NIPRO ENZYMES

MUTAROTASE (MRO)

[EC 5. 1. 3. 3]

from *Microorganism*

α-D-Glucose ↔ β-D-Glucose

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 120 U/mg protein	
Contaminants	:	(as MRO activity = 100 %)	
		NADHoxidase	< 0.01 %

PROPERTIES

Subunit molecular weight	:	ca. 39,500	
Optimum pH	:	7.0 - 9.0	(Fig. 1)
pH stability	:	3.5 - 10.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)

STORAGE

Stable at -20 °C for at least one year

APPLICATION

This enzyme is useful for enzymatic determination of glucose.

ASSAY

Principle

Acceleration of the glucose dehydrogenase reaction by Mutarotase is measured according to the following reactions.

$$\text{Sucros} \xrightarrow{\beta\text{-Fructosidase}} \alpha\text{-D-Glucose} + \text{Fructose}$$

$$\alpha\text{-D-Glucose} \xrightarrow{\text{MRO}} \beta\text{-D-Glucose}$$

$$\beta\text{-D-Glucose} + NAD^+ \xrightarrow{\text{GlucoseDH}} \text{D-Glucono-}\delta\text{-lactone} + NADH + H^+$$

Unit Definition

One unit of activity is defined as the amount of Mutarotase that forms 10 μmol of NADH per minute at 25 °C.

Solutions

Ⅰ HEPES buffer ; 50 mM (1.19 g HEPES / 100 mL distilled water, adjust pH to 7.5 with NaOH)
Ⅱ Sucrose solution ; 16.7 mM (57 mg Sucrose / 10 mL distilled water)
Ⅲ NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid / 10 mL distilled water)
Ⅳ Glucose dehydrogenase solution ; 3 kU/mL (GlcDH2, Nipro Corp. / 20 mM potassium phosphate containing 2 M NaCl, pH6.5)
Ⅴ β-Fructosidase solution ; ≧30 kU/mL (100 mg Invertase from baker's yeast, Sigma-Aldrich I4504 / 1 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 0.7 to 1.4 U/mL with 20 mM potassium phosphate pH 7.3 containing 1 mg/mL BSA.

Procedure

1. Prepare the following reaction mixture and pipette 2.70 mL of reaction mixture into a cuvette.

Solution Ⅰ	19.90 mL	Solution Ⅳ	0.166 mL
Solution Ⅱ	1.00 mL		
Solution Ⅲ	0.60 mL		

2. Add 0.015 mL of the enzyme solution into the cuvette and mix.
3. Incubate at 25 °C for about 3 minutes.
4. Add 0.06 mL of the Solution Ⅴ into the cuvette and mix.
5. Read absorbance change at 340 nm per minute (ΔAbs1) in the linear portion of curve.
6. Run the procedure 1 to 5 with the enzyme diluent instead of the enzyme solution (ΔAbs2).

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs1 - \Delta Abs2) \times (2.70 + 0.015 + 0.060)}{6.22 \times 0.015 \times 10} \times \text{d.f.}$$

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

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH ($cm^2/\mu mol$)
10 ; conversion factor
*Protein concentration ; determined by the absorbance at 280 nm (Abs280), where 1 Abs280 = 1 mg/mL

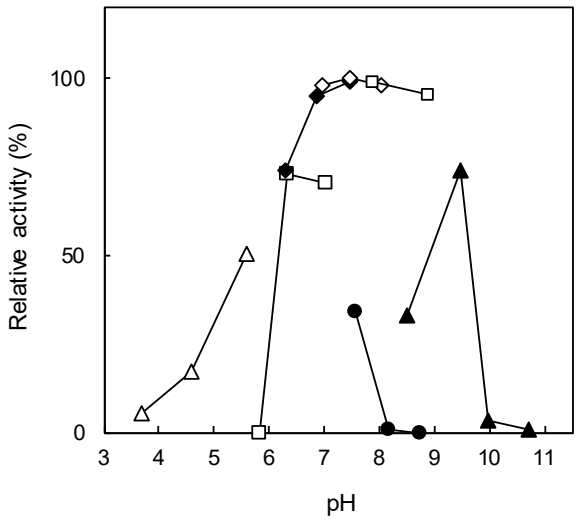

Fig. 1 pH profile

△ acetate, □ MES, ◆ PIPES,
◇ HEPES, ● Tris-HCl,
■ Bicine, ▲ Glycine-KOH

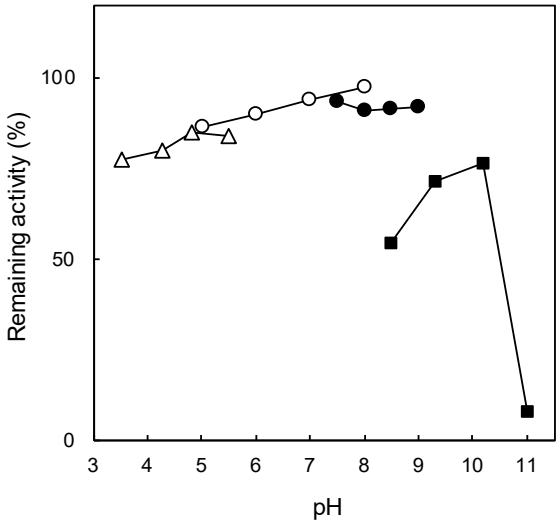

Fig. 2 pH stability

treated for 24 hr at 4 ℃ in the following buffer solution (0.1 M) containing 0.1 % BSA;
△ acetate, ○ phosphate,
● Tris-HCl, ▲ Glycine-KOH

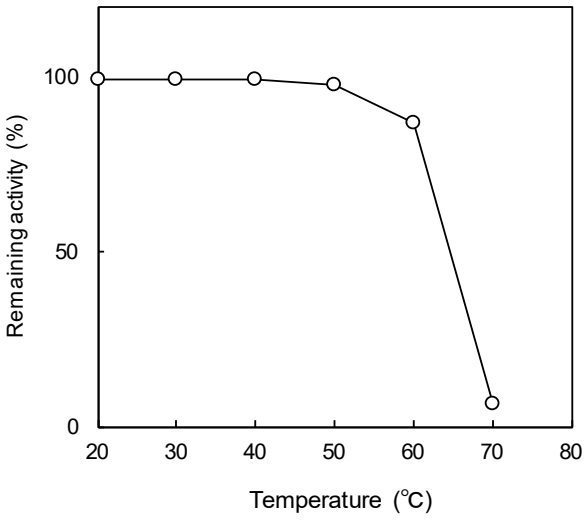

Fig. 3 Thermal stability

treated for 15 min in 0.1 M potassium phosphate buffer, pH 6.5, 0.1 % BSA.

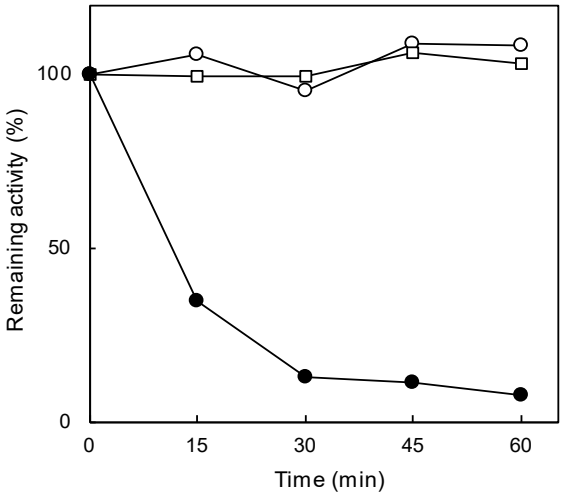

Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 6.5, 0.1 % BSA at ○ 40 ℃, □ 50 ℃, ● 60 ℃