(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Malate Dehydrogenase Assay Kit (Tissue)

Catalog No.: BC00191

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Malate Dehydrogenase Assay Kit (Tissue)
Detection Method	Colorimetric
Sample Type	Tissue
Assay Type	Quantitative
Detection Instrument	Ultraviolet spectrophotometer (340 nm)

Product Introduction

MDH is closely associated with multiple vital pathways of plant metabolism. It plays an essential role in the malate/oxaloacetate (Mal/OAA) and malate/aspartate (Mal/Asp) shuttles that mediate substance and energy transport. During photorespiration, MDH provides NAD^+ for glycine (Gly) oxidation. In mitochondria, MDH also serves as one of the regulatory enzymes that control the operating rate of the tricarboxylic acid (TCA) cycle. In the cytosol, MDH is linked to the pyruvate bypass pathway. Therefore, the MDH system is not only an ideal model for studying enzyme compartmentalization and regulation, but also facilitates research on inter-organelle communication and a wide range of plant developmental processes.

Principle

Malate Dehydrogenase (MDH) catalyzes a redox reaction accompanied by a decrease in absorbance at 340 nm. The activity of malate dehydrogenase is calculated by measuring the change in absorbance per minute.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	12 mL×5	Store at -20°C
Reagent 2	Powder	2 vials	Store at -20°C. Before use, thaw one vial of Diluent, add it to one vial of Powder, and mix thoroughly to prepare Working Reagent 2. Prepare freshly before use.
	Diluent	0.5 mL×2	

Reagent 3	Powder	5 vials	Store at -20°C. Before use, thaw one vial of Diluent and add it to one vial of Powder. Mix thoroughly to obtain Working Reagent 3 for later use.
	Diluent	1 mL×5	

Preparation of Working Solution: Prepare the solution at the ratio of Reagent 1: Working Reagent 2: Working Reagent 3 = 60: 1: 5. Prepare an appropriate amount as needed and use it immediately after preparation.

Storage

The kit shall be stored frozen at -20°C and is valid for 6 months.

Preparation

UV spectrophotometer with 340 nm wavelength, 0.5 cm optical path quartz cuvettes, 37°C water bath, bench low-speed centrifuge, pipettes of various specifications, double distilled water, normal saline (0.9%) or 0.1 M PBS, vortex mixer, centrifuge tubes, and protein assay reagents (available from our company).

Operation process

1. Preparation of 10% tissue homogenate: Accurately weigh the tissue sample. Add 9 times the volume of normal saline at a mass(g)-to-volume(mL) ratio of 1:9. Perform mechanical homogenization in an ice water bath, followed by centrifugation at 2500 rpm for 10 minutes. Collect the supernatant for determination (refer to the experimental methodology for details). According to the differences in MDH activity in various tissues, further dilute the 10% homogenate supernatant with normal saline at different ratios to prepare test samples with concentrations such as 0.2% and 0.1%.
   Reference homogenate sampling concentration: 0.2% for liver tissue and 0.5% for muscle tissue in general.
2. Adjust the UV spectrophotometer to 340 nm with a 0.5 cm optical path quartz cuvette, and set zero with double distilled water. (Prepare two quartz cuvettes: one for zero adjustment and the other for sample determination.)
3. Pre-warm the Working Solution at 37°C for more than 3 minutes.

4. Add 50 μL of test sample into the correspondingly numbered test tube, rapidly add 1 mL of Working Solution into the tube, mix immediately and start timing. (For the blank tube, add 50 μL of double distilled water and 1 mL of Working Solution; perform all other operations the same as the sample group.)
5. Transfer the mixture quickly into a quartz cuvette and measure the absorbance at 340 nm using the UV spectrophotometer. Record the absorbance value (A_1) at 20 seconds, and detect the absorbance value (A_2) again at 1 minute and 20 seconds.
6. Calculate the absorbance difference between the two measurements: $\triangle A = A_1 - A_2$.

Calculation

Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the conversion of 1 μmol of substrate into product per milligram of tissue protein per minute in this reaction system.

$$\text{MDH activity (U/mg)} = \frac{\Delta A_{\mathbf{Assay}} - \Delta A_{\mathbf{Blank}}}{6.2 \times d} \times \frac{V_{Total}}{V_{Sample}} \div Cpr$$

Annotation:

V_{Total}: Total volume of reaction system, 1.05 mL

V_{Sample}: Volume of sample added, 0.05 mL

d: Cuvette optical path, 0.5 cm

6.2: Micromolar extinction coefficient of the substrate

Cpr: Homogenate protein concentration, mg/mL

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. Only 1–2 blank tubes are required (the blank OD value is highly stable).
4. If the measured ΔA is less than 0.05, increase the sample concentration; otherwise, the test

results will be affected.

5. If the measured ΔA is greater than 0.3, dilute the sample before retesting to avoid inaccurate detection results.
6. It is essential to perform a pre-experiment with normal control samples to determine the optimal sample concentration before batch testing.