MALATE DEHYDROGENASE (MDH)

[EC 1. 1. 1. 37]

from *Microorganism*

FOR OXALATE → MALATE REACTION

SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 1,200 U/mg protein	
Contaminants	: (as MDH activity = 100 %)	
	GOT	< 0.01 %
	GPT	< 0.01 %
	NADHoxidase	< 0.01 %
	Glutamate dehydrogenase	< 0.01 %
	Fumarase	< 0.01 %

PROPERTIES

Molecular weight	: ca. 72,000	
Subunit molecular weight	: ca. 36,000	
Optimum pH	: 9.0	(Fig. 1)
pH stability	: 5.5 - 11.0	(Fig. 2)
Thermal stability	: No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)
Michaelis constants	: (90 mM Tris-HCl buffer, pH 9.0, at 30 °C)	
	Oxaloacetate	0.027 mM
	NADH	0.014 mM

STORAGE

Stable at -20 °C for at least six months

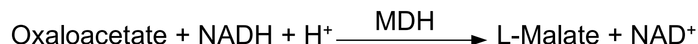
APPLICATION

This enzyme is useful for enzymatic determination of L- malate and of glutamate oxaloacetate transaminase in clinical analysis.

ASSAY

Principle

The change in absorbance is measured at 340 nm according to the following reaction.



$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}^+$$

Unit Definition

One unit of activity is defined as the amount of MDH that forms 1 μmol of NAD^+ per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 200 mM Tris-HCl, pH 9.0
Ⅱ Oxaloacetate solution ; 15 mM (0.020 g oxaloacetate/10 mL distilled water)
Ⅲ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 3 to 5 U/mL with 100 mM Tris-HCl buffer, pH 9.0.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.

Solution Ⅰ	13.50 mL
Solution Ⅱ	1.00 mL
Solution Ⅲ	0.57 mL
H_2O	14.93 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{340}) \times (3.00 + 0.01)}{6.22 \times 0.01} \times \text{d.f.}$$

$$\text{Specific activity (U/mg protein)} = \frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)*}}$$

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH ($cm^2/\mu mol$)
*Protein concentration ; determined by Bradford's method

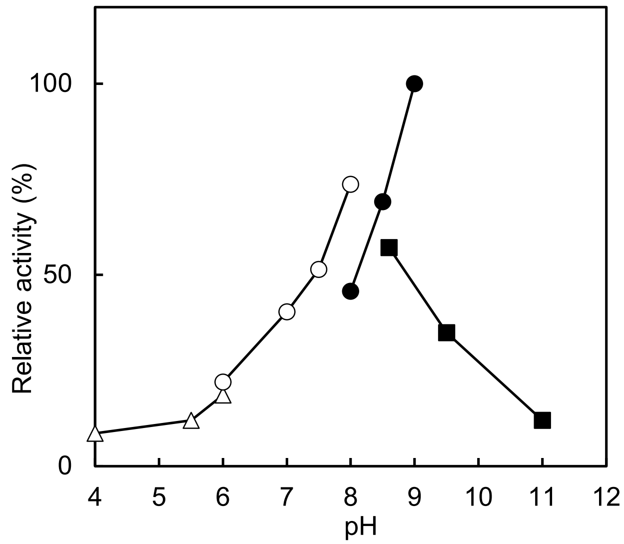


Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

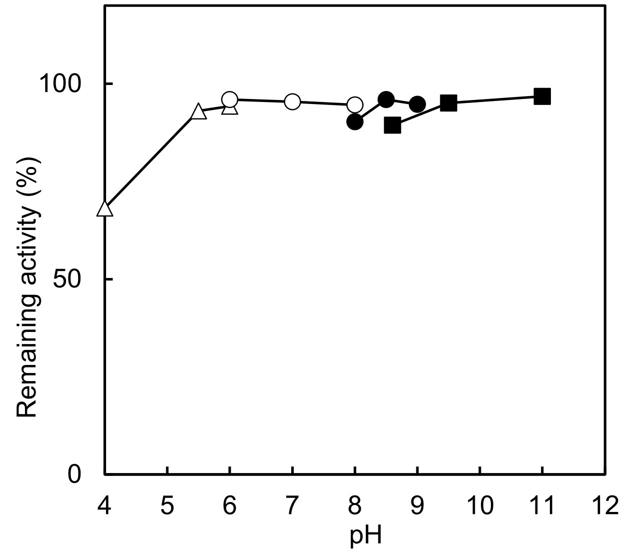


Fig. 2 pH stability

treated for 24 hr at 4 ℃ in the following buffer solution (0.1 M);
△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

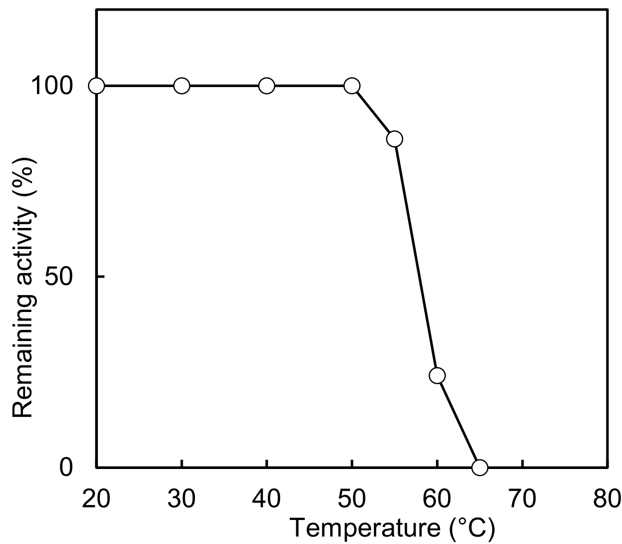


Fig. 3 Thermal stability

treated for 15 min in 0.1 M Tris-HCl buffer, pH 9.0

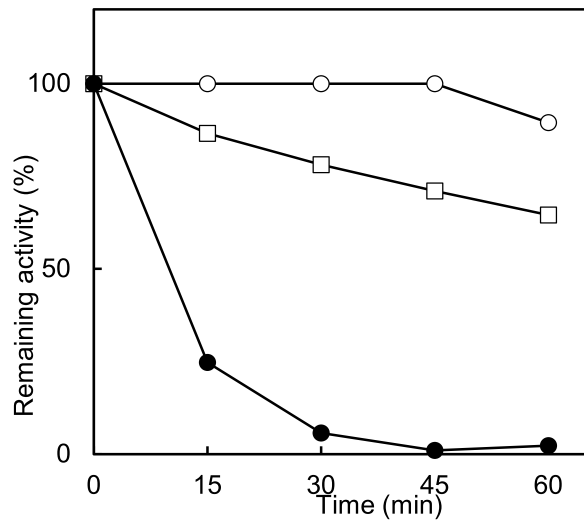


Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer, pH 9.0
○ 50 ℃, □ 55 ℃, ● 60 ℃