EZMeta™ NAD-Malate Dehydrogenase (NAD-MDH) Colorimetric Activity Kit

Cat #: D-AKC3021

Size: 48T / 96T

Storage: Stored at -20℃ for 6 months, protected from light

Product Information

Applicable samples: Animal Tissue, Plant Tissue, Cells, Bacteria, Serum, Plasma

Assay Principle

Malate dehydrogenase (MDH, EC1.1.1.37) exists widely in animals, plants, microorganisms and cultured cells. MDH in mitochondria is one of the key enzymes of tricarboxylic acid cycle (TCA), which catalyzes malate to oxaloacetic acid. On the contrary, MDH in cytoplasm catalyzes oxaloacetic acid to malate. Oxaloacetic acid is an important intermediate, which connects many important metabolic pathways. Therefore, MDH plays an important role in a variety of cellular physiological activities, including mitochondrial energy metabolism, malic acid-aspartic acid shuttle system, active oxygen metabolism and disease resistance. According to different coenzyme specificity, MDH can be divided into NAD-dependent MDH and NADP-dependent MDH. EZMeta™ NAD-Malate Dehydrogenase (NAD-MDH) Colorimetric Activity Kit provides a simple, convenient and rapid NAD-MDH activity detection method, which is suitable for the detection of animal and plant tissues, cells, bacteria, serum (plasma) and other samples. The detection principle is that NAD-MDH catalyzes NADH to reduce oxaloacetic acid to malic acid, resulting in a decrease in light absorption at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	50 mL	100 mL	4°C
Reagent Ⅱ	10 mL	20 mL	4°C
Reagent Ⅲ	1	1	-20°C, protected from light

Materials Required but Not Supplied

·Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm

·96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips

·Ice maker, refrigerated centrifuge, water bath

·Homogenizer (for tissue samples)

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Working Solution: Before use, Reagent Ⅱ poured into ReagentⅢ, fully mixed and ready for use. Unused reagents can also be stored at -20°C and protected from light after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month. All samples and reagents should be on ice to avoid denaturation and deactivation.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Reagent Ⅰ and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent Ⅰ to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and

place it on ice to be tested.

3. Serum (Plasma): Direct detection.

Note: It will be better to quantify the total protein with EZMeta™ NAD-Malate Dehydrogenase (NAD-MDH) Colorimetric Activity Kit, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Working Solution for 10 min at 37°C (mammal) or 25°C (other).

3. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

Reagent	Blank Well（μL）	Test Well（μL）
Sample	0	5
Reagent Ⅰ	5	0
Working Solution	195	195

4. After mixing quickly, record the absorbance values of 20 s and 1 min 20 s at 340 nm, mark as A_1 and A_2, and calculate $\Delta A_{Test}=(A_{Test2}-A_{Test1})-(A_{Blank2}-A_{Blank1})$.

Note: Blank well only needs to measure 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Reagent Ⅰ, the calculated result multiplied by the dilution factor. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

1. Calculation of NAD-MDH activity in serum

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by each mL of serum per min.

NAD-MDH (U/mL)=[$\Delta A_{Test} \times V_{Total} \div (\varepsilon \times d) \times 10^9$]$\div V_{Sample} \div T$=**12,860×$\Delta A_{Test}$**

2. Calculation of NAD-MDH activity in tissue of the sample

(1) Calculation according to the protein concentration of the sample

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 mg tissue proteins per min.

NAD-MDH (U/mg prot)=[$\Delta A_{Test} \times V_{Total} \div (\varepsilon \times d) \times 10^9$]$\div (V_{Sample} \times Cpr) \div T$=**12,860×$\Delta A_{Test}$÷Cpr**

(2) Calculation according to the weight of the sample

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 g tissue per min.

NAD-MDH (U/g fresh weight)=[$\Delta A_{Test} \times V_{Total} \div (\varepsilon \times d) \times 10^9$]$\div (W \div V_{Reagent\ I} \times V_{Sample}) \div T$=**12,860×$\Delta A_{Test}$÷W**

3. Calculation of NAD-MDH activity in cells or bacteria

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 10^4 cells or bacteria per min.

NAD-MDH (U/10^4)=[$\Delta A_{Test} \times V_{Total} \div (\varepsilon \times d) \times 10^9$]$\div (V_{Sample} \div V_{Reagent\ I} \times 500) \div T$=**25.72×$\Delta A_{Test}$**

Where: V_{Total}: the total volume of the reaction system, 0.2 mL=2×10^{-4} L; $V_{Reagent\ I}$: the volume of the Reagent Ⅰ, 1 mL; V_{Sample}: the volume of the supernatant in the reaction system, 0.005 mL; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; Cpr: protein concentration (mg/mL); T: reaction time, 1 min; W: sample weight, g; 500: total number of cells or bacteria, 5 million; 10^9: unit conversion factor, 1 mol=10^9 nmol.

B. Microquartz cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.