution to 0.1g of tissue, grinding on ice, boiling 5 minutes (Wrap the sealing film to prevent bursting), ice bath after cooling. Centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant 200μL and add equal volume alkaline extract solution. Mix thoroughly, centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant, preserve on ice for test. (Equivalent to 0.1g tissue+1mL extract solution)

   Extraction of NADH: Add 0.5mL of alkaline extract solution to 0.1g of tissue, grinding on ice, boiling 5 minutes (Wrap the sealing film to prevent bursting), ice bath after cooling. Centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant 200μL and add equal volume acid extract solution. Mix thoroughly, centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant, preserve on ice for test. (Equivalent to 0.1g tissue+1mL extract solution)

3. Bacteria or cell

   Extraction of NAD^+: Add 0.5mL of acid extract solution to 5 million cells or germ, ultrasonic 1 minutes (power 200W, ultrasonic 2s, interval 1s), boiling 5 minutes (Wrap the sealing film to prevent bursting), ice bath after cooling. Centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant 200μL and add equal volume alkaline extract solution. Mix thoroughly, centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant, preserve on ice for test. (Equivalent to 5 million cells / bacteria + 1mL extract solution)

   Extraction of NADH: Add 0.5mL of alkaline extract solution to 5 million cells or germ, ultrasonic 1minutes (power 200W, ultrasonic 2s, interval 1s) boiling 5 minutes(Wrap the sealing film to prevent bursting), ice bath after cooling. Centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant 200μL and add equal volume acid extract solution. Mix thoroughly, centrifuge at 10000 × g for 10 minutes at 4℃, take supernatant, preserve on ice for test. (Equivalent to 5 million cells / bacteria + 1mL extract solution)

II. Determination procedure.

1. Preheat the spectrophotometer for more than 30 minutes, adjust wavelength to 570nm, and set zero with distilled water.
2. Reagent I, Reagent II and Reagent III should be balanced to room temperature in advance.

3. Operation table (Add reagents according to the following table in 1.5mL EP tube, pay attention to avoid light throughout the process).

Reagents	Test tube (μL)	NAD or NADH	Blank tube
Supernatant	50	-	-
Standard	-	50	-
Distilled water	-	-	50
Reagent I	250	250	250
Reagent II	75	75	75
Reagent III	150	150	150
Reagent IV	35	35	35
Mix well and leave for 20 minutes at room temperature away from light			
Reagent V	500	500	500
Fully dissolved sediments, and determine absorbance at 570nm, the NAD standard tubes were recorded as ΔAS1= AS1-AB, the NADH standard tubes were recorded as ΔAS2= AS2-AB, the NAD test tubes were recorded as ΔAT1= AT1-AB, the NADH test tubes were recorded as ΔAT2= AT2-AB. The blank tube and standard tube only need to be measured 1-2 times. **Note:** (1) Reagent III is unstable under light, so the whole experimental process should be strictly away from light, after the completion of the reaction to the determination of the need to avoid light. (2) If the number of samples is large, reagent I, II and IV can be mixed in proportion as a working solution. The working solution is used up within half an hour after preparation. Reagent III cannot be added after mixing with any reagent, and must be added separately.			

III. Calculation of NAD content

1. Serum (plasma) sample
   NAD^+ content(nmol/mL) = ΔAT1÷(ΔAS1÷CS)×VE÷Vs×F=15×ΔAT1÷ΔAS1×F
2. Protein concentration
   NAD^+ content(nmol/mg prot) = ΔAT1÷(ΔAS1÷CS)×VE÷(VE×Cpr)×F = 1.25×ΔAT1÷ΔAS1÷Cpr×F
3. Sample mass
   NAD^+ content(nmol/g)=ΔAT1÷(ΔAS1÷CS)×VE÷W×F= 1.25×ΔAT1÷ΔAS1÷W×F
4. Cells or germ
   NAD^+ content(nmol/10^4cell)=ΔAT1÷(ΔAS1÷CS)×VE÷N×F=1.25×ΔAT1÷ΔAS1×F

IV. Calculation of NADH content

1. Serum (plasma) sample
   NADH content (nmol/mL) = ΔAT2÷(ΔAS2÷CS)×VE÷VSE×F=15×ΔAT2÷ΔAS2×F
2. Protein concentration
   NADH content (nmol/mg prot) =ΔAT2÷(ΔAS2÷CS)×VE÷(VS×Cpr)×F= 1.25×ΔAT2÷ΔAS2÷Cpr×F
3. Sample mass
   NADH content (nmol/g) =ΔAT2÷(ΔAS2÷CS)×VE÷W×F=1.25×ΔAT2÷ΔAS2÷W×F
4. Cells or germ
   NADH content (nmol/10^4cell)=ΔAT2÷(ΔAS2÷CS)×VE÷N×F=1.25×ΔAT2÷ΔAS2×F

CS: Concentration of NAD and NADH standard, 1.25nmol/mL;
Cpr: Protein concentration, mg/mL;
VE: Extract solution volume, 1mL;
VSE: Total volume of serum (plasma) during extraction, 1.2mL; W: Sample mass, g;
N: cell number, count by 10^6;
F: Dilution factor.

Notes

1. The NAD^+ and NADH is unstable and it is recommended to use fresh samples for determination.
2. The room temperature range of this experiment should be 20-25℃.
3. If A_T> 1, it is recommended to dilute the sample with distilled water and re-determination.
4. The protein will be denatured by the extract solution. If the protein concentration is used to calculate NAD or ANDH content, the sample needs to be re-extracted.
5. Each tube can measure NAD^+ or NADH of 1 sample. The kit 50T can measure NAD^+ or NADH of 48 samples.