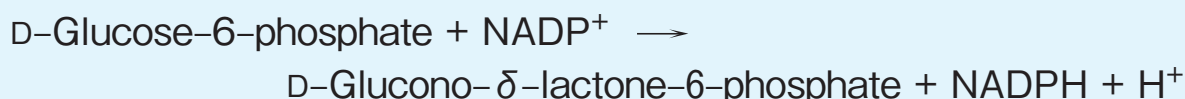


(Diagnostic Reagent Grade)

T-51

GLUCOSE-6-PHOSPHATE DEHYDROGENASE [G6PDH II]

from *Bacillus* sp.

(D-Glucose-6-phosphate: NADP⁺ 1-oxidoreductase, EC 1.1.1.49)


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°C, 15 min)	Figure 2
Optimum temperature	: 75°C	Figure 3
Thermal stability	: Stable at 65°C and below (pH 7.5, 15 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).

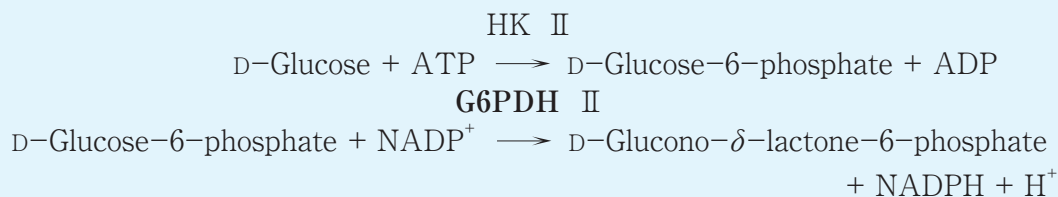


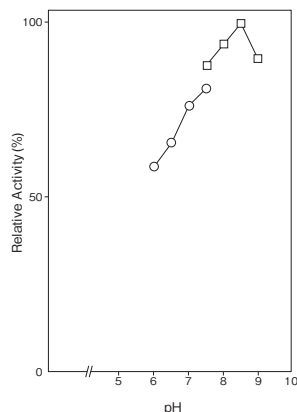
Table 1. Substrate specificity

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	10mM	100
KCl	10mM	100
LiCl	1mM	100
MgCl ₂	10mM	100
CaCl ₂	10mM	100
BaCl ₂	10mM	97
MnCl ₂	1mM	42
EDTA	1mM	100
CuCl ₂	1mM	22
Triton X-100	1%	155
Adekato PC-8	1%	161
Nikkol OP-10	1%	155
Tetronic 704	1%	117

Fig.1 pH Optimum

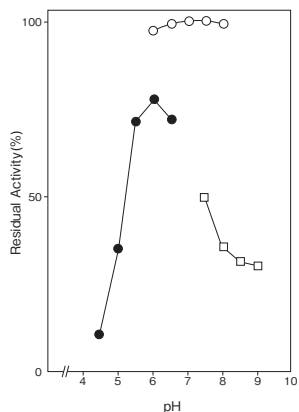


40 mM buffer, 37°C

○ : Phosphate buffer

□ : Tris-HCl buffer

Fig.2 pH Stability



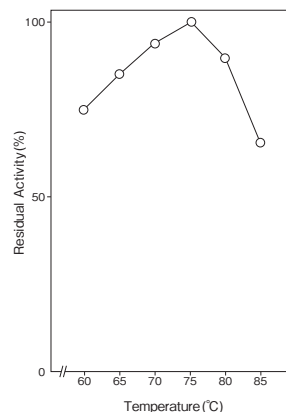
40 mM buffer, 75°C, 15 min.

○ : Phosphate buffer

● : 3,3-Dimethylglutarate-NaOH buffer

□ : Tris-HCl buffer

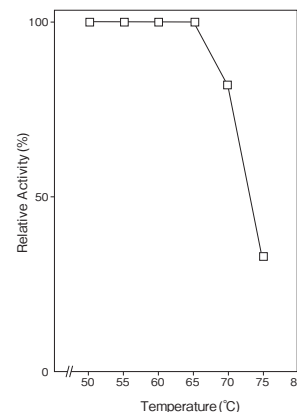
Fig.3 Optimum Temperature



pH 7.5

40mM Phosphate buffer

Fig.4 Thermal Stability



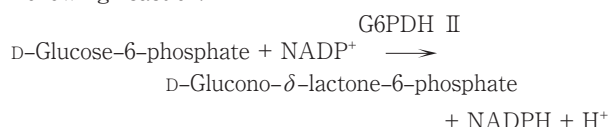
pH 7.5, 15 min.

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 reaction:



NADP: Nicotinate adenine dinucleotide phosphate

Unit definition

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

Reagents

- Reaction mixture

0.2 M KH ₂ PO ₄ -K ₂ HPO ₄ buffer pH 7.5	1.5 ml
2.0% (W/V) BSA solution	0.3 ml
10 mM NADP solution	0.3 ml
0.1 M D-Glucose-6-phosphate solution	0.3 ml
Distilled water	0.6 ml
- Enzyme dilution buffer

10 mM KH ₂ PO ₄ -K ₂ HPO ₄ buffer pH 7.5
--
- Reagents

NADP (oxidized form):	
FUJIFILM Wako Pure Chemical Corporation #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°C.
2. After 5 min, add exactly 50 μ l of enzyme solution and mix to start the reaction at 37°C.
※ In the case of a test blank, add 50 μ l 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.

$$\begin{aligned} \text{Absorbance} \quad & \text{sample} : A_s/\text{min} \\ & \text{blank} : A_b/\text{min} \\ \Delta A/\text{min} &= A_s/\text{min} - A_b/\text{min} \\ 0.030 \text{ Abs/min} &\leq \Delta A/\text{min} \leq 0.050 \text{ Abs/min} \end{aligned}$$

■ Calculation

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

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

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

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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	1.5 ml
2.0% (W/V) BSA 溶液	0.3 ml
10mM NADP 溶液	0.3 ml
0.1M G-6-P 溶液	0.3 ml
精製水	0.6 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・リン酸化型):
富士フイルム和光純薬製 #308-50463

G-6-P (D-Glucose-6-phosphate):
シグマ製 #G-7250

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°C で予備加温する。
2. 5 分経過後、酵素試料液 50 μ l 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 μ l 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 A_s/min 、盲検液は A_b/min とする。
 $0.030 \text{ Abs/min} \leq \Delta A/\text{min} = (A_s/\text{min} - A_b/\text{min}) \leq 0.050 \text{ Abs/min}$

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
($\text{cm}^2/\mu\text{mole}$)

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)