PHOSPHOGLUCOSE ISOMERASE (PGI)

[EC 5. 3. 1. 9]

from *Bacillus stearothermophilus*

D-Glucose 6-phosphate ↔ D-Fructose 6-phosphate

**SPECIFICATION**

State : Lyophilized
Specific activity : more than 400 U/mg protein
Contaminants : (as PGI activity = 100 %)
  Phosphofructokinase < 0.01 %
  6-Phosphogluconate dehydrogenase < 0.01 %
  Phosphoglucomutase < 0.01 %
  NADPH oxidase < 0.01 %
  Glutathione reductase < 0.01 %

**PROPERTIES**

Molecular weight : ca. 200,000
Subunit molecular weight : ca. 54,000
Optimum pH : 9.0 - 10.0 (Fig. 1)
P pH stability : 6.0 - 10.5 (Fig. 2)
Isoelectric point : 4.2
Thermal stability : No detectable decrease in activity up to 60 °C. (Fig. 3, 4)
Michaelis constants : (95mM Tris-HCl buffer, pH 9.0, at 30 °C)
  Fructose 6-phosphate 0.27 mM

**STORAGE**

Stable at -20 °C for at least one year
ASSAY

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

\[
\text{Fructose 6-phosphate} \xrightarrow{\text{PGI}} \text{Glucose 6-phosphate}
\]
\[
\text{Glucose 6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{Gluconolactone 6-phosphate} + \text{NADPH} + \text{H}^+
\]

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

Solutions
I  Buffer solution ; 100 mM Tris-HCl, pH 9.0
II Fructose 6-phosphate (F6P) solution ; 100 mM (0.310 g F6P disodium salt/10 mL distilled water)
III NADP⁺ solution ; 22.5 mM (0.188 g NADP⁺ sodium salt·4H₂O/10 mL distilled water)
IV Glucose-6-phosphate dehydrogenase (G6PDH) ; (from yeast, Roche Diagnostics K.K., No. 127 671) suspension in 3.2 M (NH₄)₂SO₄ solution (10 mg/2 mL) approx. 140 U/mg at 25 °C

Preparation of Enzyme Solution
Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50mM Tris-HCl buffer, pH 8.5.

Procedure
1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution I  28.44 mL  Solution III  0.60 mL
   Solution II  0.90 mL  Solution IV  0.06 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 (ΔAbs₃₄₀) in the linear portion of the curve.

Calculation
Volume activity (U/mL) = \(\frac{(\Delta \text{Abs}_{340}) \times (3.00 + 0.01)}{6.22 \times 0.01}\) X d.f.

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

*Protein concentration ; determined by Bradford’s method

REFERENCE
Fig. 1  pH profile
\[\Delta\text{acetate}, \quad \bigcirc\text{phosphate}, \quad \bullet\text{Tris-HCl}, \quad \blacksquare\text{Gly-KOH}\]

Fig. 2  pH stability
\[\text{treated for 24 hr at 4 °C in the following buffer solution (0.1 M);} \]
\[\Delta\text{acetate}, \quad \bigcirc\text{phosphate}, \quad \bullet\text{Tris-HCl}, \quad \blacksquare\text{Gly-KOH}\]

Fig. 3  Thermal stability
\[\text{treated for 15 min in 50 mM Tris-HCl buffer, pH 8.5}\]

Fig. 4  Thermal stability
\[\text{treated in 50 mM Tris-HCl buffer, pH 8.5} \]
\[\bigcirc 60 ^\circ\text{C}, \quad \square 65 ^\circ\text{C}, \quad \bullet 70 ^\circ\text{C}\]