(Diagnostic Reagent Grade) ASAHI KASEI ENZYMES T–51

GLUCOSE-6-PHOSPHATE DEHYDROGENASE [G6PDH Ⅱ]

from *Bacillus* sp.
(D–Glucose–6–phosphate: $NADP^+$ 1–oxidoreductase, EC 1.1.1.49)

D–Glucose–6–phosphate + $NADP^+$ ⟶
D–Glucono–δ–lactone–6–phosphate + NADPH + H^+

Preparation and Specification

Appearance : White amorphous powder, lyophilized
Specific activity : More than 100 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 342 kDa (gel filtration)	
Isoelectric point	: pH 6.13	
Michaelis constants	: $NADP^+$ 8.3×10^{-6}M G–6–P 1.2×10^{-4}M	
Optimum pH	: pH 8.4 (Tris–HCl)	Figure 1
pH stability	: pH 6.0–8.0 (75℃, 30 min)	Figure 2
Optimum temperature	: 75℃	Figure 3
Thermal stability	: Stable at 65℃ and below (pH 7.5, 10 min)	Figure 4
Effect of various chemicals	: See Table 2	
Inhibitors	: Mn^{2+}, Cu^{2+}, Al^{3+}	
Stabilizer	: BSA	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **glucose or ATP** when coupled with hexokinase (T–50).

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

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

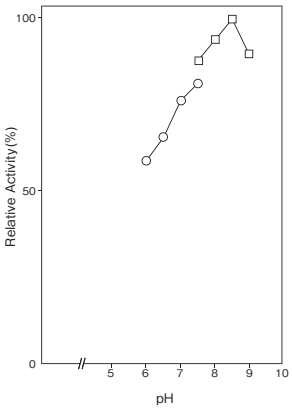
Table 1. Substrate specificity

Substrate	Relative activity (%)
Glucose-6-phosphate	100
Galactose-6-phosphate	16
Mannose-6-phosphate	33
Fructose-6-phosphate	0
Glucose-1-phosphate	0

Table 2. Effect of various chemicals on G6PDH II activity

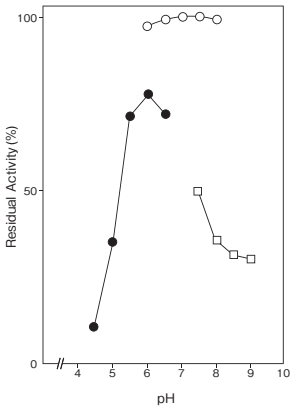
Additive	Concentration	Relative activity (%)
None		100
NaCl	10 mM	100
KCl	10	100
LiCl	1	100
$MgCl_2$	10	100
$CaCl_2$	10	100
$BaCl_2$	10	97
$MnCl_2$	1	42
EDTA	1	100
$CuCl_2$	1	22
Triton X-100	1%	155
Adekatol PC-8	1	161
Nikkol OP-10	1	155
Tetronic 704	1	117

Fig.1 pH Optimum



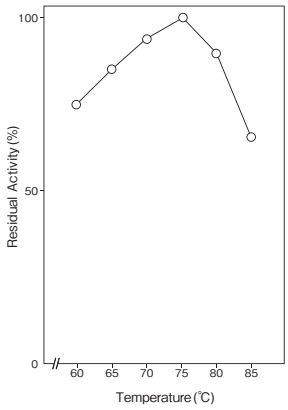
40 mM buffer, 37℃
○ : Phosphate buffer
□ : Tris-HCl buffer

Fig.2 pH Stability



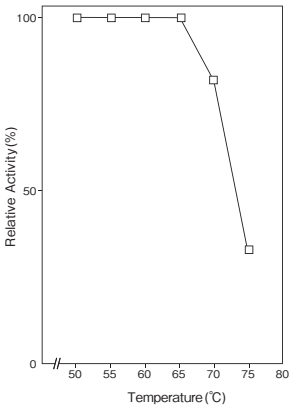
40 mM buffer, 75℃, 15 min.
○ : Phosphate buffer
● : 3,3-Dimethylglutarate-NaOH buffer
□ : Tris-HCl buffer

Fig.3 Optimum Temperature



40 mM Phosphate buffer (pH 7.5)

Fig.4 Thermal Stability



pH 7.5, 15 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:

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

NADP: Nicotineamide adenine dinucleotide phosphate

■ Unit definition

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

■ Reagents

1. Reaction mixture

0.2 M KH_2PO_4–K_2HPO_4 buffer pH 7.5	1.50 ml
2.0% (W/V) BSA solution	0.30 ml
10 mM NADP solution	0.30 ml
0.1 M D-Glucose-6-phosphate solution	0.30 ml
Distilled water	0.60 ml

2. Enzyme dilution buffer
   10 mM KH_2PO_4–K_2HPO_4 buffer pH 7.5
3. Reagents
   NADP (oxidized form):
   Wako Pure Chemical Industries, Ltd. #308-50463
   D-Glucose-6-phosphate: Sigma Chemical Co. #G-7250
   BSA: Millipore Fraction V pH5.2 #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 ≦ △A/min ≦ 0.050 Abs/min

■ **Calculation**

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

6.22 : millimolar extinction coefficient of NADPH at 340 nm (cm²/ 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

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G6PDH Ⅱ活性測定法（Japanese）

Ⅰ．試薬液

1. 反応試薬混合液

0.2M KH_2PO_4–K_2HPO_4 緩衝液 pH7.5	1.50 ml
2.0%（W/V）BSA 溶液	0.30 ml
10mM NADP 溶液	0.30 ml
0.1M G–6–P 溶液	0.30 ml
精製水	0.60 ml

2. 酵素溶解希釈用液
   10mM KH_2PO_4–K_2HPO_4 緩衝液 pH7.5
3. 試薬
   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）：和光純薬工業製　#308–50463
   G–6–P（D–Glucose–6–phosphate）：シグマ社製　#G–7250
   BSA: Millipore 社製　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。
   0.030 Abs/min ≦ Δ A/min =（As/min − Ab/min）≦ 0.050 Abs/min

Ⅳ．計算

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

6.22 : NADPH の 340nm におけるミリモル分子吸光係数 (cm²/ 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）