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

Malate Dehydrogenase Assay Kit (Serum)

Catalog No.: BC00190

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 (Serum)
Detection Method	Colorimetric
Sample Type	Serum
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	3 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×3	

Reagent 3	Powder	2 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×2	

Preparation of Working Solution: Prepare the solution at the ratio of Reagent 1: Working Reagent 2: Working Reagent 3 = 50: 1: 1. 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 a 340 nm wavelength, 0.5 cm path-length quartz cuvettes, 37°C water bath, bench-top low-speed centrifuge, pipettes of various specifications, double distilled water, normal saline (0.9%) or 0.1 M PBS, vortex mixer, test tubes or centrifuge tubes.

Operation process

1. 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.)
2. Pre-warm the Working Solution at 37°C for more than 3 minutes.
3. Add 100 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 100 μL of double distilled water and 1 mL of Working Solution; perform all other operations the same as the sample group.)
4. 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.
5. 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 milliliter of serum (plasma) within one minute in this reaction system.

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

Annotation:

V_{Total}: Total volume of reaction system, 1.1 mL

V_{Sample}: Volume of sample added, 0.1 mL

N: Dilution multiple of sample before testing

d: Cuvette optical path, 0.5 cm

6.2: Micromolar extinction coefficient of the substrate

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.