(Diagnostic Reagent Grade) 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% (U/U)
	: NADPHox Less than 0.010% (U/U)

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℃, 1 hr.)	Figure 2
Thermal stability	: Stable at 55℃ and below (pH8.5, 30min)	Figure 3
Activators	: Mg^{2+}	

Applications for Diagnostic Test

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

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

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

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

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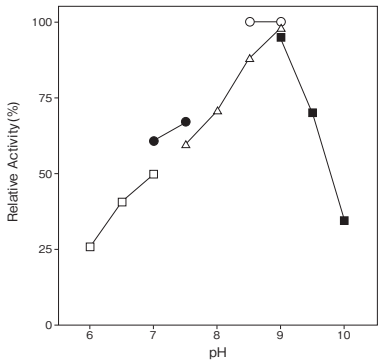
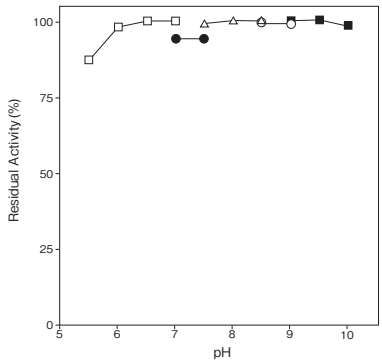
Fig.1 pH Optimum



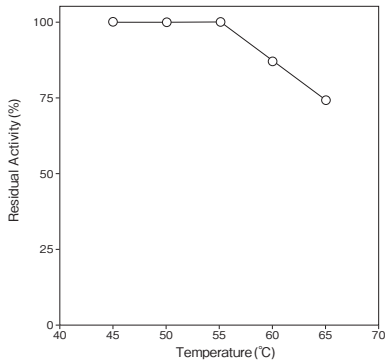
□ : MES buffer
● : PIPES buffer
△ : Tris-HCl buffer
○ : TAPS buffer
■ : CHES buffer

Fig.2 pH Stability



100 mM buffer, 37℃,1hr.
□ : MES buffer
● : PIPES buffer
△ : Tris-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 sulfate suspension	0.03 ml
550U/ml LDH/ Ammonium sulfate suspension	0.06 ml

2. Enzyme dilution buffer
   50mM KH_2PO_4 - NaOH buffer pH8.0 (25℃)
3. Reagents

Tris(hydroxymethyl)aminomethane: Sigma Chemical Co. #T-1503
Fructose-6-phosphate·2Na:
FUJIFILM Wako Pure Chemical Corporation #066-05341
ATP(Adenosine triphosphate·2Na·3H_2O):
Kyowa Hakko Co.,Ltd.
PEP [Phospho(enol)pyruvic acid tri(cyclohexylammonium) salt]: Sigma Chemical Co. #P-7252
NADH·(2Na·3H_2O·Reduced form):
Kyowa Hakko Co.,Ltd
PK(Pyruvate kinase) Ammonium sulfate suspension:
Roche Diagnostics GmbH. #128163
LDH(Lactate dehydrogenase)
Ammonium sulfate 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
   △A/min = (As/min - Ab/min) ≦ 0.25 Abs/min

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■ 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 KH_2PO_4–NaOH 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：
シグマ製　#T–1503

フルクトース –6– リン酸・2Na：
富士フイルム和光純薬製　#066–05341

ATP（アデノシン三リン酸・2Na・3H2O）：
協和発酵製

PEP［ホスホ（エノール）ピルビン酸トリ（シクロヘキシルアンモニウム）塩］：シグマ製　#P–7252

NADH（2Na・3H_2O・還元型）：協和発酵製

PK（ピルビン酸キナーゼ）硫安懸濁液：
ロシュ製　#128163

LDH（乳酸脱水素酵素）硫安懸濁液：
ロシュ製　#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）≦ 0.25Abs/min

Ⅳ. 計算

以下の計算式に従い、PFKW Ⅱ活性（U/mg）を計算する。

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

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）

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