NIPRO ENZYMES

6-PHOSPHOGLUCONATE DEHYDROGENASE (DECARBOXYLATING) (6PGDH)

[EC 1. 1. 1. 44]

from *Microorganism*

6-Phospho-D-gluconate + NAD^+ ↔ D-Ribulose 5-phosphate + CO_2 + NADH + H^+

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 40 U/mg protein	
Contaminants	:	(as 6PGDH activity = 100 %)	
		Glucokinase	< 0.01 %
		Phosphoglucomutase	< 0.01 %
		Hexose-6-phosphate isomerase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 132,000	
Subunit molecular weight	:	ca. 33,000	
Optimum pH	:	7.0 - 7.5	(Fig. 1)
pH stability	:	5.0 - 10.0	(Fig. 2)
Isoelectric point	:	ca. 4.5	
Thermal stability	:	(50 mM MES-NaOH buffer, pH 6.8, containing 0.5 M KCl)	
		No detectable decrease in activity up to 40 °C.	(Fig. 3, 4)
Michaelis constants	:	(80 mM GIycylglycine buffer, pH 7.5, at 30 °C)	
		6-Phospho-D-gluconate	0.95 mM
		NAD^+	0.32 mM
Stabilizer	:	KCl, $MgCl_2$, Sorbitol, BSA	
Activators	:	Mg^{2+}, Mn^{2+}, Ca^{2+}, K^+, Na^+	
Inhibitors	:	Fructose 1,6-bisphosphate, Erythrose 4-phosphate, NADH	

STORAGE

Stable at -20 °C for at least six months

ASSAY

Principle

The change in absorbance is measured at 340 nm according to the following reaction.

$$\text{6-Phosphogluconate} + NAD^+ \xrightarrow{\text{6PGDH}} \text{Ribulose 5-phosphate} + CO_2 + NADH + H^+$$

Unit Definition

One unit of activity is defined as the amount of 6PGDH that forms 1 μmol of NADH per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Glycylglycine-NaOH, pH 7.5
Ⅱ 6-Phospho-D-gluconate (6PG) solution ; 100 mM (0.378g 6PG trisodium salt·$2H_2O$/10 mL distilled water)
Ⅲ NAD^+ solution ; 50 mM (0.332 g NAD^+ free acid/10 mL distilled water)
Ⅳ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 100 mM MES-NaOH buffer containing 1 mg/mL BSA, pH 6.8.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution Ⅰ 24.6mL
   Solution Ⅱ 3.0mL
   Solution Ⅲ 2.1mL
   Solution Ⅳ 0.3mL
2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{340}) \text{ X } (3.00 + 0.01)}{6.22 \text{ X } 0.01} \text{ X d.f.}$$

$$\text{Specific activity (U/mg protein)} = \frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)*}}$$

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH (cm^2/μmol)
*Protein concentration ; determined by Bradford's method

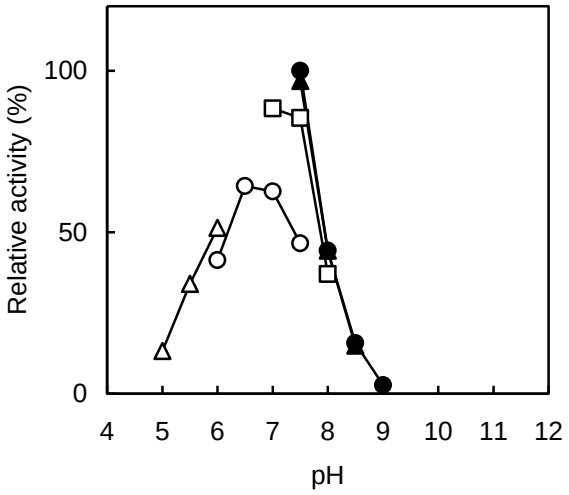

Fig. 1 pH profile

△ acetate, ○ phosphate,
□ TEA-NaOH, ▲ GlyGly-NaOH,
● Tris-HCl

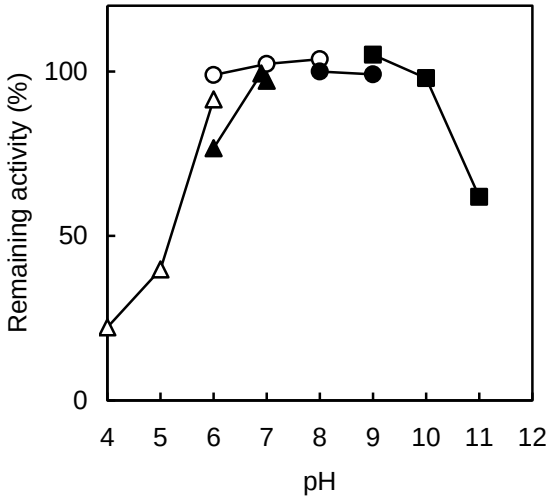

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, ○ phosphate,
▲ MES-NaOH, ● Tris-HCl,
■ Gly-KOH

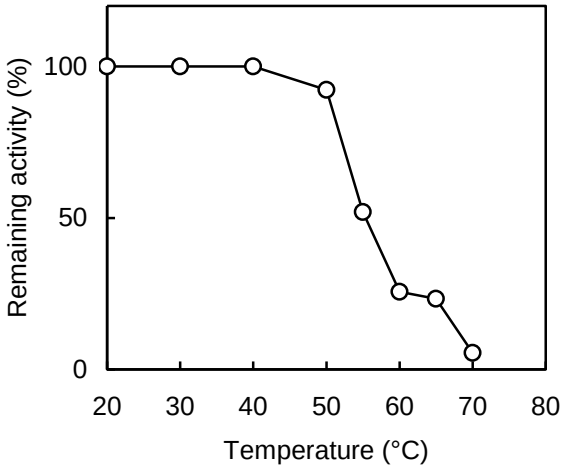

Fig. 3 Thermal stability

treated for 15 min in 50 mM MES-NaOH buffer, pH 6.8, containing 0.5 M KCl

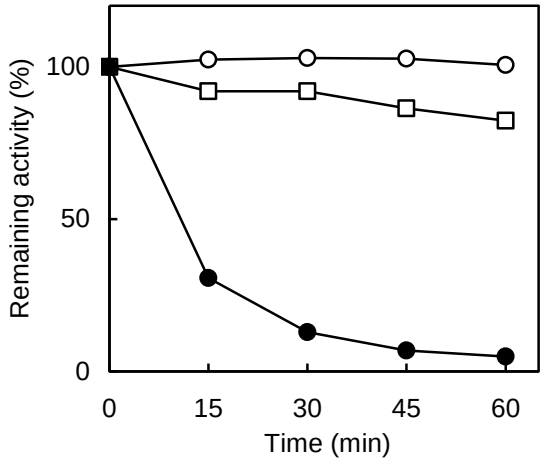

Fig. 4 Thermal stability

treated in 50 mM MES-NaOH buffer, pH 6.8, containing 0.5 M KCl
○ 40 °C, □ 50 °C, ● 60 °C