NIPRO ENZYMES

GLUCOKINASE (ZM-GlcK)

[EC 2. 7. 1. 2]

from *Zymomonas mobilis*

ATP + D-Glucose ↔ ADP + D-Glucose-6-phosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 150 U/mg protein	
Contaminants	:	(as ZM-GlcK activity = 100 %)	
		Glucose-6-phosphate dehydrogenase	< 0.02 %
		Phosphoglucomutase	< 0.01 %
		6-Phosphogluconate dehydrogenase	< 0.01 %
		Hexose-6-phosphate isomerase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 66,000	
Subunit molecular weight	:	ca. 33,000	
Optimum pH	:	7.0 - 8.0	(Fig. 1)
pH stability	:	6.0 - 8.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 40 °C.	(Fig. 3, 4)
Michaelis constants	:	(60 mM Phosphate buffer, pH 7.0, at 30 °C)	
		Glucose	0.10 mM
		ATP	0.65 mM
Activator	:	Pi	

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.
Tris-HCl buffer is not suitable for the practical use of ZM-GlcK.

ASSAY

Principle

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

$$\text{ATP + Glucose} \xrightarrow{\text{ZM-GlcK}} \text{ADP + Glucose-6-phosphate}$$

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

Unit Definition

One unit of activity is defined as the amount of ZM-GlcK that forms 1 μmol of glucose-6-phosphate per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Triethanolamine - NaOH and 3 mM K_2HPO_4, pH 7.5
Ⅱ ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
Ⅲ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
Ⅳ NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid/10 mL distilled water)
Ⅴ Glucose solution ; 40mM (0.072 g glucose (anhyd.)/10 mL distilled water)
Ⅵ Glucose-6-phosphate dehydrogenase (G6PDH) ; 2000 U/mL (from *Zymomonas mobilis*, Nipro Corp., Dissolve with Buffer solution Ⅰ)

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	20.07 mL	SolutionⅣ	0.60 mL
Solution Ⅱ	1.50 mL	Solution Ⅴ	7.50 mL
SolutionⅢ	0.30 mL	SolutionⅥ	0.03 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

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

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

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)

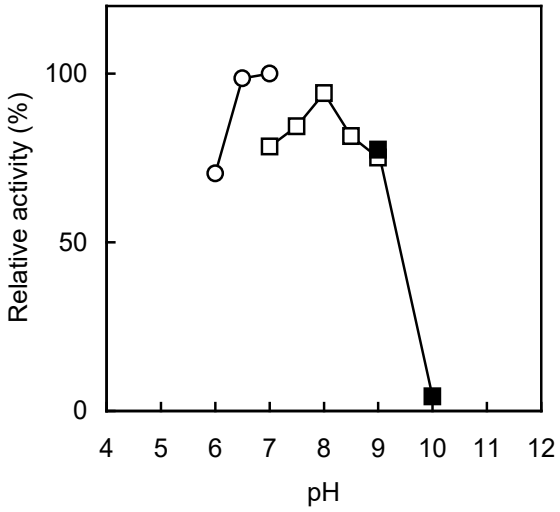

Fig. 1 pH profile

○ MES-KOH, □ TEA-NaOH,
■ Gly-KOH

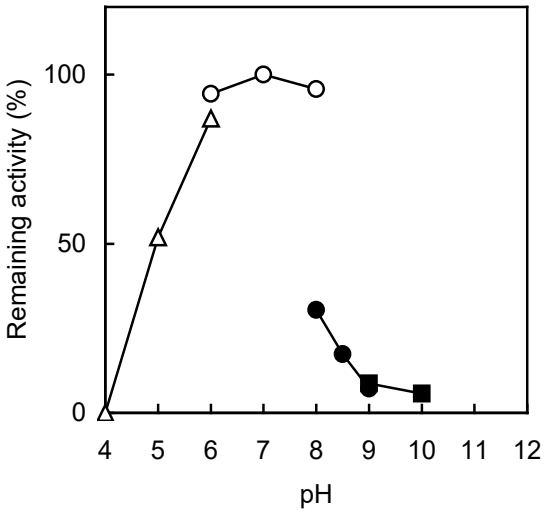

Fig. 2 pH stability

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

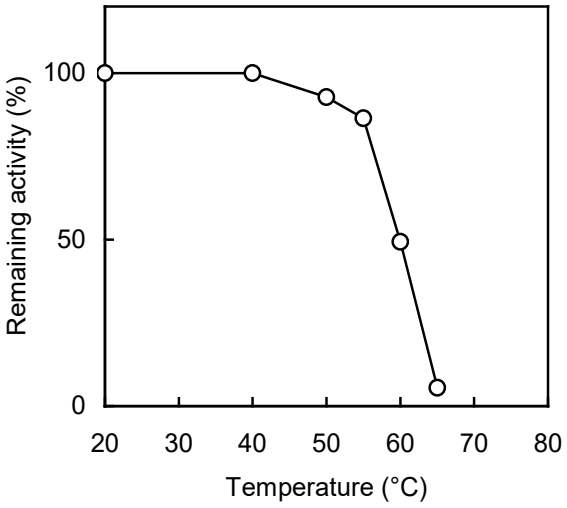

Fig. 3 Thermal stability

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

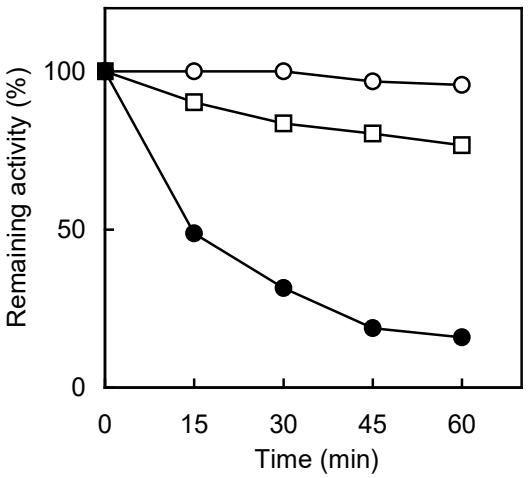

Fig. 4 Thermal stability

treated in 0.1 M phosphate buffer , pH 7.0
○ 40 °C, □ 50 °C, ● 60 °C