NADP-Malate Dehydrogenase (NADP-MDH) Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC1055

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℃. Add 500 μL distilled water when the solution will be used. The rest of reagent store at -20℃;

Reagent III: Powder×1, store at -20℃. Add 600 μL distilled water when the solution will be used. The rest of reagent store at -20℃;

Product Description:

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. NADP-MDH is mainly present in eukaryotic cells.

NADP-MDH catalyzes NADPH to reduce oxaloacetic acid into malic acid, resulting in a decrease in absorption at 340 nm.

Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, water-bath, adjustablepipette, micro quartz cuvette/96-well flat-bottomUV plate and distilled water.

Protocol

I. Preparation:

1. Cells or bacterial

Collect bacteria or cells into the centrifuge tube. Discard the supernatant after centrifugation. It is suggested to take about 2 million bacteria/cell and add 400 μL ofExtractsolution. Bacteria/cell is split by ultrasonic (power 20%, ultrasonic 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 g 4℃ for 10 minutes. Take the supernatant on ice for test.

2. Tissue:

Add 1 mL of Extract solutionto 0.1 g of tissue. Homogenate on ice. Centrifuge at 8000 g and4℃ for 10 minutes. Take the supernatant on ice for test.

3. Serum (plasma): detect directly.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set the counter to zero with distilled water.

2. Preheat reagent I in 37℃ for 15 minutes.

3. Operation table:

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Sample	5	-
Distilled water	-	5
Reagent I	185	185
Reagent II	5	5
Reagent III	5	5

The above reagents are added into the micro quartz cuvette/96 well UV plate in sequence. Mix thoroughly. The initial absorbance A1 and the absorbance A2 after reaction 1 minute are recorded at 340 nm wavelength, and keep the reaction temperature at 37℃ as far as possible. $\Delta A_B=A1_B-A2_B$. $\Delta A_T=A1_T-A2_T$. $\Delta A=\Delta A_T-\Delta A_B$.

III. NADP-MDH Calculation:

a. Micro quartz cuvette

1. Serum

Unit definition: One unit of enzyme activityis defined as the amount of enzyme catalyzes the consumes of 1 nmol NADPH per minute in the reaction systemevery milliliter serum.

$NADP\text{-}MDH(U/mL)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div V_{SA}\div T\times 10^9=6430\times\Delta A$

2. Tissue

1) Protein concentration:

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

$NADP\text{-}MDH(U/mg\ prot)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(Cpr\times V_{SA})\div T\times 10^9=6430\times\Delta A\div Cpr$

2) Sample weight:

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

$NADP\text{-}MDH(U/g\ weight)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(W\div V_E\times V_{SA})\div T\times 10^9=6430\times\Delta A\div W$

3. Cells or germ

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumes of 1 nmol NADPH per minute in the reaction system every 10^4 cells or germ.

$NADP\text{-}MDH(U/10^4\ cell)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(200\times V_{SA}\div V_E)\div T\times 10^9=12.8\times\Delta A$

ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d: Light path of cuvette, 1 cm;

V_{RT}: Total reaction volume, 0.0002 L;

V_{SA}: Sample volume, 0.005 mL;
V_E: Extract solution volume of cells, 1 mL;
200: Cells or germ, 2 million;
T: Reaction time, 1 minute;
Cpr: Protein concentration, mg/mL;

b. 96 well flat-bottom plate
The optical diameter d=1 cm of the cuvette in the above formula is changed to 0.6 cm of the 96 well flat-bottom UV plate.

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. Reagent I should be placed at 37℃.
3. It is suggested that one person add samples and one person compare colors. Because of the small time monitoring range, it is not recommended to use 96 wellflat-bottom UV plate to measure multiple samples at the same time.
4. Blank tube just test 1-2 times.
5. When the initial value is less than 0.7 or ΔA is greater than 0.5, it is recommended to measure after dilution.

Experimental instances：
1. Take 0.1g of heart tissue, 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 calculateΔA_T $=A1_T-A2_T=0.7173-0.6399=0.0774$, $\Delta A_B=A1_B-A2_B=0.6583-0.6562=0.0021$, $\Delta A=\Delta A_T-\Delta A_B=0.0753$, calculate the enzyme activity according to sample weight:
NADP-MDH（U/g weight）$=6430\times\Delta A\div W=6430\times0.0753\div0.1=4841.79$ U/g weight.
2. Take 5μL serum to detect directly according to the measured steps, $\Delta A_T=A1_T-A2_T=0.6934-0.6854=0.008$，$\Delta A_B=A1_B-A2_B=0.6583-0.6562=0.0021$, $\Delta A=\Delta A_T-\Delta A_B=0.0059$, calculate the enzyme activity according to volume of serum:
NAD-MDH（U/mL）$=6430\times\Delta A=6430\times0.0059=37.937$ 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