Malate dehydrogenase (MDH) assay kit

(Cat/No. BC053 Size:50T/48S)

1. Composition and preparation (The kit is valid for 6 months)

	Reagent composition	Reagent volume	Storage conditions
Reagent 1	liquid	12mL×5 bottle	-20℃
Reagent 2	powder	Powder x 2 vials	-20℃
	diluent	0.5mL×2 vials	-20℃
Preparation of Reagent 2 Application Solution: Before use, dissolve one vial of No. 2 diluent and add it to one vial of No. 2 powder. Mix thoroughly to obtain the Reagent 2 Application Solution. Prepare fresh before use.			
Reagent 3	powder	Powder x 5 vials	-20℃
	diluent	1mL x 5 vials	-20℃
Preparation of Reagent 3 Application Solution: Just before use, dissolve one vial of No. 3 diluent and add it to one vial of No. 3 powder. Mix thoroughly to obtain the Reagent 3 Application Solution.			
Preparation of working solution: Prepare the solution according to the ratio of Reagent 1: Reagent 2 working solution: Reagent 3 working solution = **60:1:5**. Prepare only the amount needed and prepare immediately before use.			

2. Measurement principle (tissue testing Ultraviolet method)

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

3. Significance of measurement

MDH is closely related to several important pathways of plant metabolism. It plays a crucial role in the Mal/OAA (malate/oxaloacetate) and Mal/Asp (malate/aspartate) shuttles that transport substances and energy. In photorespiration, MDH provides NAD+ for Gly oxidation. In mitochondria, MDH is also one of the regulatory enzymes that determine the rate of TCA transport. In the cytosol, MDH is linked to the pyruvate pathway. Therefore, the MDH system is not only a good system for studying enzyme regionalization and enzyme regulation, but also provides convenience for studying the connections between various organelles and many developmental questions.

According to recent literature reports, this enzyme is not only related to plant pathology, but also to frost resistance and salt resistance. The relationship between MDH and heat resistance has only been reported in thermophilic bacteria.

4. Operating steps

1.Preparation of 10% homogenate: Accurately weigh the tissue and add 9 times its volume of physiological saline at a weight (g):volume (mL) ratio of 1:9. Homogenize mechanically under ice-water bath conditions, centrifuge at 2500 rpm for 10 minutes, and collect the supernatant for analysis (refer to the experimental methodology for details). Depending on the MDH activity in different tissues, further dilute the 10% homogenate supernatant with physiological saline to different concentrations such as 0.2% and 0.1% for analysis.

2.Recommended homogenate sampling concentrations: 0.2% for liver and 0.5% for muscle.

3.Operation process:

Set the UV spectrophotometer to 340 nm and use a 0.5 cm optical path quartz cuvette to zero the instrument with double-distilled water (prepare two quartz cuvettes, one for zeroing and one for measurement).

Preheat the working solution to 37°C for at least 3 minutes.

Add 50 μL of the sample to the corresponding numbered test tube, then quickly flush 1 mL of working solution into the test tube, mix immediately, and start timing. (For the blank tube, add 50 μL of double-distilled water and 1 mL of working solution; other procedures are the same as for the determination.)

Quickly pour the sample into a quartz cuvette and measure the absorbance at 340 nm using a UV spectrophotometer. Read the absorbance value (A1 value) after 20 seconds, and measure the absorbance value again at 1 minute and 20 seconds (A2 value).

Calculate the difference in absorbance between the two measurements (ΔA = A1 - A2).

[Note]: Only 1 to 2 blank tubes need to be tested (blank OD is very stable); if ΔA measurement/minute < 0.05, the sample concentration needs to be increased; otherwise, the test results will be affected; if ΔA measurement/minute > 0.3, the sample concentration needs to be diluted before testing; otherwise, the test results will be affected.

Before batch testing, please take a normal control group sample to perform a preliminary test to determine the optimal concentration of this sample.

5. Calculation formula

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

2.Calculation formula:

$$\text{MDH activity (U/mgprot)} = \frac{\Delta A_{sample} - \Delta A_{blank}}{6.2 \times d} \times \frac{V_{total}}{V_{sample}} \div C_{prot}$$

6.2: Micromolar extinction coefficient of the substrate;

d: Colorimetric path length, 0.5 (cm);

V reaction total: Total volume of reaction solution, 1050 (μL);

V sample: Sample volume, 50 (μL);

C pr>: Protein concentration in homogenate, mgprot/mL (prot refers to protein).

3.Calculation Example:

Example 1: A 0.2% homogenate of mouse liver tissue was taken, and 50 μL was analyzed according to the procedure table. The results were as follows: OD1 of the test tube was 1.088, and OD2 was 0.905; OD1 of the blank tube was 1.100, and OD2 was 1.098. The protein concentration of the 0.2% homogenate was also measured to be 0.255 mg/mL. The calculation results are as follows:

$$\text{Mouse liver MDH activity (U/mgprot)} = \frac{0.183-0.002}{6.2\times0.5} \times \frac{1050}{50} \div 0.255$$

$$= 4.81\ \text{U/mgprot}$$

Example 2: A 0.5% homogenate of mouse muscle tissue was taken, and 50 μL was tested according to the procedure table. The results were as follows: OD1 of the test tube was 1.291, and OD2 was 1.051; OD1 of the blank tube was 1.100, and OD2 was 1.098. The protein concentration of the 0.5% muscle homogenate was also measured to be 0.220 mg/mL. The calculation results are as follows:

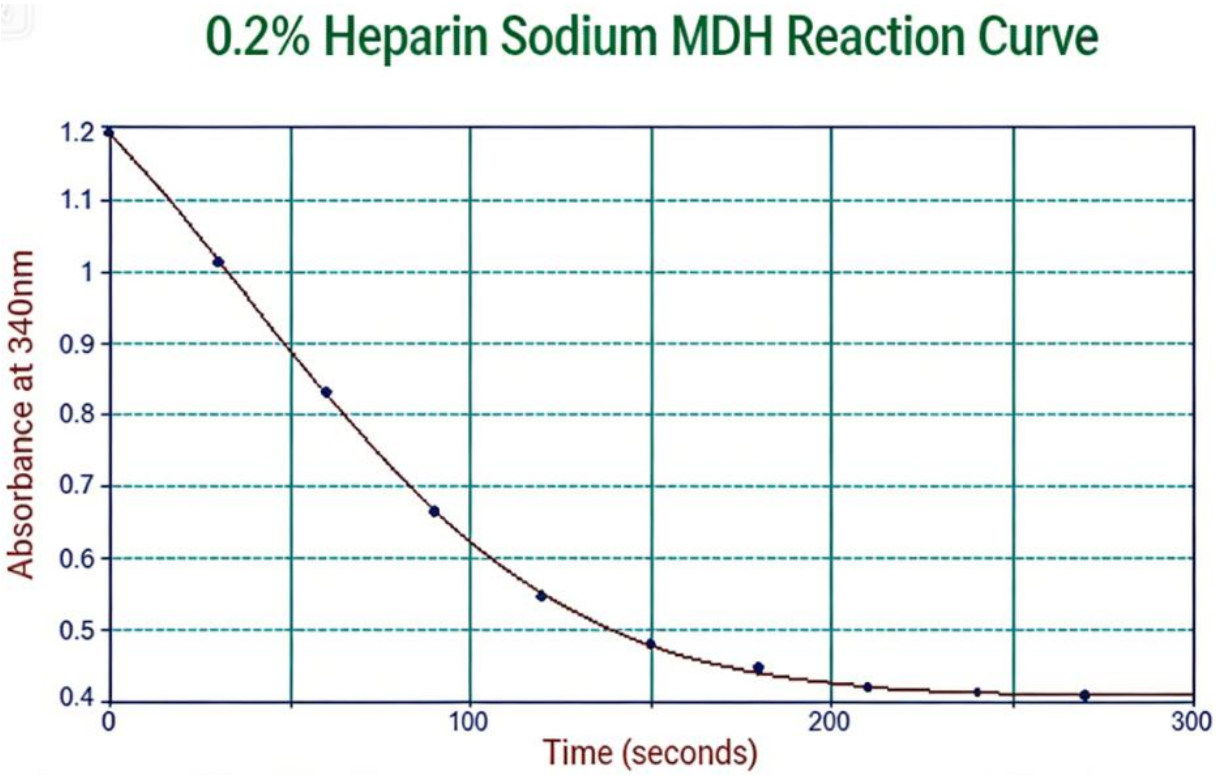
$$\text{Mouse muscle MDH activity (U/mgprot)} = \frac{0.240-0.002}{6.2\times0.5} \times \frac{1050}{50} \div 0.220$$

$$= 7.33\ \text{U/mgprot}$$

Appendix I: Reaction curve of mouse liver tissue homogenate

1.Detection steps: Take fresh mouse livers, weigh them, add physiological saline to make a 10% homogenate, then dilute with physiological saline to a concentration of 0.2% and test according to the procedure.

2.The reaction time curve of 0.2% liver tissue homogenate MDH is shown in the figure below:



3.Based on the MDH response curve of liver tissue, we can conclude that the change in OD is linearly related to time within 2 minutes.