(Diagnostic Reagent Grade) ASAHI KASEI ENZYMES T-50

HEXOKINASE [HK Ⅱ]

from *Bacillus* sp.
(ATP : D-hexose-6-phosphotransferase, EC 2.7.1.1)

D-Glucose + ATP ⟶ D-Glucose-6-phosphate + ADP

Preparation and Specification

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

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 68 kDa (gel filtration)	
Isoelectric point	: pH 5.64	
Michaelis constants	: Glucose 8.2×10^{-4}M	
	ATP 8.7×10^{-5}M	
	$MgCl_2$ 1.6×10^{-3}M	
Optimum pH	: 7.5–8.0	Figure 1
pH stability	: 7.0–8.5 (Phosphate buffer) (55℃, 10 min)	Figure 2
Optimum temperature	: 50℃	
Thermal stability	: Stable at 55℃ and below (pH 8.0, 10 min)	Figure 3
Effect of various chemicals	: See Table 2	
Stabilizer	: ATP, albumin, KCl, NaCl	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **glucose or creatinine kinase activity** when coupled with glucose-6-phosphate dehydrogenase (T-51)

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{HK Ⅱ}} \text{D-Glucose-6-phosphate} + \text{ADP}$$

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

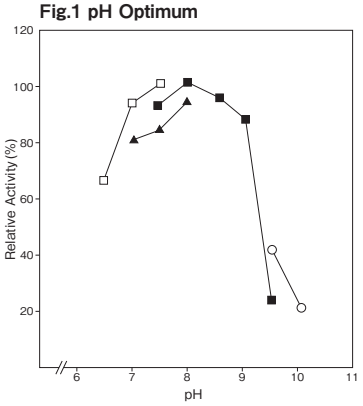
Table 1. Substrate specificity

Substrate	Relative activity (%)
Glucose	100
Xylose	11
Mannose	41
Fructose	0
Sorbitol	0
Saccharose	0
Mannitol	0

Table 2. Effect of various chemicals on HK Ⅱ activity

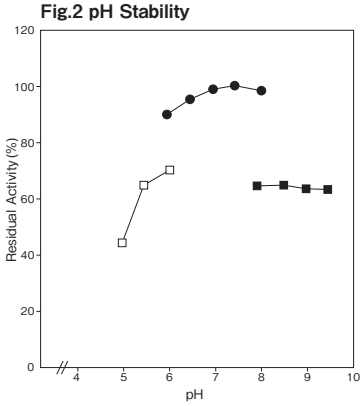
Additive	Concentration	Relative activity (%)
None		100
KCl	10mM	100
NaCl	10mM	100
$CaCl_2$	1mM	99
$BaCl_2$	1mM	100
EDTA	1mM	0
Triton X-100	1%	100
Adekatol PC-8	1%	100
Nikkol OP-10	1%	97
Pluronic P-103	1%	99

Fig.1 pH Optimum



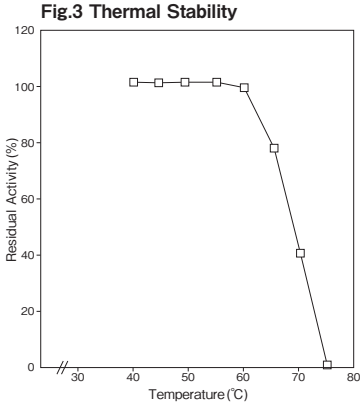
□ : PIPES-NaOH buffer
▲ : TES-NaOH buffer
■ : Tris-HCl buffer
○ : Glycine-NaOH

Fig.2 pH Stability



55℃, 10 min.
□ : Acetate buffer
● : Phosphate buffer
■ : Tris-HCl buffer

Fig.3 Thermal Stability



pH 8.0, 10 min.
40 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 reactions:

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{HK Ⅱ}} \text{Glucose-6-phosphate} + \text{ADP}$$

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

ATP: Adenosine triphosphate
NADP: Nicotineamide adenine dinucleotide phosphate
G6PDH Ⅱ : Glucose-6-phosphate dehydrogenase

■ Unit definition

One unit is defined as the amount of enzyme which generates 1 μmole of NADPH per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture

0.2 M Tris-HCl buffer pH 8.0	0.6 ml
0.1 M Glucose solution	0.3 ml
40 mM ATP solution pH 7.0	0.3 ml
100 U/ml G6PDH Ⅱ solution [1)]	0.3 ml
10 mM NADP solution	0.3 ml
0.1 M $MgCl_2$ solution	0.3 ml
Distilled water	0.9 ml

1): 100 U/ml G6PDH Ⅱ solution
Disolve 1,000 U of G6PDH with 10 ml of 10 mM Tris-HCl buffer pH 8.0.

2. Enzyme dilution buffer
0.1 M KH_2PO_4-NaOH buffer pH 7.0 containing 0.1% (W/V) BSA and 0.1% (W/V) Triton X-100.

3. Reagents
Triton X-100: The Dow Chemical Company
NADP (oxidized form):
FUJIFILM Wako Pure Chemical Corporation #308-50463
G6PDH Ⅱ : Asahi Kasei Pharma Corporation #T-51
ATP ($2Na \cdot 3H_2O$): Kyowa Hakko Co., Ltd.
BSA: Millipore Fraction V pH 5.2 #81-053

■ Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette accurately 3.0 ml of reaction mixture into a small test tube and preincubate at 37℃.
2. After 5 min, add exactly 50 μl of enzyme solution and mix to start the reaction at 37℃.
   ※ In the case of a test blank, add 50 μl of enzyme dilution buffer in place of enzyme solution.
3. After starting the reaction, measure the rate of increase per minute in absorbance at 340 nm. The rate must be measured within the linear portion of the absorbance curve.

Absorbance sample : As/min
blank : Ab/min
△A/min = (As/min − Ab/min) ≦ 0.030 Abs/min

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle\ \text{A/min}}{6.22} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

6.22 : millimolar extinction coefficient of NADPH at 340 nm (cm^2/ μmole)
3.05 : final volume (ml)
0.05 : 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.

References

1. Colowick, S.P. (1973) The Enzymes (3rd Ed.) , **4**, 1–48.
2. Barnard, E.A. (1975) Methods Enzymol., **42**, 6–25.
3. Wright, C.L. and Warsy, A.S. (1978) Biochem. J., **175**, 125–135.
4. Li, S.J., Umena, Y., Matsuoka, T., Kita, A., Fukui, K. and Morimoto, Y. (2007) Biochem. Biophys. Res. Commun., **358**, 1002–1007.

HK Ⅱ活性測定法 (Japanese)

Ⅰ. 試薬液

1. 反応試薬混合液

0.2M トリス –HCl 緩衝液 pH8.0	0.6 ml
0.1M グルコース溶液	0.3 ml
40mM ATP 溶液 pH7.0	0.3 ml
100U/ml G6PDH Ⅱ溶液 1)	0.3 ml
10mM NADP 溶液	0.3 ml
0.1M 塩化マグネシウム溶液	0.3 ml
精製水	0.9 ml

1): 100U/ml G6PDH Ⅱ溶液
G6PDHⅡ 1,000 単位 (U) を 10mM トリス –HCl 緩衝液 pH8.0 10ml で溶解する。

2. 酵素溶解希釈用液
0.1% (W/V) BSA と 0.1% (W/V) トリトン X–100 を含む 0.1M KH_2PO_4–NaOH 緩衝液 pH7.0

3. 試薬
トリトン X–100 : Dow Chemical 製
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):
富士フイルム和光純薬製 #308–50463
G6PDH Ⅱ (グルコース–6–リン酸脱水素酵素):
旭化成ファーマ製 #T–51
ATP (アデノシン三リン酸・2Na・$3H_2O$):
協和発酵製
BSA: Millipore 製 Fraction V pH5.2 #81–053

Ⅱ. 酵素試料液
検品約 20mg を精密に量り、酵素溶解希釈用液で溶解して全容 20ml とする。
その液を酵素溶解希釈用液で適宜希釈する。

Ⅲ. 測定操作法

1. 小試験管に反応試薬混合液 3.0ml を正確に分注し、37℃で予備加温する。
2. 5 分経過後、酵素試料液 50 μl を正確に加えて混和し、37℃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希釈用液 50 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。
   求められた吸光度変化の試料液は As/min、盲検液は Ab/min とする。
   ΔA/min = (As/min − Ab/min) ≦ 0.030 Abs/min

Ⅳ. 計算

$$\text{活性 (U/mg)} = \frac{\Delta\ \text{A/min}}{6.22} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

6.22 : NADPH の 340nm におけるミリモル分子吸光係数 (cm^2/ μmole)
3.05 : 反応総液量 (ml)
0.05 : 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)