

ADP-DEPENDENT HEXOKINASE [ADP-HKT II]

from *Thermococcus litoralis*
(ADP-specific glucokinase, EC 2.7.1.147)



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°C) ADP 0.057 mM (at 37°C)	
Optimum pH	: 7-7.5	Figure 1
pH stability	: 6.5-10.5 (37°C, 1 hr)	Figure 2
Optimum temperature	: 100°C	Figure 3
Thermal stability	: Stable at 95°C 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).

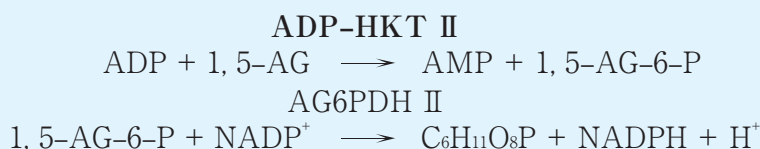


Table 1. Substrate specificity

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

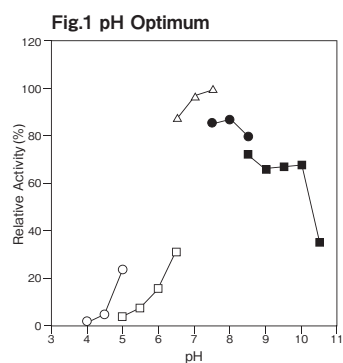


Fig.1 pH Optimum

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

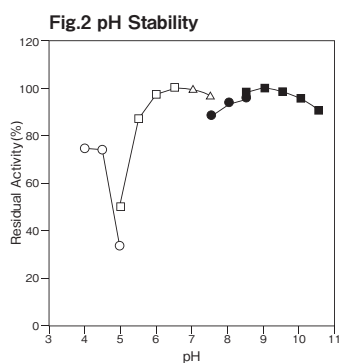


Fig.2 pH Stability

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

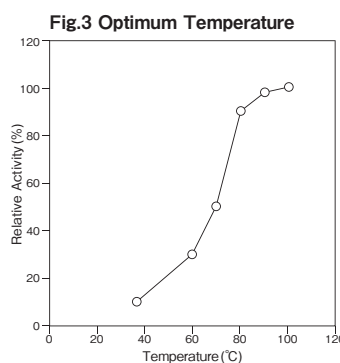


Fig.3 Optimum Temperature

pH 7.5
 50 mM Tris-HCl buffer

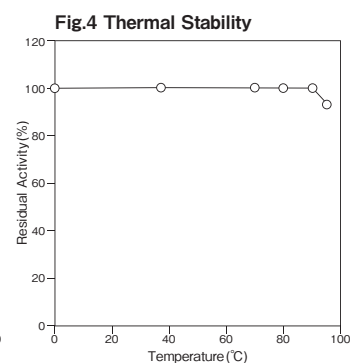


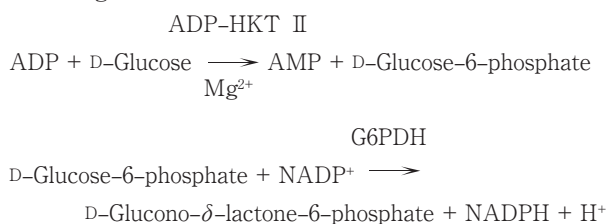
Fig.4 Thermal Stability

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 reaction.



Unit definition

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

Reagents

- 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 |
- Enzyme dilution buffer

10mM Tris-HCl Buffer pH7.5 (25°C)

 - Reagents

Tris (hydroxymethyl) aminomethane:Sigma Chemical Co. #T-1503
Glucose:Wako Pure Chemical Industries, Ltd. #041-00595
ADP (Adenosine diphosphate·2Na):Oriental yeast Co.,Ltd.
MgCl ₂ ·6H ₂ O:Wako Pure Chemical Industries, Ltd. #131-00162
NADP (Nicotinamide adenine dinucleotide oxidized form): Wako Pure Chemical Industries, Ltd. #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

- Pipette accurately 3.0 ml of reaction mixture into a small test tube and preincubate at 37°C.
- After 5 min. add accurately 50 μl of enzyme solution and mix to start the reaction at 37°C.
※ In the case of a test blank, add 50 μl of enzyme dilution buffer in place of enzyme solution.
- 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.

$$\Delta A/\text{min.} = (As/\text{min.} - Ab/\text{min.}) \leq 0.120 \text{ Abs/min.}$$

■ Calculation

$$\begin{aligned} \text{Activity (U/mg of powder)} &= \frac{\Delta A/\text{min.}}{6.3} \times \frac{3.05}{0.05} \times \frac{1}{X} \\ &= \Delta A/\text{min.} \times 9.68 \div X \end{aligned}$$

6.3 : millimolar extinction coefficient of NADPH at 340nm
($\text{cm}^2/\mu\text{mol}$)

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°C in the presence of a desiccant is recommended.

References

- Koga, S., Yoshioka, I., Sakuraba, H., Takahashi, M., Sakasegawa, S., Shimizu S. and Ohshima T. (2000) J. Biochem. (Tokyo) , **128**, 1079-1085.
- 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

- 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

- 酵素溶解希釈用液
10mM トリス-HCl 緩衝液 pH7.5 (25°C)

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
グルコース:和光純薬工業製 特級 #041-00595
ADP (アデノシン二リン酸・2Na):
オリエンタル酵母社製
塩化マグネシウム ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$):
和光純薬工業製 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):和光純薬工業社製
#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

- 小試験管に反応試薬混合液 3.0ml ずつを正確に分注し、37°C で予備加温する。
- 5分経過後、酵素試料液 50 μl を正確に加えて混和し、37°C 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。

- 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 (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

$$\Delta A/\text{min.} = (As/\text{min.} - Ab/\text{min.}) \leq 0.120 \text{ Abs/min.}$$

IV. 計算

以下の計算式に従い、活性 (U/mg) を計算する。

$$\begin{aligned} \text{活性 (U/mg)} &= \frac{\Delta A/\text{min.}}{6.3} \times \frac{3.05}{0.05} \times \frac{1}{X} \\ &= \Delta A/\text{min.} \times 9.68 \div X \end{aligned}$$

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

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)