NIPRO ENZYMES

SORBITOL DEHYDROGENASE (SorDH)

[EC 1.1.1.14]

from *Microorganism*

D-Sorbitol + NAD^+ ↔ D-Fructose + NADH + H^+

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 30 U/mg protein	
Contaminants	:	(as SorDH activity = 100 %)	
		NADH oxidase	<0.01 %

PROPERTIES

Molecular weight	:	ca. 68,000	
Subunit molecular weight	:	ca. 26,000	
Optimum pH	:	11.0	(Fig. 1)
pH stability	:	6.0 - 10.0	(Fig. 2)
Optimum temperature	:	40 °C	
Thermal stability	:	No detectable decrease in activity up to 35 °C.	(Fig. 3, 4)
Michaelis constants	:	(100 mM Tris-HCl buffer, pH 9.0, at 30°C)	
		D-Sorbitol	3.4 mM
		NAD^+	0.13 mM
Substrate specificity	:	D-Sorbitol	100 %
		Galactitol	27 %
		L-Iditol	42 %
		Xylitol	1 %
		D-Arabitol	0 %
		D-Mannitol	0 %
		D-Glucose	0 %
		D-Galactose	0 %
		Maltose	0 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

This enzyme is useful for determination of D-Sorbitol in clinical analysis and food analysis.

ASSAY

Principle

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

$$\text{D-Sorbitol} + NAD^+ \xrightarrow{\text{SorDH}} \text{D-Fructose} + NADH + H^+$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl buffer, pH 9.0
Ⅱ NAD^+ solution ; 20 mM (133 mg NAD^+ free acid /10 mL distilled water)
Ⅲ D-Sorbitol solution ; 500mM (911 mg D-Sorbitol/10 mL 100 mM Tris-HCl buffer, pH 9.0)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50mM Tris-HCl buffer containing 1 mg/mL BSA, pH 8.0.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   solution Ⅰ 24.00mL
   solution Ⅱ 3.00mL
   solution Ⅲ 3.00mL
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 the curve.

Calculation

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

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

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

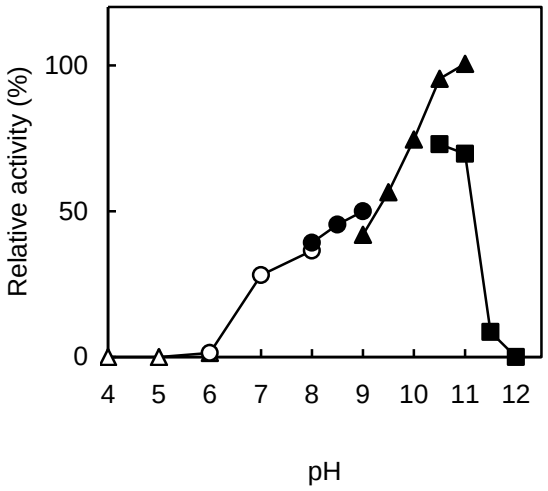

Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl, ▲Gly-KOH,
■ Na_2HPO_4-NaOH

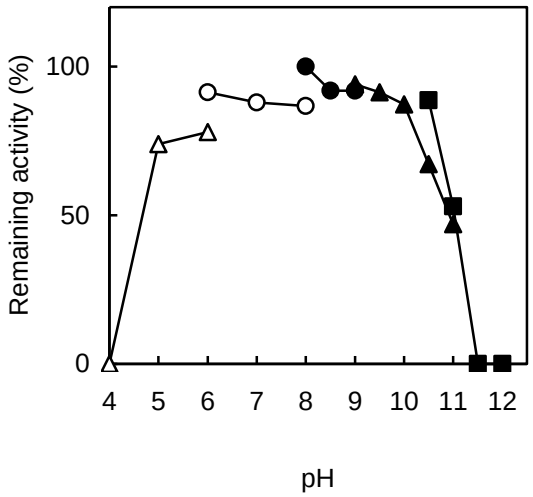

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, ○ phosphate,
● Tris-HCl, ▲ Gly-KOH,
■ Na_2HPO_4-NaOH

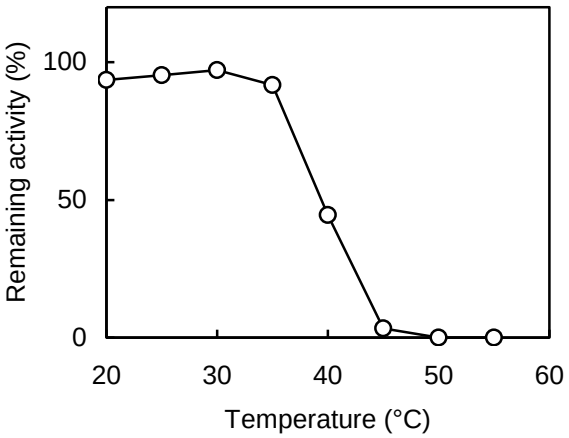

Fig. 3 Thermal stability

treated for 15 min in 0.1 M Tricine buffer, pH 8.0

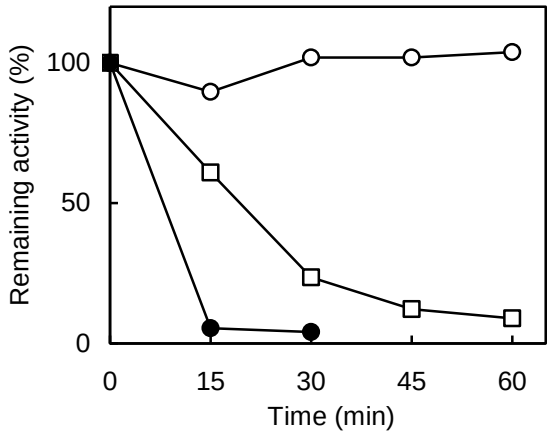

Fig. 4 Thermal stability

treated in 0.1 M Tricine -NaOH buffer, pH 8.0
○ 35 °C, □ 40 °C, ● 45 °C