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/ Microplate reader

Cat No: BC1045

Size: 100T/96S

Components:

Extract solution: Liquid 100 mL×1, store at 4℃.

Reagent I: Liquid 20 mL×1, store at 4℃.

Reagent II: Powder×1, store at -20℃. Working solution: add 360 μL of distilled water when the solution will be used. The rest of reagent store at -20℃.

Reagent III: Powder×1, store at -20℃. Working solution: add 327 μL of distilled waterwhen the solution will be used. The rest of reagent store at -20℃.

Product Description:

Malate Dehydrogenase (MDH, EC 1.1.1.37）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, resulting in the decrease of light absorption at 340nm.

Required but Not Provided:

Spectrophotometer/ Microplate reader, desk centrifuge, water-bath, adjustablepipette, micro quartz cuvette/ 96 well flat-bottom plate (UV plate) and distilled water.

Protocol

I. Preparation:

1. Cells or bacterial

Collecting 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 400 μLof Extractsolution. Bacteria and cell is split by ultrasonic (power 20%, ultrasonic 3s，interval 10s，repeat for 30 times). Centrifuge at 8000 ×g and 4℃ for 10 minutes. Take the supernatant and placed on ice for test.

2. Tissue:

It is suggested to take about 0.1 g of tissue and add 1 mL of Extract solution. Then ice bath

homogenization should be carried out. Centrifuge at 8000 ×g and 4℃ for 10 minutes. Take the supernatant and placed on ice for testing.

3. Serum: Directly detect.

II. Procedure:

Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 340 nm, set zero with distilled water. Preheat Reagent I at 37℃ for 15 minutes.

Name of Reagent(μL)	Test tube	Blank tube
Sample	5	-
Distilled water	-	5
Reagent I	190	190
Reagent II	2.5	2.5
Reagent III	2.5	2.5

The above reagents are added into a micro quartz colorimetric dish/96 well flat-bottom plate (UV plate) in sequence. After full mixing, the initial absorbance A1 and the absorbance A2 after 1 minute of reaction are 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:

A. Micro quartz cuvette

1. Serum

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

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

2. Tissue

1) Protein concentration:

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

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

2) Sample weight:

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

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

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.

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

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, 0.2 mL;
V_{SA}: Sample volume, 0.005 mL;
ε: NADH molar extinction coefficient, 6.22×10^{-3} mL/nmoL/cm;
d: Light path of cuvette, 1 cm;
T: Reaction time, 1 minute;
Cpr: Protein concentration, mg/mL;
500: Cells or germ, 10^4/mL

B: 96 well flat-bottom plate(UV plate)

1. Serum

Unit defenition: One unit of enzyme activity is defined as theamountof enzyme consumes 1 nmol of NADH per minute in the reaction system every milliliter serum.

NAD-MDH(U/mL)=(ΔA_T-ΔA_B)÷(ε×d)×V_{RT}÷V_{SA}÷T =12860×(ΔA_T-ΔA_B)

2. Tissue

1) Protein concentration

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

NAD-MDH(U/mg prot)=(ΔA_T-ΔA_B)÷(ε×d)×V_{RT}÷(V_{SA}×Cpr)÷T=12860×(ΔA_T-ΔA_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)=(ΔA_T-ΔA_B)÷(ε×d)×V_{RT}÷(W÷V_E×V_{SA})÷T =12860×(ΔA_T-ΔA_B) ÷W

3. Cells or germ

1) Protein concentration:

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

NAD-MDH(U/mg prot)=(ΔA_T-ΔA_B)÷(ε×d)×V_R÷(V_{SA}×Cpr)÷T= 12860 ×(ΔA_T-ΔA_B) ÷Cpr

2) Cells or germ:

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

NAD-MDH(U/10^4 cell)=(ΔA_T-ΔA_B)÷(ε×d)×V_{RT}÷(500×V_{SA})÷T=26×(ΔA_T-ΔA_B)

V_{RT}: Total reaction volume, 0.2 mL;
V_{SA}: Sample volume, 0.005 mL;
ε: NADH molar extinction coefficient, 6.22×10^{-3} mL/nmoL/cm;
d: Light path of 96-well-plate, 0.5 cm;
T: Reaction time, 1 minute;
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 sample must 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 the 96 well flat-bottom plate (UV plate) is used, if the absorbance less than 0.4 or ΔA>0.3, dilute the liquid before recommending.
4. It is suggested 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, according to the measured steps, put it in added into a micro quartz colorimetric dish to measure and calculate $\Delta A_T=A1_T-A2_T=0.8484-0.3068=0.5416$, $\Delta A_B=A1_B-A2_B=0.7628-0.7583=0.0045$, calculate the enzyme activity according to sample weight:

NAD-MDH（U/g weight）$=6431\times(\Delta A_T-\Delta A_B)\div W=6431\times0.5371\div0.05=69082$ 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 $\Delta A_T=A1_T-A2_T=0.7796-0.6917=0.087$, $\Delta A_B=A1_B-A2_B=0.7628-0.7583=0.0045$, calculate the enzyme activity according to sample weight:

NAD-MDH（U/g weight）$=6431\times(\Delta A_T-\Delta A_B)\div W=6431\times0.0834\div0.05=10727$ U/g weight.

3. Take 5μL serum to detect directly according to the measured steps, calculate $\Delta A_T=A1_T-A2_T=0.8194-0.7959=0.0235$， $\Delta A_B=A1_B-A2_B=0.7628-0.7583=0.0045$, calculate the enzyme activity according to volume of serum:

NAD-MDH（U/mL）$=6431\times(\Delta A_T-\Delta A_B)=6431\times0.019=122$ 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