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Coenzyme Ⅰ NAD(H) Content Assay Kit

Note： It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Microplate reader/ Spectrophotometer

Cat No: AK0559

Size: 100T/48S

Components:

Acid extract solution：Liquid 25 mL×1. Storage at 4℃.

Alkaline extract solution：Liquid 25 mL×1. Storage at 4℃.

Reagent I：Liquid 5mL×1. Storage at 4℃.

Reagent II：Liquid 1.5mL×1. Storage at 4℃.

Reagent III： Powder×1. Storage at -20℃. Add 1.6 mL of double distilled water before use, mix thoroughly, store at 4℃ for one week；

Reagent IV：Powder××1. Storage at 4℃. Add 1.9 mL of double distilled water before use, mix thoroughly, store at 4℃ for one week；

Reagent V：Liquid 0.7mL×1. Storage at 4℃.

Reagent VI：Liquid 15mL×1. Storage at 4℃.

Reagent VII：Self-provided, take 19.2 mL of alcohol and 0.8 mL of distilled water, mix thoroughly.

NAD standard： Powder×1. Storage at -20℃. Add 3 mL of distilled water before use to prepare as 1μmol/mL, then dilute it to 1.25nmol/mL NAD standard solution.

NADH standard：Powder×1. Storage at -20℃. Add 2.8 mL of distilled water before use to prepare as 1 μmol/mL, then dilute it to 1.25 nmol/mL NADH standard solution.

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 NAD+.

Required but Not Provided:

Microplate reader/Spectrophotometer, desk centrifuge, desk centrifuge, transferpettor, micro glass cuvette/ 96 well flat-bottom plate, mortar/ homogenizer, ice and distilled water.

Protocol

I. Extraction of NAD^+ and NADH:

1. Serum

Extraction of NAD^+: Add 0.5 mL of acid extract solution to 0.1 mL of serum, boiling 5 minutes, ice bath after cooling. Centrifuge at 10000 ×g for 10 minutes at 4℃, take supernatant 200 μL 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.

Extraction of NADH: Add 0.5 mL of alkaline extract solution to 0.1 mL of serum, boiling 5 min, ice bath after cooling. Centrifuge at 10000 ×g for 10 minutes at 4℃, take supernatant 200 μL 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.

2. Tissue

Extraction of NAD^+: Add 0.5 mL of acid extract solution to 0.1 g of tissue, grinding on ice, boiling 5 minutes, ice bath after cooling. Centrifuge at 10000 ×g for 10 minutes at 4℃, take supernatant 200 μL 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.

Extraction of NADH: Add 0.5 mL of alkaline extract solution to 0.1 g of tissue, grinding on ice, boiling 5 minutes, ice bath after cooling. Centrifuge at 10000 ×g for 10 minutes at 4℃, take supernatant 200 μL 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.

3. Cells or microorganism:

Extraction of NAD^+: Add 0.5 mL of acid extract solution to 5 million cells or germ, ultrasonic 1min(power 20% or 200W, ultrasonic 2s, interval 1s), boiling 5 minutes, ice bath after cooling. Centrifuge at 10000 ×g for 10 minutes at 4℃, take supernatant 200 μL 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.

Extraction of NADH: Add 0.5 mL of alkaline extract solution to 5 million cells or germ, ultrasonic 1min (power 20% or 200W, ultrasonic 2s, interval 1s) boiling 5 minutes, ice bath after cooling. Centrifuge at 10000 ×g for 10 minutes at 4℃, take supernatant 200 μL 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.

II. Procedure:

1.Preheat spectrophotometer/ microplate reader for 30 minutes, adjust wavelength to 570 nm, set zero with distilled water.

2.Add reagents according to the following table.

Reagents	Contrast tube (μL)	Test tube (μL)	NAD or NADH	空白管
Supernatant	10	10	-	-
Standard	-	-	10	-
Distilled water				10

Reagent VI	100	-	-	-
Reagent I	50	50	50	50
Reagent II	15	15	15	15
Reagent III	15	15	15	15
Reagent IV	15	15	15	15
Reagent V	7	7	7	7
Mix thoroughly, incubate prevent from light at room temperature for 20 minutes.				
Reagent VI	-	100	100	100
Mix thoroughly, place for 5 minutes, centrifuge at 15000 rpm at 25℃ for 15 minutes, discard supernatant.				
Reagent VII	200	200	200	200

Mix thoroughly, take 200 μL into a micro glass cuvette or 96 well plate, colorimetric at 570 nm, record the absorbance $\Delta A=A_T-A_C$, the standard tube of NAD, record $\Delta A_{S1}=A_{S1}-A_B$. The standard tube of NADH, record $\Delta A_{S2}=As_2-A_B$. Blank tube just need test one to twice.

Calculation

The content of NAD^+

1. Serum (plasma) sample

$NAD+(nmol/mL) =\Delta A_T \div (\Delta A_{S1} \div C_S) \times V_E \div Vs = 12.5 \times \Delta A_T \div \Delta A_{S1}$

2. Tissue, germ or cells

1) Protein concentration

$NAD+ (nmol/mg\ prot) =\Delta A_T \div (\Delta A_{S1} \div C_S) \times V_E \div (V_E \times Cpr) = 1.25 \times \Delta A_T \div \Delta A_{S1} \div Cpr$

2) Sample weight

$NAD+ (nmol/g) = \Delta A_T \div (\Delta A_{S1} \div C_S) \times V_E \div W = 1.25 \times \Delta A_T \div \Delta A_{S1} \div W$

3) Cells or germ

$NAD+ (nmol/10^4 cell) = \Delta A_T \div (\Delta A_{S1} \div C_S) \times V_E \div 500 = 0.0025 \times \Delta A_T \div \Delta A_{S1}$

The content of NADH

1. Serum (plasma) sample

$NADH\ (nmol/mL) = \Delta A_T \div (\Delta AS2 \div CS) \times V_E \div V_{SE} == 12.5 \times \Delta A_T \div \Delta A_{S2}$

2. Tissue, germ or cells

1) Protein concentration

$NADH\ (nmol/mg\ prot) = \Delta A_T \div (\Delta A_{S2} \div C_S) \times V_E \div (V_S \times Cpr) = 1.25 \times \Delta A_T \div \Delta A_{S2} \div Cpr$

2) Sample weight

$NADH\ (nmol/g) = \Delta A_T \div (\Delta A_{S2} \div C_S) \times V_E \div W = 1.25 \times \Delta A_T \div \Delta A_{S2} \div W$

3) Cells or germ

$NADH\ (nmol/10^4 cell) = \Delta A_T \div (\Delta A_{S2} \div C_S) \times V_E \div 500 = 0.0025 \times \Delta A_T \div \Delta A_{S2}$

CS： Concentration of NAD and NADH standard, 1.25 nmol/mL；

Cpr: Protein concentration, mg/mL;

V_E：Extract solution volume, 1 mL;
V_{SE}：Serum volume, 0.1 mL;
W：Sample weight, g;
500: 5 million cells.

Note:
1. Reagents I, II, III, and IV need add separately, must not be mixed ahead of time.
2. Avoid light during reaction.
3. If the absorbance more than 1, measure the sample after diluting, multiply dilution times during equation.

Experimental examples:
1. Extraction of NAD^+: weigh about 0.1 g of lung, add 0.5 mL of Acid extract solution, grind in ice bath, boil for 5 min (cover tightly to prevent water loss), cool in ice bath, centrifuge at 4℃ and 10000g for 10 min, take 200 μL supernatant, add equal volume of Alkaline extract solution, mix well, After centrifugation at 4℃ and 10000g for 10 min, the supernatant is taken out, and then the operation is carried out according to the determination steps. The results showed that $\Delta A_T = A_T - A_C = 0.507-0.2=0.307$, $\Delta A_{S1}=A_{S1}-A_B=0.612-0.144=0.468$, the content of NAD^+ is calculated according to the sample mass: NAD^+ (nmol/g mass) $= 1.25 \times \Delta A_T \div A_{S1} \div W = 1.25\times0.307\div0.468\div0.1=8.1998$ nmol/g mass.
Extraction of NADH: weigh about 0.1g lung, add 0.5ml Alkaline extract solution, grind in ice bath, boil for 5min (cover tightly to prevent moisture), cool in ice bath, centrifuge at 4℃ and 10000g for 10 min, take 200 μL supernatant, add equal volume of Acid extract solution, mix well, After centrifugation at 4℃ and 10000g for 10 min, the supernatant is taken out, and then the operation is carried out according to the determination steps. The results showed that $\Delta A_T = A_T-A_C=0.183-0.077=0.106$, $\Delta A_{S2} =A_{S2}-A_B = 0.399-0.143=0.256$, the content of NADH is calculated according to the sample mass: NADH (nmol/g mass) $= 1.25 \times \Delta A_T \div A_{S2} \div W=1.25\times0.106\div0.256\div0.1=5.1758$ nmol/g mass.
2. Extraction of NAD^+: weigh about 0.1 mL of horse serum, add 0.5 mL of Acid extract solution, grind in ice bath, boil for 5 min (cover tightly to prevent water loss), cool in ice bath, centrifuge at 10000g and 4℃ for 10min, take 200 μL of supernatant, add equal volume of Alkaline extract solution, mix well, After centrifugation at 4℃ and 10000g for 10 min, the supernatant is taken out, and then the operation is carried out according to the determination steps. The results showed that $\Delta A_T = A_T - A_C =0.120-0.071=0.049$, $\Delta A_{S1} =A_{S1}-A_B =0.612-0.144=0.468$, NAD^+ content (nmol/mL) $=12.5\times\Delta A_T\div\Delta A_{S1} =12.5\times0.049\div0.468 =1.3088$ nmol/mL.
Extraction of NADH: weigh about 0.1 mL of horse serum, add 0.5 mL of Alkaline extract solution, grind in ice bath, boil for 5min (cover tightly to prevent moisture), cool in ice bath, centrifuge 10000g at 4 ℃ for 10min, take 200 μ l supernatant, add equal volume of Acid extract solution, mix well, After centrifugation at 4℃ and 10000g for 10 min, the supernatant is taken out, and then the operation is carried out according to the determination steps. The results showed that ΔAT = AT - AC =0.089-0.065=0.024, $\Delta A_{S2} =A_{S2}-A_B=0.399-0.143=0.256$, the NADH content (nmol/mL) $= 12.5\times\Delta A_T\div A_{S2}=12.5\times0.024\div0.256=1.1719$ nmol/mL.

Recent Product Citation:

[1] Xiaofen Fu,Pengsong Li,Lei Zhang,et al. Understanding the stress responses of Kluyveromyces marxianus after an arrest during high-temperature ethanol fermentation based on integration of RNA-Seq and metabolite data. Applied Microbiology and Biotechnology. March 2019;103(6) :2715–2729.(IF3.67)

[2] Mengqing Tao,Jia Jiang,Lin Wang,et al. α-Mangostin Alleviated Lipopolysaccharide Induced Acute Lung Injury in Rats by Suppressing NAMPT/NAD Controlled Inflammatory Reactions. Evidence-Based Complementary and
Alternative Medicaine. 2018;(IF1.984)

[3] Bin Zhang,Dongmei Shi, Xiangyu Zhang,et al. FK866 inhibits the epithelial-mesenchymal transition of hepatocarcinoma MHCC97-H cells. Oncology Letters. October 2018;(IF1.871)

[4] Yang K, Yin Q, Mao Q, et al. Metabolomics analysis reveals therapeutic effects of α-mangostin on collagen-induced arthritis in rats by down-regulating nicotinamide phosphoribosyltransferase[J]. Inflammation, 2019, 42(2): 741-753.

References：

[1] Ying W. NAD^+/NADH and $NADP^+$/NADPH in cellular functions and cell death: regulation and biological consequences[J]. Antioxidants & redox signaling, 2008, 10(2): 179-206.

[2] Gibon Y, Larher F. Cycling assay for nicotinamide adenine dinucleotides: NaCl precipitation and ethanol solubilization of the reduced tetrazolium[J]. Analytical biochemistry, 1997, 251(2): 153- 157.

Related Products：

AK0548/AK0547	Rubisco-Bisphosphate Carboxylase/Oxygenase (Rubisco) Activity Assay Kit
AK0528/AK0527	NADH Oxidase(NOX) Activity Assay Kit
AK0500/AK0499	NAD Kinase(NADK) Activity Assay Kit
AK0498/AK0497	NAD-Malate Dehydrogenase(NAD-MDH) Activity Assay Kit
AK0496/AK0495	NADP-Malate Dehydrogenase(NAD-MDH) Activity Assay Kit
AK0494/AK0493	Citrate Synthase(CS) Activity Assay Kit
AK0484/AK0483	NAD Malic Enzyme(NAD- ME) Activity Assay Kit