(Diagnostic Reagent Grade) ASAHI KASEI ENZYMES T-142

PHOSPHOFRUCTOKINASE [PFKW Ⅱ]

from *Geobacillus stearothermophilus*
(ATP: D-fructose-6-phosphate-1-phosphotransferase, EC 2.7.1.11)

ATP + D-Fructose-6-phosphate ⟶ ADP + D-Fructose-1,6-bisphosphate

Preparation and Specification

Appearance	: White to pale yellowish amorphous powder, lyophilized
Specific activity	: More than 150 U/mg solid
Contaminants	: ATPase Less than 0.005
	: NADPHox Less than 0.010

Properties

Molecular weight	: 72 kDa (gel filtration) 35 kDa (SDS-PAGE)	
Isoelectric point	: pH 5.9	
Michaelis constants	: D-Fructose-6-phosphate (D-F-6-P) 5.8 mM (at 37℃) ATP 0.07 mM (at 37℃)	
Optimum pH	: 9.0	Figure 1
pH stability	: 6.0-10.0 (37℃, lhr)	Figure 2
Thermal stability	: Stable at 55℃ and below	Figure 3
Activators	: Mg^{2+}	

Applications for Diagnostic Test

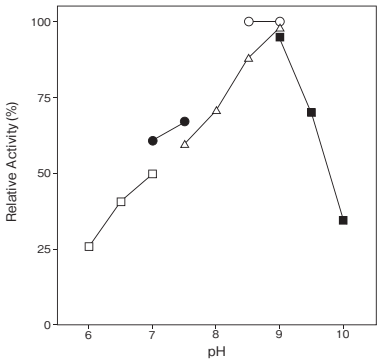
This enzyme is useful for enzymatic determination of F-6-P.

$$\text{ATP+D-F-6-P} \xrightarrow{\text{PFKW Ⅱ}} \text{ADP+D-Fructose-1, 6-bisphosphate}$$

$$\text{ADP+D-Glucose} \xrightarrow{\text{ADP-HKT Ⅱ}} \text{AMP+D-Glucose-6-phosphate}$$

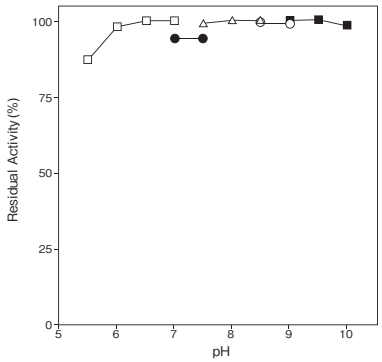
$$\text{D-Glucose-6-phosphate+NADP}^{+} \xrightarrow{\text{G6PDH}} \text{D-Glucono-}\delta\text{-lactone-6-phosphate+NADPH+H}^{+}$$

Fig.1 pH Optimum



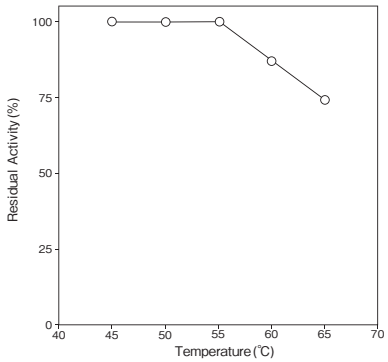
□ : MES buffer
● : PIPES buffer
△ : Trsi-HCl buffer
○ : TAPS buffer
■ : CHES buffer

Fig.2 pH Stability



100 mM buffer, 37℃,1hr.
□ : MES buffer
● : PIPES buffer
△ : Trsi-HCl buffer
○ : TAPS buffer
■ : CHES buffer

Fig.3 Thermal Stability



pH 8.5, 30 min.
100 mM TAPS buffer

Assay

■ Principle

The assay is based on the decrease in absorbance at 340 nm of NADH in the following reaction

$$\text{Fructose-6-P} + \text{ATP} \xrightarrow{\text{PFKW II}} \text{Fructose-1,6-bisphosphat} + \text{ADP}$$

$$\text{ADP} + \text{PEP} \xrightarrow{\text{PK}} \text{ATP} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^{+} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^{+}$$

■ Unit definition

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

■ Reagents

1. Reaction mixture (10test)

0.1M Tris-HCl Buffer pH9.0	27.36 ml
0.5M Fructose-6-P solution	0.30 ml
0.1M ATP solution	0.39 ml
56mM PEP solution	0.60 ml
13.1mM NADH solution	0.60 ml
2.5M KCl solution	0.06 ml
0.1M $MgSO_4$ solution	0.60 ml
5,000U/ml PK/ Ammonium sulphate suspension	0.03 ml
550U/ml LDH/ Ammonium sulphate suspension	0.06 ml

2. Enzyme dilution buffer
   50mM phosphate buffer pH8.0 (25℃)
3. Reagents (10test)
   Tris(hydroxymethyl)aminomethane: Sigma Chemical Co. #T-1503
   Fructose-6-phosphate・2Na:
   Wako Pure Chemical Industries, Ltd. #066-05341
   ATP(Adenosine triphosphate・2Na・$3H_2O$):
   Kyowa Hakko Co.,Ltd.
   PEP・TCA(Phosphoenolpyruvate): Sigma Chemical Co. #P-7252
   NADH・2Na・$3H_2O$(Nicotinamide adenine dinucleotide reduced form): Kyowa Hakko Co.,Ltd
   PK(Pyruvate kinase) Ammonium sulphate suspension:
   Roche Diagnostics GmbH. #128163
   LDH(Lactate dehydrogenase)
   Ammonium sulphate suspension
   Roche Diagnostics GmbH #107085

■ 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 diluti on buffer in place of enzyme solution.
3. After starting the reaction, measure the rate of decrease 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.
   $\triangle A/\text{min.} = (As/\text{min.} - Ab/\text{min.}) \leqq 0.25$ Abs/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 NADPH at 340nm ($cm^2/\ \mu mol$)

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.

PFKW 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	27.36 ml
0.5M フルクトース –6–P 溶液	0.30 ml
0.1M ATP 溶液	0.39 ml
56mM PEP 溶液	0.60 ml
13.1mM NADH 溶液	0.60 ml
2.5M KCl 溶液	0.06 ml
0.1M $MgSO_4$ 溶液	0.60 ml
5,000U/ml PK/ 硫安懸濁液	0.03 ml
550U/ml LDH/ 硫安懸濁液	0.06 ml

2. 酵素溶解用液
   50mM リン酸緩衝液 pH8.0 (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:
   和光純薬工業製 #066–05341
   ATP(アデノシン三リン酸·2Na·3H2O):
   協和発酵社製
   PEP(フォスフォエノールピルビン酸·TCA):
   シグマ社製 #P–7252
   NADH·2Na·3H2O : 協和発酵社製
   PK(ピルビン酸キナーゼ)硫安懸濁液:
   ロシュ社製 #128163
   LDH(乳酸脱水素酵素) 硫安懸濁液:
   ロシュ社製 #107085

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。
   Δ A/min. = (As/min. − Ab/min.) ≦ 0.25/min

Ⅳ. 計算

以下の計算式に従い、PFKW Ⅱ活性 (U/mg) を計算する。

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

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

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

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)