β-Phosphoglucomutase (βPGM-EP)

from recombinant *E. coli*

β-D-Glucose 1,6-phosphomutase, EC 5.4.2.6

β-D-Glucose 1-phosphate ⟶ β-D-Glucose 6-phosphate

SPECIFICATION

Appearance	white lyophilizate	
Activity	≧20 U/mg lyophilizate	
Contaminants	α-amylase	$\leqq 5.0\times10^{-3}$%
	α-glucosidase	$\leqq 5.0\times10^{-2}$%
	NADPH oxidase	$\leqq 5.0\times10^{-2}$%
Stabilizers	lactose, EDTA	
Storage	at −20°C	

PROPERTIES

Molecular weight	*ca*. 34 kDa (gel filtration)	
Structure	monomer of *ca*. 25 kDa (SDS-PAGE)	
Michaelis constant	2.3×10^{-4} M (β-D-glucose-1-phosphate)	
pH Optimum	*ca*. 7.0	(Fig. 1)
pH Stability	5.0–9.5	(Fig. 2)
Optimum temperature	40°C	(Fig. 3)
Thermal stability	below 45°C	(Fig. 4)
Stability (liquid form)	stable at 37°C for at least one week	(Fig. 5)
Stability (powder form)	stable at 30°C for at lest one month	(Fig. 6)
Activators	Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}	
Inhibitors	Hg^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}	

ASSAY PROCEDURE

Principle

$$\beta\text{-D-Glucose-1-phosphate} \xrightarrow{\beta\text{-phosphoglucomutase}} \beta\text{-D-Glucose-6-phosphate}$$

$$\text{D-Glucose-6-phosphate} + NADP^+ \xrightarrow{\text{glucose-6-phosphate dehydrogenase}} \text{D-Glucono-1,5-lactone-6-phosphate} + NADPH + H^+$$

The appearance of NADPH is measured spectrophotometrically at 340 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which converts 1 μmol of β-D-glucose-1-phosphate to β-D-glucose-6-phosphate per min at 37°C and pH 7.0 under the conditions described below.

Reagents

A. HEPES–NaOH buffer, 0.3 M; pH 7.0, containing 40 mM KCl, 4 mM $MgCl_2$ and 1.6% (w/v) Triton X-100: dissolve 7.15 g of HEPES, 298 mg of KCl, 81.3 mg of $MgCl_2 \cdot 6H_2O$ and 1.6 g of Triton X-100 in 75 ml of distilled water, adjust to pH 7.0 with 4 N NaOH and dilute with distilled water to 100 ml.
B. D-Glucose-1,6-bisphosphate (G-1,6-P_2) solution, 3.0 mM: 60.7 mg of G-1,6-P_2 cyclohexylammonium·$4H_2O$/25 ml of distilled water.
C. $NADP^+$ solution, 12 mM: 230 mg of $NADP^+ \cdot Na$/25 ml of distilled water.
D. β-D-Glucose-1-phosphate (β-G-1-P) solution, 22 mM:167 mg of β-G-1-P disodium salt/25 ml of distilled water.
E. Glucose-6-phosphate dehydrogenase (G6PDH) solution: 1750 U/ml.
F. Enzyme dilution buffer: mix 10 mM KH_2PO_4 solution and 10 mM K_2HPO_4 solution to make a pH 7.0 solution.

Sample: dissolve the lyophilized enzyme to a volume activity of 1.0–3.0 U/ml with ice-cold enzyme dilution buffer (Reagent F) immediately before measurement.

Procedure

1. Pipette the following reagents into a cuvette (light path: 1 cm).

1.5 ml	HEPES–NaOH buffer	(Reagent A)
0.3 ml	G-1,6-P_2 solution	(Reagent B)
0.3 ml	$NADP^+$ solution	(Reagent C)
0.3 ml	β-G-1-P solution	(Reagent D)
0.02 ml	G6PDH solution	(Reagent E)
0.6 ml	Distilled water	

2. Equilibrate at 37°C for about 5 min.
3. Add 0.03 ml of sample and mix.
4. Record the increase of absorbance at 340 nm in a spectrophotometer thermostated at 37°C, and calculate the ΔA per min using the linear portion of the curve (ΔA_S).
   The blank solution is prepared by adding enzyme dilution buffer (Reagent F) instead of sample (ΔA_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_S - \Delta A_0) \times 3.05\,(\text{ml}) \times df}{6.2 \times 0.03\,(\text{ml})} = \Delta A \times 16.4 \times df$$

Weight activity (U/mg) = (U/ml) × 1/*C*

6.2 : Millimolar extinction coefficient of NADPH at 340 nm ($cm^2/\mu mol$)
df : Dilution factor
C : Content of β-phosphoglucomutase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of α-amylase and inorganic phosphate in clinical analysis.

$$\text{Maltopentaose} \xrightarrow{\alpha\text{-amylase}} \text{Maltotriose} + \text{Maltose}$$

$$\beta\text{-Maltose} \xrightarrow{\text{maltose 1-epimerase}} \alpha\text{-Maltose}$$

$$\alpha\text{-Maltose} + \text{Phosphate} \xrightarrow{\text{maltose phosphorylase}} \alpha\text{-D-Glucose} + \beta\text{-D-Glucose-1-phosphate}$$

$$\beta\text{-D-Glucose-1-phosphate} \xrightarrow{\beta\text{-phosphoglucomutase}} \beta\text{-D-Glucose-6-phosphate}$$

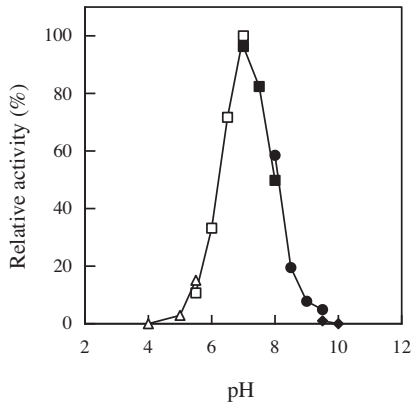
$$\text{D-Glucose-6-phosphate} + NADP^+ \xrightarrow{\text{glucose-6-phosphate dehydrogenase}} \text{D-Glucono-1,5-lactone-6-phosphate} + NADPH + H^+$$

REFERENCES

Nakamura, K. *et al*., *J. Ferment. Bioeng*., **85**, 350–353 (1998).
Shirokane, Y. *et al.*, *Carbohydr. Res.*, **329**, 699–702 (2000).

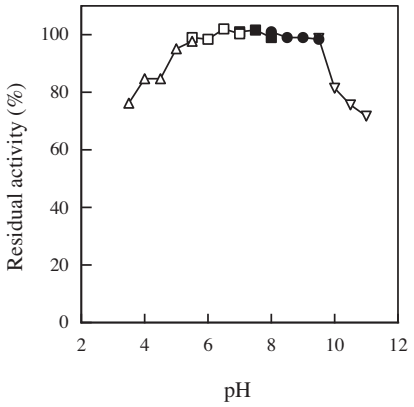
EXPERIMENTAL DATA

Fig. 1 pH Optimum



△: 50 mM acetate buffer
□: 50 mM MES–NaOH buffer
■: 50 mM HEPES–NaOH buffer
●: 50 mM Tris–HCl buffer
◆: 50 mM CHES–NaOH buffer

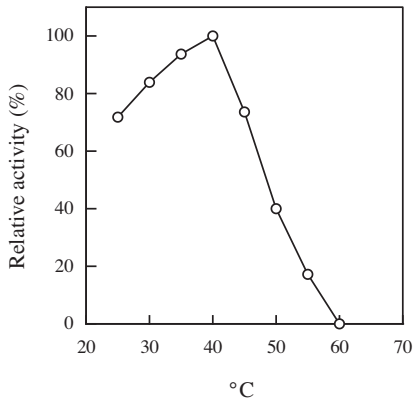
Fig. 2 pH Stability



Treatment: 30°C, 30 min

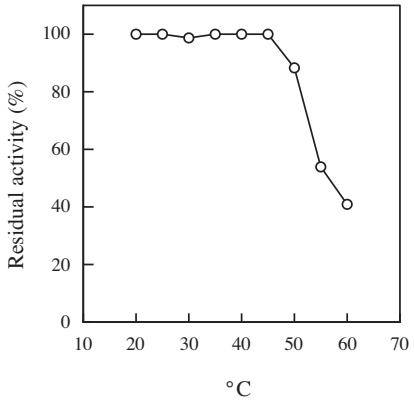
△: 50 mM acetate buffer
□: 50 mM MES–NaOH buffer
●: 50 mM Tris–HCl buffer
■: 50 mM HEPES–NaOH buffer
▽: 50 mM CAPS–NaOH buffer

Fig. 3 Optimum temperature



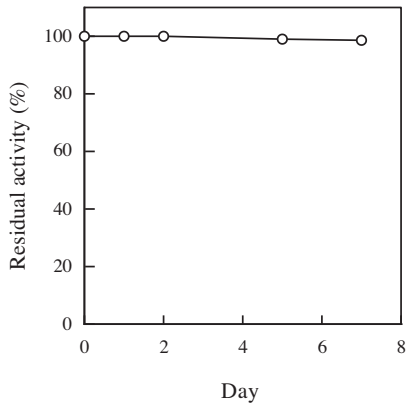
Buffer: 50 mM phosphate buffer, pH 7.0

Fig. 4 Thermal stability



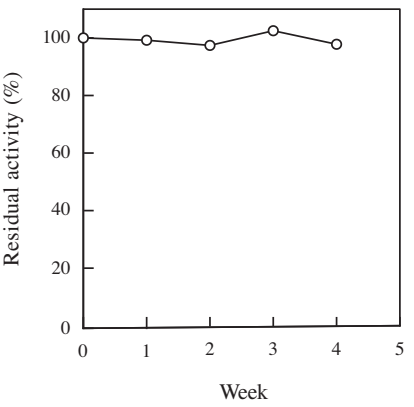
Treatment: 50 mM phosphate buffer, pH 7.0, containing 2 mM EDTA, 15 min

Fig. 5 Stability (liquid form) at 37°C



(Kept in 50 mM phosphate buffer, pH 7.0, containing 2 mM EDTA)

Fig. 6 Stability (powder form) at 30°C



(Kept under dry conditions)