(Diagnostic Reagent Grade) ASAHI KASEI ENZYMES T-93

ADP-DEPENDENT HEXOKINASE [ADP-HKT Ⅱ]

from *Thermococcus litoralis*
(ADP: D-glucose 6-phosphotransferase, EC 2.7.1.147)

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

Preparation and Specification

Appearance : White amorphous powder, lyophilized
Specific activity : More than 25 U/mg solid

Properties

Substrate specificity	: See table 1	
Molecular weight	: 50 kDa (gel filtration) 50 kDa (SDS-PAGE)	
Isoelectric point	: pH 4.1	
Michaelis constants	: Glucose 0.4 mM (at 37℃) ADP 0.057 mM (at 37℃)	
Optimum pH	: 7-7.5	Figure 1
pH stability	: 6.5-10.5 (37℃, 1 hr)	Figure 2
Optimum temperature	: 100℃	Figure 3
Thermal stability	: Stable at 95℃ and below	Figure 4
Activators	: Mg^{2+}	
Effect of chemicals	: See Table 2	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of 1, 5-Anhydroglucitol (1, 5-AG) when coupled with 1, 5-Anhydroglucitol-6-phosphate dehydrogenase (T-95).

ADP-HKT Ⅱ
ADP + 1, 5-AG ⟶ AMP + 1, 5-AG-6-P
AG6PDH Ⅱ
1, 5-AG-6-P + $NADP^+$ ⟶ $C_6H_{11}O_8P$ + NADPH + H^+

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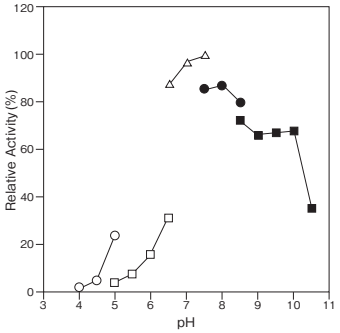
Table 1. Substrate specificity

Substrate (20mM)	Relative activity (%)
D-Glucose	100
D-Mannose	13
D-Galactose	9
D-Fructose	2
2-deoxy-D-Glucose	4
D-Glucosamine	67
D-1,5-Anhydroglucitol	166
D-Sorbitol	0
D-Myoinocitol	0
D-Mannitol	0
Sucrose	0

Table 2. Effect of chemicals on ADP-HKTII activity

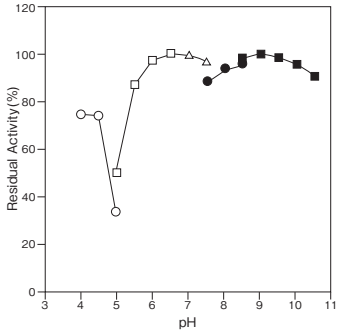
Chemical	Concentration	Relative activity (%)
None	-	100
NaF	1mM	107
NaN_3	1mM	107
EDTA	10mM	0
Cholic acid	0.1%	97
Tween-20	0.1%	103
Tween-40	0.1%	104
Tween-60	0.1%	104
Tween-80	0.1%	103

Fig.1 pH Optimum



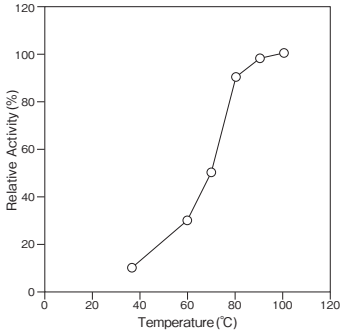
○ : Acetate buffer
□ : Citrate buffer
△ : Phosphate buffer
● : Tris-HCl buffer
■ : Glycine-NaOH buffer

Fig.2 pH Stability



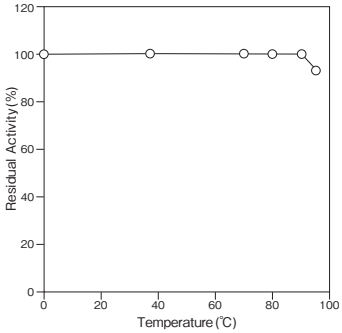
37℃, 60 min.
○ : Acetate buffer
□ : Citrate buffer
△ : Phosphate buffer
● : Tris-HCl buffer
■ : Glycine-NaOH buffer

Fig.3 Optimum Temperature



pH 7.5
50 mM Tris-HCl buffer

Fig.4 Thermal Stability



pH 7.5, 10min.
50 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:

$$\text{ADP + D-Glucose} \xrightarrow[Mg^{2+}]{\text{ADP-HKT II}} \text{AMP + D-Glucose-6-phosphate}$$

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

■ Unit definition

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

■ Reagents

1. Reaction mixture

0.1M Tris-HCl Buffer pH7.5	1.50 ml
0.2M Glucose solution	0.30 ml
20mM ADP solution	0.30 ml
20mM $MgCl_2$ solution	0.30 ml
10mM NADP solution	0.30 ml
50U/ml G6PDH solution	0.30 ml

2. Enzyme dilution buffer
   10mM Tris-HCl Buffer pH7.5 (25℃)
3. Reagents
   Tris (hydroxymethyl) aminomethane:Sigma Chemical Co. #T-1503
   Glucose:FUJIFILM Wako Pure Chemical Corporation #041-00595
   ADP (Adenosine diphosphate・2Na):Oriental yeast Co.,Ltd.
   $MgCl_2 \cdot 6H_2O$:
   FUJIFILM Wako Pure Chemical Corporation #131-00162
   NADP (Nicotinamide adenine dinucleotide oxidized form):
   FUJIFILM Wako Pure Chemical Corporation #308-50463
   G6PDH II (Glucose-6-phosphate dehydrogenase) :
   Asahi Kasei Pharma Corporation #T-51

■ 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 accurately 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 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 6 min)

   Absorbance sample : As/min
   blank : Ab/min
   △ A/min= (As/min − Ab/min) ≦ 0.120Abs/min

■ **Calculation**

$$\text{Activity (U/mg of powder)} = \frac{\triangle\ \text{A/min}}{6.3} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

6.3 : millimolar extinction coefficient of NADPH at 340nm ($cm^2/\mu mol$)
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. Koga, S., Yoshioka, I., Sakuraba, H., Takahashi, M., Sakasegawa, S., Shimizu S. and Ohshima T. (2000) J. Biochem. (Tokyo), **128**, 1079–1085.
2. Ito, S., Fushinobu, S., Yoshioka, I., Koga, S., Matsuzawa, H. and Wakagi, T. (2001) Structure, **9**, 205–214.

ADP-HKT Ⅱ活性測定法 (Japanese)

Ⅰ. 試薬液

1. 反応試薬混合液

0.1M トリス－HCl 緩衝液 pH7.5	1.50 ml
0.2M グルコース溶液	0.30 ml
20mM ADP 溶液	0.30 ml
20mM 塩化マグネシウム溶液	0.30 ml
10mM NADP 溶液	0.30 ml
50U/ml G6PDH 溶液	0.30 ml

2. 酵素溶解希釈用液
   10mM トリス－HCl 緩衝液 pH7.5 (25℃)
3. 試薬
   トリス (ヒドロキシメチル) アミノメタン:
   シグマ製 #T-1503
   グルコース:富士フイルム和光純薬製 特級
   #041-00595
   ADP (アデノシン二リン酸･2Na):
   オリエンタル酵母製
   塩化マグネシウム ($MgCl_2 \cdot 6H_2O$):
   富士フイルム和光純薬製 特級 #131-00162
   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

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 ずつを正確に分注し、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。(直線範囲例:2 分目から 6 分目まで)
   求められた吸光度変化を
   試料液については As/min
   盲検液については Ab/min とする。
   ※吸光度範囲
   Δ A/min=(As/min − Ab/min) ≦ 0.120Abs/min

Ⅳ. 計算

以下の計算式に従い、活性 (U/mg) を計算する。

$$\text{活性 (U/mg)} = \frac{\Delta\ \text{A/min}}{6.3} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

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