NIPRO ENZYMES

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (ZM-G6PDH)

[EC 1. 1. 1. 49]

from *Zymomonas mobilis*

D-Glucose-6-phosphate + $NAD(P)^+$ ↔ D-Gluconolactone-6-phosphate + $NAD(P)H + H^+$

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 250 U/mg protein	
Contaminants	:	(as ZM-G6PDH activity = 100 %)	
		Glucokinase	< 0.02 %
		Phosphoglucomutase	< 0.01 %
		6-Phosphogluconate dehydrogenase	< 0.02 %
		Hexose-6-phosphate isomerase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 208,000	
Subunit molecular weight	:	ca. 52,000	
Optimum pH	:	8.0	(Fig. 1)
pH stability	:	5.0 - 10.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)
Michaelis constants	:	(30 mM Tris-HCl buffer, pH 8.0, at 30 °C)	
		Glucose-6-phosphate	0.14 mM
		$NADP^+$	0.02 mM
		NAD^+	0.14 mM
Substrate specificity	:	$NADP^+$	70 %
		NAD^+	100 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for diagnostic reagent, for example, glucose determination or CK determination, and for the specific determination of glucose.

ASSAY

Principle

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

$$\text{Glucose-6-phosphate} + NAD^+ \xrightarrow{\text{ZM-G6PDH}} \text{Gluconolactone-6-phosphate} + NADH + H^+$$

Unit Definition

One unit of activity is defined as the amount of ZM-G6PDH that forms 1 μmol of NADH per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 50 mM Tris-HCl, pH 8.0
Ⅱ NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid/10 mL distilled water)
Ⅲ Glucose-6-phosphate (G6P) solution ; 33 mM (0.112 g G6P disodium salt $2H_2O$/10 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50 mM potassium phosphate buffer containing 1 mg/mL BSA, pH 7.0.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution Ⅰ 26.40 mL
   Solution Ⅱ 0.90 mL
   Solution Ⅲ 2.70 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/μmol)
*Protein concentration ; determined by Bradford's method

REFERENCE

1. Scopes, R.K., Testolin, V., Stoter, A., Griffiths-Smith, K., and Algar. E.M.; *Biochem. J.*, **228**. 627 (1985)

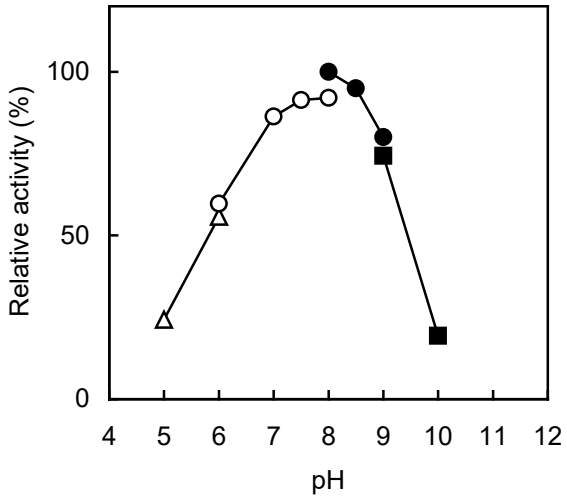

Fig. 1 pH profile

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

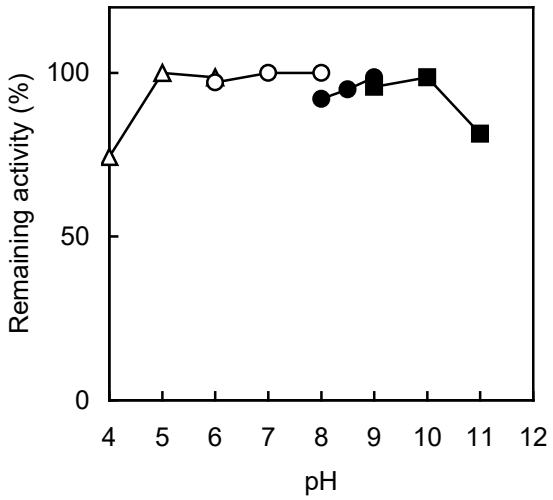

Fig. 2 pH stability

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

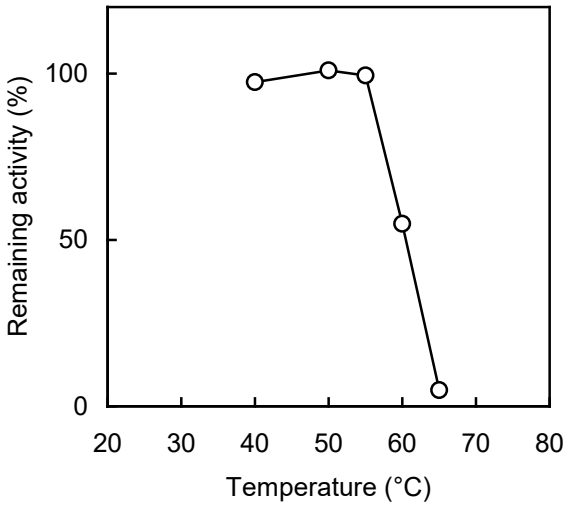

Fig. 3 Thermal stability

treated for 15 min in 0.1 M phosphate buffer, pH 7.0

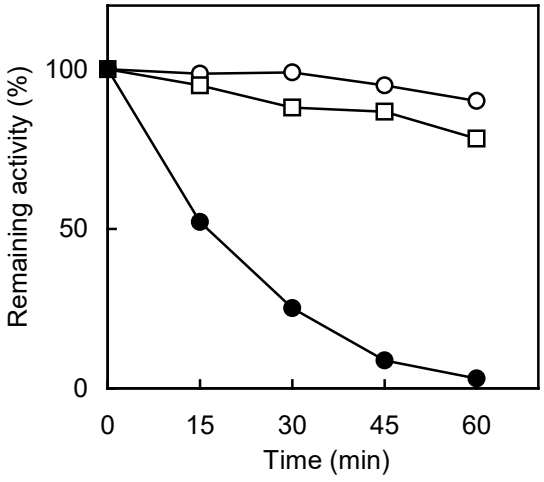

Fig. 4 Thermal stability

treated in 0.1 M phosphate buffer, pH 7.0
○ 50 °C, □ 55 °C, ● 60 °C