β-Phosphoglucomutase (βPGM-EP)

B1374176

Storage conditions

-20℃,Avoid freeze/thaw cycle.

Introduction:

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

PROPERTIES

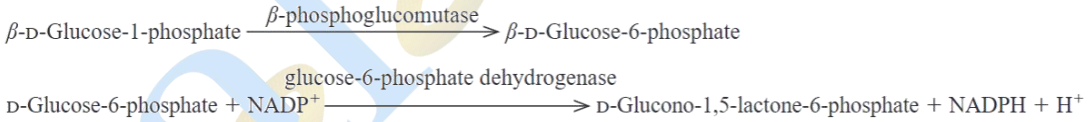
Molecular weight:	ca. 34 kDa (gel filtration)	
Structure:	monomer of ca. 25 kDa (SDS-PAGE)	
Michaelis constant:	2.3×10^{-4} M (β-d-glucose-1-phosphate)	
pH Optimum:	ca. 7.0	(Fig. 1)
pH Stability:	5.0-9.5	(Fig. 2)
Optimum temperature:	40℃	(Fig. 3)
Thermal stability:	below 45℃	(Fig. 4)
Stability (liquid form):	stable at 37℃ for at least one week	(Fig. 5)
Stability (powder form):	stable at 30℃ 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+}	

STABILIZERS

lactose,EDTA

ASSAY PROCEDURE

Principle



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-glucos e-1-phosphate to β-d-glucose-6-phosphate per min at 37°C and pH 7.0 under the condit ions 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$\cdot 4H_2O$/ 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:

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

$$\text{Weight activity (U/mg)} = (\text{U/ml}) \times 1/C$$

6.2: Millimolar extinction coefficient of NADPH at 340 nm (cm2/μmol)

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.

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

β-Maltose $\xrightarrow{\text{maltose 1-epimerase}}$ α-Maltose

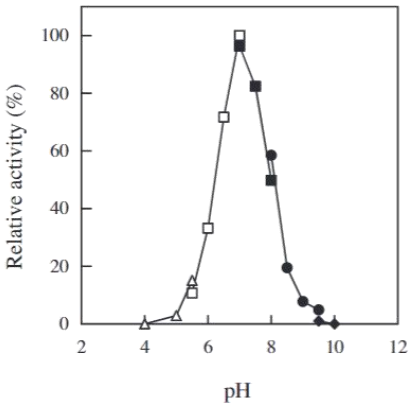
α-Maltose + Phosphate $\xrightarrow{\text{maltose phosphorylase}}$ α-D-Glucose + β-D-Glucose-1-phosphate

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

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

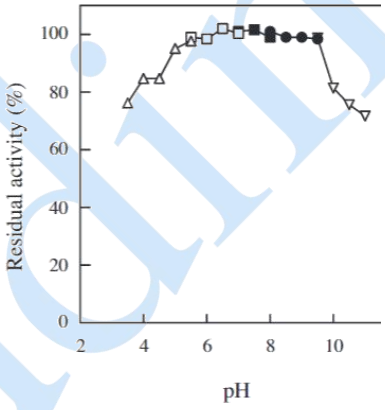
EXPERIMENTAL DATA

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

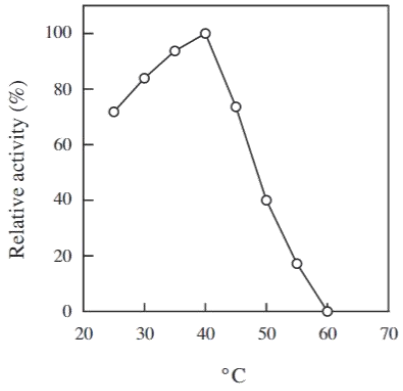
Fig. 2 pH Stability



Treatment: 30°C, 30 min

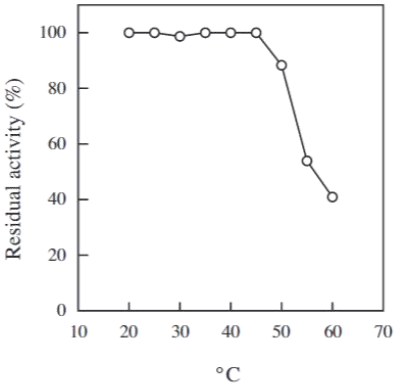
△: 50 mM acetate buffer
□: 50 mM MES–NaOH buffer
●: 50 mM Tris–HCl buffer
■: 50 mM HEPES–NaOH buffer
▽: 50 mM CAPS–NaOH buffer

Fig. 3 Optimum temperature



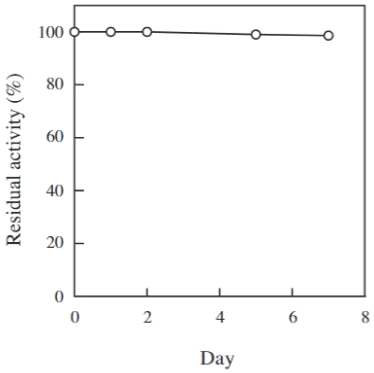
Buffer: 50 mM phosphate buffer, pH 7.0

Fig. 4 Thermal stability



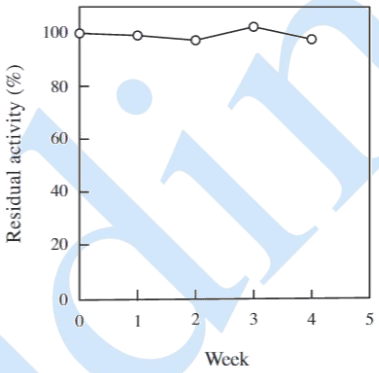
Treatment: 50 mM phosphate buffer, pH 7.0, containing 2 mM EDTA, 15 min

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)