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

Cat No: BC1050

Size: 50T/48S

Components:

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

Reagent I: Liquid 40 mL×1, store at 4℃;

Reagent II: Powder×2, store at -20℃. Add 250 μL distilled water when the solution will be used. The rest of reagent store at -20℃;

Reagent III: Powder×2, store at -20℃. Add 300 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, desk centrifuge, water-bath, adjustablepipette, 1 mL quartz cuvette 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 Extractsolutionto 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 30 minutes.

3. Operation table:

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

The above reagents are added into the 1 mL quartz cuvette in sequence. Mix thoroughly. The initial absorbance A1 and the absorbance A2 after reaction 1 minute are recorded at 340 nm wavelength. $\Delta A_B=A1_B-A2_B$. $\Delta A_T=A1_T-A2_T$. $\Delta A=\Delta A_T-\Delta A_B$.

III. NADP-MDH Calculation:

1. Serum

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 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, 0.0008 L;

V_{SA}: Sample volume, 0.02 mL;

V_E: Extract solution volume of cells, 0.4 mL;

200: Cells or germ, 2 million;
T: Reaction time, 1 minute;
Cpr: Protein concentration, mg/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. Reagent I should be placed at 37℃.

3. It is suggested that one person add samples and one person compare colors. Try to keep the reaction temperature at 37℃.

4. Blank tube just test 1-2 times.

Experimental instances：

1. Take 0.1g of rat heart tissue, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. Calculate $\Delta A_T=A1_T-A2_T=0.694-0.628=0.066$， $\Delta A_B=A1_B-A2_B=0.652-0.643=0.009$， $\Delta A=\Delta A_T-\Delta A_B=0.057$, calculate the enzyme activity according to sample weight:

NAD-MDH（U/g weight）=6430×ΔA÷W=6430×0.057÷0.1=3665.1 U/g weight.

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