(Diagnostic Reagent Grade) ASAHI KASEI ENZYMES T-120

PHOSPHOGLUCOSE ISOMERASE [PGI Ⅱ]

from *Geobacillus stearothermophilus*
(D-Glucose-6-phosphate ketol-isomerase, EC 5.3.1.9)

D-Glucose-6-phosphate ⟶ D-Fructose-6-phosphate

Preparation and Specification

Appearance : White amorphous powder, lyophilized
Specific activity : More than 250 U/mg solid

Properties

Molecular weight	: 200 kDa (gel filtration) 50 kDa (SDS-PAGE)	
Isoelectric point	: pH 4.3	
Michaelis constant	: D-Fructose-6-phosphate 0.21 mM (at 37 ℃)	
Optimum pH	: 9.5	Figure 1
pH stability	: 6.5-10.5 (37℃, 1hr)	Figure 2
Thermal stability	: Stable at 60℃ and below	Figure 3

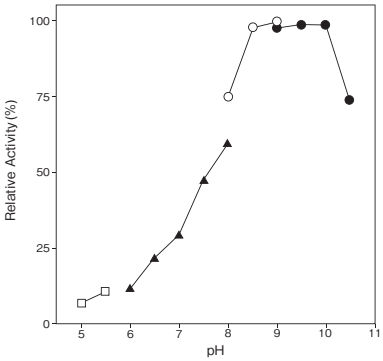
Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of F-6-P.

$$\text{D-Fructose-6-phosphate} \xrightarrow{\text{PGI Ⅱ}} \text{D-Glucose-6-phosphate}$$

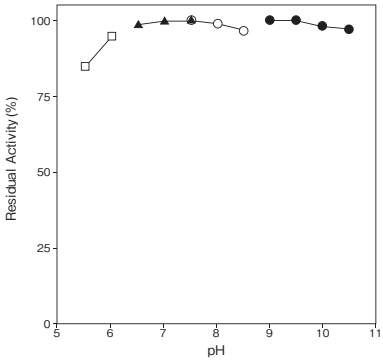
$$\text{D-Glucose-6-phosphate} + NADP^+ \xrightarrow{\text{G6PDH}} \text{D-Glucono-}\delta\text{-lactone-6-phosphate} + NADPH + H^+$$

Fig.1 pH Optimum



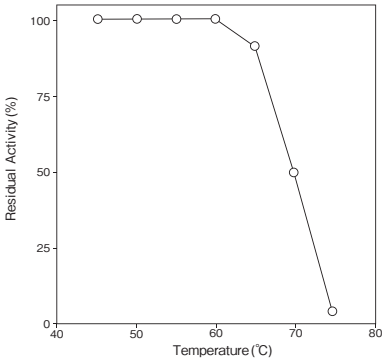
□ : Acetate buffer
▲ : Phosphate buffer
○ : Trsi-HCl buffer
● : Glycine-NaOH buffer

Fig.2 pH Stability



37℃,1hr.
□ : Acetate buffer
▲ : Phosphate buffer
○ : Trsi-HCl buffer
● : Glycine-NaOH buffer

Fig.3 Thermal Stability



pH 7.5, 15 min.
100 mM Tris-HCl buffer

Assay

■ Principle

The assay is based on the increase in absorbance at 340 nm as the formation of NADPH proceeds in the following reaction

$$\text{Fructose-6-P} \xrightarrow{\text{PGI II}} \text{D-Glucose-6-P}$$

$$\text{D-Glucose-6-P} + \text{NADP}^{+} \xrightarrow{\text{G6PDH}} \text{D-Glucono-}\delta\text{-6-P} + \text{NADPH} + \text{H}^{+}$$

■ Unit definition

One unit is defined as the amount of enzyme which converts 1 μmole of fructose-6-phosphate to D-Glucose-6-phosphate per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture (10test)

0.1M Tris-HCl Buffer pH9.0	28.44 ml
0.1M Fructose-6-P solution	0.90 ml
22.5mM NADP solution	0.60 ml
700U/ml G6PDH suspension	0.06 ml

※ Use G6PDH 10mg/2ml (700U/ml): market product

2. Enzyme dilution buffer
   50mM Tris-HCl buffer pH8.5 (25℃)
3. Reagents
   Tris(hydroxymethyl)aminomethane:
   Sigma Chemical Co. #T-1503
   Fructose-6-phosphate·2Na:
   Wako Pure Chemical Industries, Ltd. #066-05341
   NADP(Nicotinamide adenine dinucleotide phosphate oxidized form): Wako Pure Chemical Industries, Ltd. #308-50463
   G6PDH(Glucose-6-phosphate dehydrogenase):
   Ammonium sulphate suspension Roche Diagnostics GmbH #10 127 671 001

■ Enzyme solution

Accurately weigh about 10 mg of the sample and add enzyme dilution buffer to make a total of 10 ml. Dilute it with enzyme dilution buffer to adjust the concentration to 5.0-10.0 U/ml.

■ Procedure

1. Pipette accurately 3.0 ml of reaction mixture into a small test tube and preincubate at 30℃.
2. After 5 min. add accurately 10 μl of enzyme solution and mix to start the reaction at 30℃.
   ※ In the case of a test blank, add 10 μl of enzyme dilution buffer in place of enzyme solution.
3. After starting the reaction, measure the rate of increase per minutes in absorbance at 340 nm. The rate must be measured within the linear portion of the absorbance curve. (Ex. Linear range from 2 min. to 5 min.)
   Absorbance sample : As/min.
   blank : Ab/min.
   △A/min.=(As/min.−Ab/min.)≦0.05−0.10Abs/min.

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle \text{ A/min.}}{6.22} \times \frac{3.01}{0.01} \times \frac{1}{X}$$

$$= \triangle \text{ A/min.} \times 48.39 \div X$$

6.22 : millimolar extinction coefficient of NADH at 340nm (cm^2/ μmol)
3.01 : final volume (ml)
0.01 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at −20℃ in the presence of a desiccant is recommended.

PGI Ⅱ活性測定法（Japanese）

Ⅰ．試薬液

1. 反応試薬混合液（10 テスト用）

0.1M トリス –HCl 緩衝液 pH9.0	28.44 ml
0.1M フルクトース –6–P 溶液	0.90 ml
22.5mM NADP 溶液	0.60 ml
700U/ml G6PDH 懸濁液	0.06 ml

※ロシュ社製の G6PDH 10mg/2ml（700U/ml）3.2M 硫安懸濁液をそのまま使用する。

2. 酵素溶解用液

50mM トリス –HCl 緩衝液 pH8.5（25℃）

3. 試薬

トリス（ヒドロキシメチル）アミノメタン：シグマ社製　#T–1503

フルクトース–6– リン酸·2Na：和光純薬工業社製　#066–05341

NADP（ニコチンアミドアデニンジヌクレオチド·リン酸酸化型）：和光純薬工業社製　#308–50463

G6PDH（グルコース–6–リン酸脱水素酵素）：ロシュ社製　#10 127 671 001

Ⅱ．酵素試料液

検品約 10mg を精密に計り、酵素溶解用液にて溶解し全容 10ml とする。

その液を更に酵素溶解用液にて 5〜10U/ml の濃度に適宜希釈する

Ⅲ．測定操作法

1. 小試験管に反応試薬混合液 3.00ml を正確に分注し、30℃で予備加温する。
2. 5 分経過後、酵素試料液 10 μl を正確に加え混和し、30℃で反応を開始する。

※盲検は酵素試料液の代わりに酵素溶解用液 10 μl を加える。

3. 反応開始後、340nm における吸光度を測定して直線的に反応している 1 分間当たりの吸光度変化を求める。（直線範囲例：2 分目から 5 分目）

求めた吸光度変化を

酵素試料液については As/min.

盲検液については　　Ab/min.　とする。

ΔA/min. = (As/min. − Ab/min.) = 0.05 〜 0.10/min

Ⅳ．計算

以下の計算式に従い、PGI Ⅱ活性（U/mg）を計算する。

$$\text{活性 (U/mg)} = \frac{\Delta A/\text{min.}}{6.22} \times \frac{3.01}{0.01} \times \frac{1}{X}$$

$$= \Delta A/\text{min.} \times 48.39 \div X$$

6.22 : NADPH の 340nm におけるミリモル分子吸光係数　（cm^2/ μmol）

3.01 : 反応総液量（ml）

0.01 : 反応に供した酵素試料液量（ml）

X　: 酵素試料液中の検品濃度（mg/ml）