NAD-Malate Dehydrogenase (NAD-MDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC1040

Size: 50T/48S

Components:

Extract solution: 50 mL×1, store at 4℃.

Reagent I: 40 mL×1, store at 4℃.

Reagent II: Powder×2, store at -20℃. Add 360 μL of distilled water when the solution will be used. The rest of reagentstore at -20℃.

Reagent III: Powder×2, store at -20℃. Add 327 μL of distilled water when the solution will be used. The rest of reagentstore at -20℃.

Product Description:

Malate Dehydrogenase (MDH, EC 1.1.1.37） is widely exist in animal, plant, microbe and cells culture. MDH in mitochondria is one of the key enzymes of TCA cycle, which catalyzes the formation of oxaloacetic acid from malic acid. In contrast, MDH in serum catalyzes the formation of malic acid from oxaloacetic acid. Oxaloacetic acid is an important intermediate that connects several important metabolic pathways. MDH plays an important role in many physiological activities of cells, including mitochondrial energy metabolism, malic acid-aspartic acid shuttle system, reactive oxygen species metabolism and disease resistance. According to the different coenzyme specificity, MDH is divided into NAD- dependent MDH and NADP- dependent MDH. Bacteria usually only contain NAD-MDH. NAD-MDH is distributed in the cytoplasm and mitochondria in eukaryotic cells.

NAD-MDH catalyzes NADH to reduce oxaloacetic acid into malic acid, which resulting in a decrease in absorption at 340nm.

Required but Not Provided:

Ultraviolet spectrophotometer,desk centrifuge, water-bath, adjustablepipette, 1mL quartzcuvette, distilled water.

Protocol

I. Preparation:

1. Cells or bacterial

Collect bacteria or cells into the centrifuge tube. The liquid in the upper layer is discarded after centrifugation. It is suggested to take about 2 million bacteria/cell and add 400μLof Extractsolution. Bacteria/cell is splitby ultrasonic(power 20%, ultrasonic 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 ×g and4℃ for 10minutes. Take the supernatant and placed it on ice for testing.

2. Tissue:

It is suggested to take about 0.05 g of tissue and add 1 mL of Extract solution.Then ice bath

homogenization should be carried out. Centrifuge at 8000 ×gand4℃ for 10 minutes. The supernatant is placed on ice for testing.

3. Serum: detect directly.

II. Procedure:

Preheat ultraviolet spectrophotometer for 30minutes, adjust wavelength to 340nm, set zero with Distilled water. PreheatReagent I in 37℃ for 15minutes.

Reagent (μL)	Test tube (T)	Blank tube (B)
Sample	20	-
Distilled water	-	20
Reagent I	760	760
Reagent II	10	10
Reagent III	10	10

The above reagents are added into a 1mL of quartzcuvette in sequence. After fully mixing, the initial absorbance A1 and the absorbance A2 after 1minuteofreactionare recorded at 340 nm wavelength, and the reaction temperature is kept at 37 ℃ as far as possible.ΔA=A1-A2. Record ΔA_T, ΔA_B.

III. NAD-MDH Calculation:

1. Serum

   Unit definition: One unit ofenzyme activity is defined as theamountofenzymeconsumes 1 nmol of NADH per minute in the reaction system every milliliter serum.

   NAD-MDH(U/mL)=$(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RT}\div V_{SA}\div T$ =6430 ×$(\Delta A_T-\Delta A_B)$

2. Tissue

1) Protein concentration:

   Unit definition: One unit ofenzyme activity is defined as theamountofenzymeconsumes 1 nmol of NADH per minute in the reaction system every milligram tissue protein.

   NAD-MDH(U/mg prot)=$(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RT}\div(V_{SA}\times Cpr)\div T$ = 6430 ×$(\Delta A_T-\Delta A_B)$ ÷Cpr

2) Sample weight:

   Unit definition: One unit of enzyme activity is defined as the amount of enzyme consumes 1 nmol of NADH per minute in the reaction system every gram tissue.

   NAD-MDH(U/g)=$(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RT}\div(W\div V_E\times V_{SA})\div T$=6430×$(\Delta A_T-\Delta A_B)$ ÷W

3. Cells or germ

1) Protein concentration:

   Unit definition: One unit of enzyme activity is defined as the amount of enzyme consumes 1 nmol of NADH per minute in the reaction system every milligram tissue protein.

   NAD-MDH(U/mg prot)=$(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RT}\div(V_{SA}\times Cpr)\div T$ = 6430×$(\Delta A_T-\Delta A_B)$ ÷Cpr

2) Cells or germ

   Unit definition: One unit of enzyme activity is defined as the amount of enzyme consumes 1 nmol of NADH per minute in the reaction system every 10 thousand cells or germ.

NAD-MDH(U/10^4 cell)=(ΔA_T-ΔA_B)÷(ε×d)×V_{RT}÷(500×V_{SA})÷T=13×(ΔA_T-ΔA_B)

V_{RT}: Total reaction volume, 0.8mL;
V_{SA}: Sample volume, 0.02mL;
ε: NADH molar extinction coefficient, 6.22×10^{-3}mL/nmoL/cm;
d: Light path of cuvette, 1cm;
T: Reaction time, 1minute;
Cpr: Protein concentration, mg/mL;
500: Cells or germ, 10^4/mL

Note:

1. The extraction of crude enzyme must be done at 0℃-4℃ to prevent enzyme denaturation and inactivation.
2. Reagent II, III and samplemust keep on ice during experiment in order to avoiding denaturation and inactivation.
3. When micro quartz cuvette with the light diameter of 1 cm is used, if the absorbance less than 0.7 or ΔA>0.5, dilute the liquid before recommending.When 96 well flat-bottomplate(UV plate) is used, if the absorbance less than 0.4 or ΔA>0.3, dilute the liquid before recommending.
4. It is suggestted that two people to cooperate in the experiment, one adding the sample and the other colorimetric.
5. Blank tube only needs to measure 1-2 times.

Experimental instances:

1. Take 0.05g of rat liver, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. Calculate ΔA_T=$A1_T$ –$A2_T$ =0.917-0.293=0.624, ΔA_B =$A1_B$-$A2_B$=0.789-0.784=0.005, calculate the enzyme activity according to sample weight:
NAD-MDH（U/g weight）=6431×(ΔA_T-ΔA_B) ÷W=6431×0.619 ÷0.05=79616 U/g weight.
2. Take 0.05g Willow leaf, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. Calculate ΔA_T= $A1_T$-$A2_T$ =0.812-0.741=0.071, ΔA_B =$A1_B$-$A2_B$=0.789-0.784=0.005, calculate the enzyme activity according to sample weight:
NAD-MDH（U/g weight）=6431×(ΔA_T-ΔA_B) ÷W=6431×0.066 ÷0.05=8489 U/g weight.
3. Take 20μL serum of mouse to detect directly, calculate ΔA_T= $A1_T$-$A2_T$ =0.855-0.789=0.066, ΔA_B =$A1_B$-$A2_B$=0.789-0.784=0.005, calculate the enzyme activity according to volume of serum:
NAD-MDH（U/mL）=6431×(ΔA_T-ΔA_B)=6431×0.061=392 U/mL.

References:

[1] Yao Y X, Li M, Zhai H, et al. Isolation and characterization of an apple cytosolic malate dehydrogenase gene reveal its function in malate synthesis[J]. Journal of plant physiology, 2011, 168(5): 474-480.

Related products:

BC0310/BC0315	CoenzymeINAD (H) Content Assay Kit
BC1030/BC1035	NAD Kinase (NADK) Assay Kit
BC0630/BC0635	NADH oxidase (NOX) Activity Assay Kit
BC1130/BC1135	NAD Malic Enzyme (NAD-ME) Assay Kit