NIPRO

NIPRO ENZYMES

LEUCINE DEHYDROGENASE (LeuDH)

[EC 1. 4. 1. 9]

from *Bacillus stearothermophilus*

SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 40 U/mg protein	
Contaminants	: (as LeuDH activity = 100 %)	
	NADH oxidase	< 0.01 %
	Lactate dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	: ca. 300,000	
Subunit molecular weight	: ca. 49,000	
Optimum pH	: 10.6	(Fig. 1)
pH stability	: 6.0 - 11.5	(Fig. 2)
Thermal stability	: No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	: (125 mM Sodium phosphate buffer, pH 10.5, at 30 °C)	
	L-Leucine	3.4 mM
	NAD^+	0.3 mM
Substrate specificity	: L-Leucine	100 %
	L-Valine	86 %
	L-Isoleucine	73 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for determination of L-leucine, L-valine or L-isoleucine.

ASSAY

Principle

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

$$\text{L-Leucine} + NAD^{+} + H_2O \xrightarrow{\text{LeuDH}} \alpha\text{-Ketoisocaproate} + NH_4^{+} + NADH$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 250 mM Sodium phosphate, pH 10.5
Ⅱ L-Leucine solution ; 60 mM (0.787 g L-leucine/80 mL distilled water, adjusted to pH 10.5 with 1 N-NaOH and filled up to 100 mL with distilled water)
Ⅲ NAD^{+} solution ; 100mM (0.663 g NAD^{+}/ 10mL with distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 100 mM sodium phosphate buffer, pH 9.5.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette,

Solution Ⅰ	15.00 mL	Solution Ⅲ	0.93 mL
Solution Ⅱ	10.00 mL	H_2O	4.07 mL

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

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

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

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

REFERENCE

1. Ohshima, T., Nagata, S., and Soda, K.; *Arch. Microbiol.*, **141**, 407 (1985)

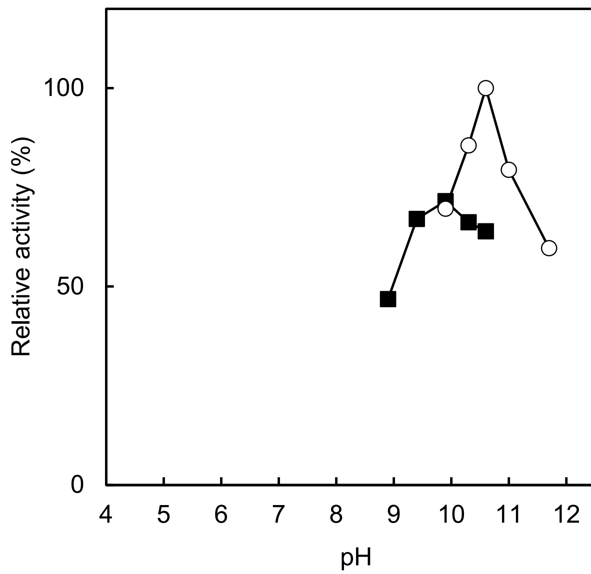

Fig. 1 pH profile

(■ Gly-KOH, ○ phosphate)

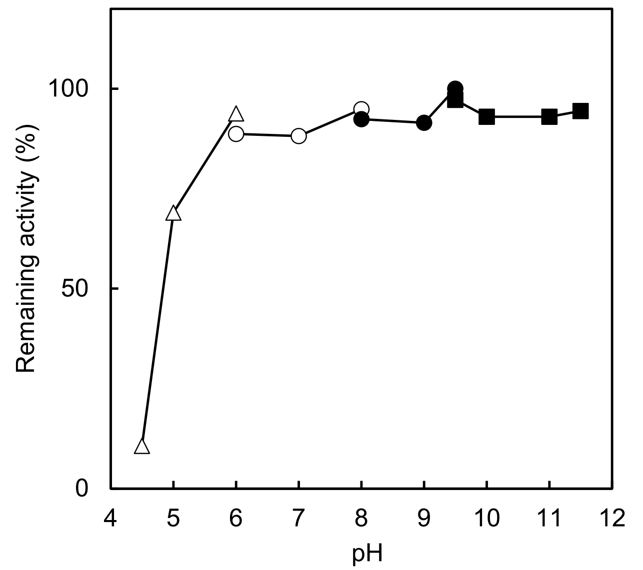

Fig. 2 pH stability

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

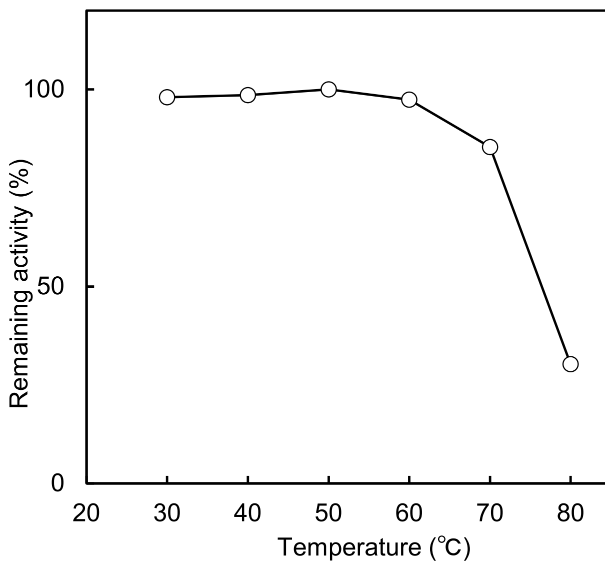

Fig. 3 Thermal stability

(treated for 15 min in 0.1 M Gly-KOH buffer, pH 9.0)

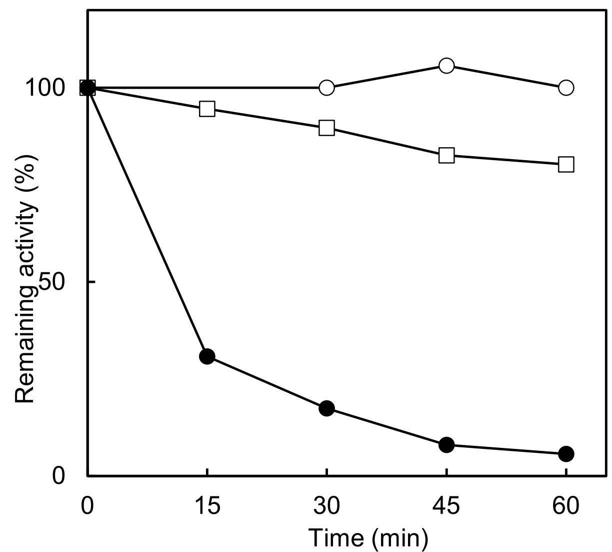

Fig. 4 Thermal stability

(treated in 0.1M Gly-KOH buffer, pH 9.0 ○ 60 ℃, □ 70 ℃, ● 80 ℃)