Malate dehydrogenase (MDH) assay kit

(Cat/No.:BC107 Size:50T/48S For Tissue)

1. Composition and Preparation (Shelf life of the kit is 6 months)

	Reagent composition	reagent volume	Storage conditions
Reagent 1	liquid	12mL x 5 bottles	-20℃
Reagent 2	powder	Powder x 2 tubes	-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 tubes	-20℃
	diluent	1mL x 5 vials	-20℃
Preparation of **Reagent 3 Application Solution :** 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 fluid :** Prepare the solution according to the following ratio: **Reagent 1: Reagent 2 working solution: Reagent 3 working solution = 60: 1: 5. Prepare only the amount needed and use immediately.**			

2. Measurement Principle

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 the 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 Procedures

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 different MDH activities in various tissues, further dilute the 10% homogenate supernatant with physiological

saline at different ratios to different concentrations such as 0.2% and 0.1% for analysis.

2. Reference homogenate sampling concentration : generally 0.2% for liver; generally 0.5% for muscle.

3. Operation process:

a . 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).

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

c. 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.)

d. Quickly pour 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).

e. Calculate the difference between the two absorbance values (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:

$$\text{MDH Activity}\ (\text{U/mgprot}) = \frac{\Delta A_{sample} - \Delta A_{blank}}{6.2 \times d} \times \frac{V_{Total}}{V_{sample}} \div \text{Cpr}$$

6.2: Micromolar extinction coefficient of the substrate;

d : Colorimetric path length, 0.5 (cm);

V_{Total} : Total volume of reaction solution, 1050 (μL);

V_{sample} : Sample volume, 50 (μL);

Cpr : homogenate protein concentration, 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 tested 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:

$$\text{Mouse liver MDH activity (U/mgprot)} = \frac{0.183 - 0.002}{6.2 \times 0.5} \times \frac{1050}{50} \div 0.255$$
$$= 4.81 \text{U/mgprot}$$

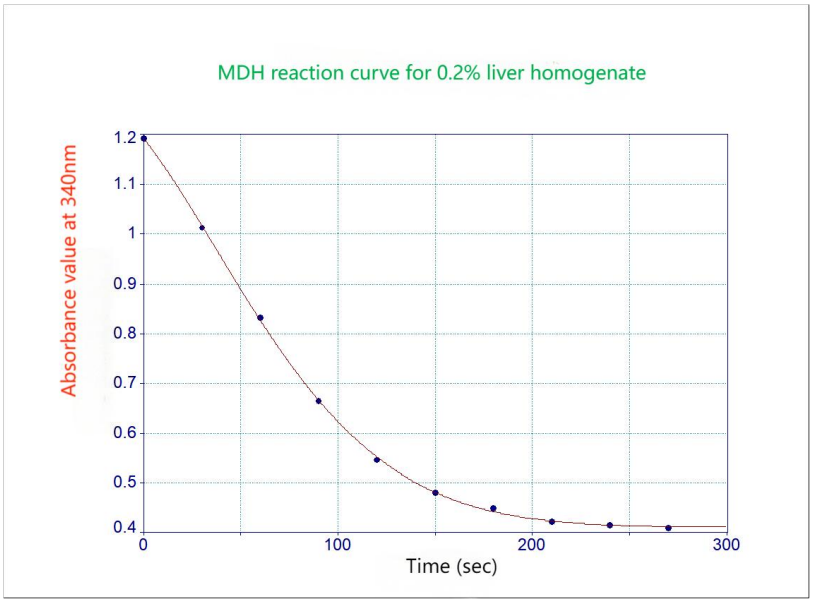
Example 2: A 0.5% homogenate of mouse muscle tissue was taken, and 50 μL was tested according to the procedure table. The results were as follows: OD1 of the test tube $_{was}$ 1.291, and OD2 $_{was}$ 1.051; OD1 of the blank tube was 1.100, and OD2 was 1.098. The protein concentration of the 0.5% muscle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

$$\text{Mouse muscle MDH activity (U/mgprot)} = \frac{0.240 - 0.002}{6.2 \times 0.5} \times \frac{1050}{50} \div 0.220$$
$$= 7.33 \text{U/mgprot}$$

Appendix I: Reaction curve of mouse liver tissue homogenate

1. Detection steps: Weigh fresh mouse liver, add physiological saline to make a 10% homogenate, then dilute with physiological saline to a concentration of 0.2% and test according to the operating steps.

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.