NIPRO

NIPRO ENZYMES

GLUCOSE DEHYDROGENASE (GlcDH2)

[EC 1. 1. 1. 47]

from recombinant *E. coli*

SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 900 U/mg protein	
Contaminants	: (as GlcDH2 activity = 100 %)	
	NADH oxidase	< 0.01 %

PROPERTIES

Molecular weight	: ca. 126,000	
Subunit molecular weight	: ca. 31,500	
Optimum pH	: 8.5	(Fig. 1)
pH stability	: 5.0 - 10.0 (with 3M NaCl)	(Fig. 2)
Thermal stability	: No significant decrease in activity up to 70 °C.	
	(with 3M NaCl and 0.1% BSA)	(Fig. 3, 4)
Michaelis constants	: D-Glucose	3.7 mM
	NAD$^+$	0.06 mM
	NADP$^+$	0.02 mM
Substrate specificity (100mM)	: D-Glucose	100 %
	D-Maltose	1.1 %
	D-Galactose	0.1 %
	D-Xylose	3.0 %
	D-Fructose	0.3 %
	D-Mannose	4.8 %
	D-Arabinose	0 %
	Trehalose	0 %
	D-Lactose	1.3 %
	D-Sucrose	0 %
	2-Deoxy-D-Glucose	100 %
	D-Glucose-1-Phosphate	0 %
	D-Glucose-6-Phosphate	0 %
	D-Sorbitol	0 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

This enzyme is useful for determination of glucose.

ASSAY

Principle

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

Unit Definition

One unit of activity is defined as the amount of GlcDH2 that forms 1 μmol of NADH per minute at 37 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH 8.5 (at 25 °C)
Ⅱ NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid/10 mL distilled water)
Ⅲ D-Glucose solution ; 1 M (1.802 g glucose (anhyd.)/10 mL distilled water)
Ⅳ NaCl solution ; 5 M (2.92 g NaCl/10 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 15 U/mL with 20 mM potassium phosphate buffer containing 1 mg/mL BSA and 2 M NaCl, pH 6.5.

Procedure

1. Prepare the following reaction mixture and pipette 2.70 mL of reaction mixture into a cuvette.

Solution Ⅰ	17.22 mL
Solution Ⅱ	0.50 mL
Solution Ⅲ	2.00 mL
Solution Ⅳ	0.28 mL

2. Incubate at 37 °C for about 3 minutes.
3. Add 0.015 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

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{340}) \times (2.70 + 0.015)}{6.22 \times 0.015} \times \text{d.f.}$$

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

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH ($cm^2/\mu mol$)
*Protein concentration ; determined by the absorbance at 280nm (Abs280), where 1 Abs280 = 1 mg/mL

REFERENCE

1. Ramaley, R.F. and Vasantha, N.; *J. Biol. Chem.* **258**, 12558-12565 (1983)

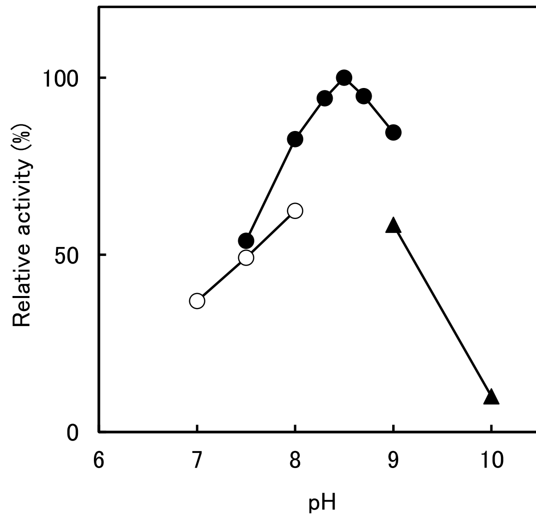

Fig. 1 pH profile

[○ phosphate, ● Tris-HCl, ▲ glycine]

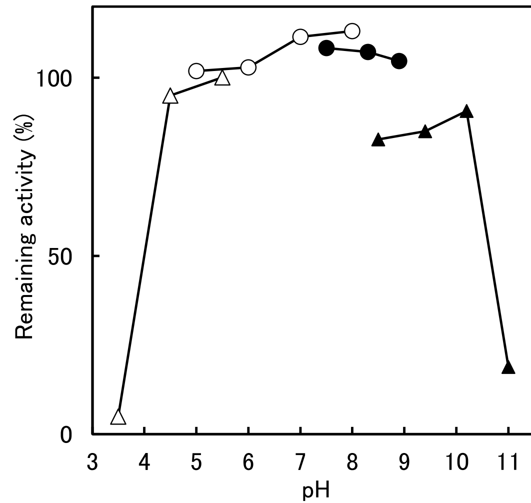

Fig. 2 pH stability

(treated for 24 hr at 4 ℃ in the following buffer solution (0.1 M) containg 3 M NaCl : △ acetate, ○ phosphate, ● Tris-HCl, ▲ glycine)

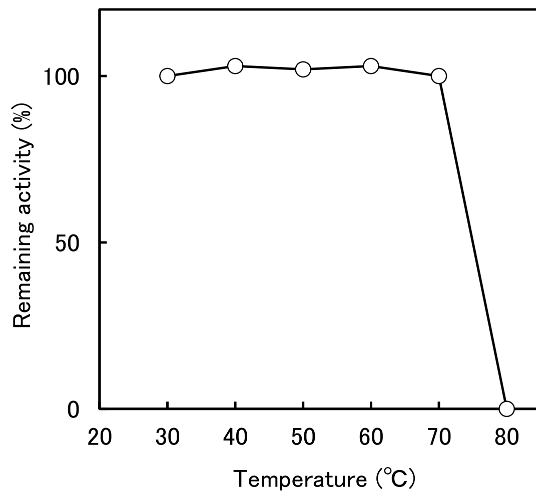

Fig. 3 Thermal stability

(treated for 15 min in 0.1M phosphate buffer, pH 6.5, containing 3 M NaCl and 0.1 % BSA)

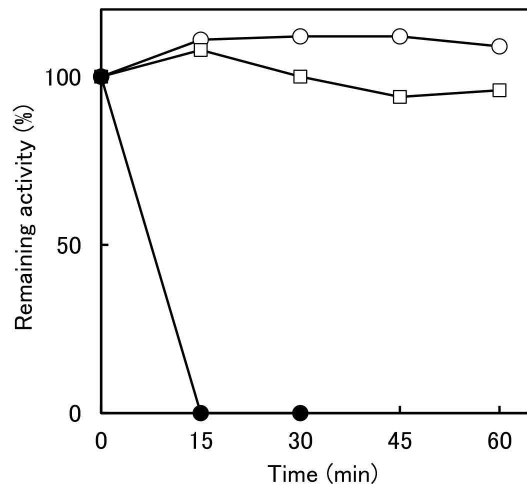

Fig. 4 Thermal stability

(treated for in 0.1 M phosphate buffer, pH 6.5, containing 3M NaCl and 0.1 % BSA, ○ 60℃, □ 70℃, ● 80℃)