NIPRO ENZYMES

ALANINE RACEMASE (AlaR)

[EC 5. 1. 1. 1]

from *Bacillus stearothermophilus*

D-Alanine ↔ L-Alanine

SPECIFICATION

State	:	Liquid	
Specific activity	:	more than 950 U/mg protein	
Contaminants	:	(as AlaR activity = 100 %)	
		Lactate dehydrogenase	< 0.01 %
		NADH oxidase	< 0.01 %
		Alanine dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 78,000	
Subunit molecular weight	:	ca. 39,000	
Optimum pH	:	10.5 - 12.0	(Fig. 1)
pH stability	:	5.5 - 11.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 70 °C.	(Fig. 3, 4)
Michaelis constants	:	(100 mM Carbonate buffer, pH 10.5, at 30 °C)	
		D-Alanine	31 mM
Substrate specificity	:		

STORAGE

Stable at least one year at -25 °C.

ASSAY

Principle

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

$$\text{D-Alanine} \xrightarrow{\text{AlaR}} \text{L-Alanine}$$

$$\text{L-Alanine} + NAD^+ + H_2O \xrightarrow{\text{AlaDH}} \text{Pyruvate} + NH_4^+ + \text{NADH}$$

Unit Definition

One unit of activity is defined as the amount of AlaR that forms 1 μmol of L-alanine per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 200 mM Sodium hydrogencarbonate, pH 10.5
Ⅱ D-Alanine solution ;1 M (0.891 g D-alanine/10 mL distilled water)
Ⅲ NAD^+ solution ; 100 mM (0.663 g NAD^+/10 mL distilled water)
Ⅳ L-Alanine dehydrogenase (AlaDH) ; 1000 U/mL (from *Bacillus stearothermophilus*, Nipro Corp., Dissolve with distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50 mM potassium phosphate buffer, pH 7.5.

Procedure

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

Solution Ⅰ	16.50 mL	SolutionⅣ	1.50 mL
Solution Ⅱ	3.00 mL	H_2O	8.25 mL
SolutionⅢ	0.75 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

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

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

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

REFERENCE

1. Inagaki, K., Tanizawa, K., Badet, B., Walsh, C.T., Tanaka, H., and Soda, K.; *Biochemistry*, **25**, 3268 (1986)

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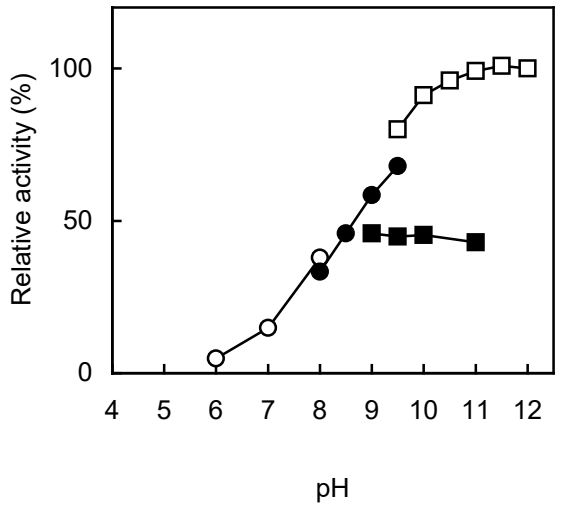

Fig. 1 pH profile

○ phosphate, ● Tris-HCl,
■ Gly-KOH, □ $NaHCO_3$-NaOH

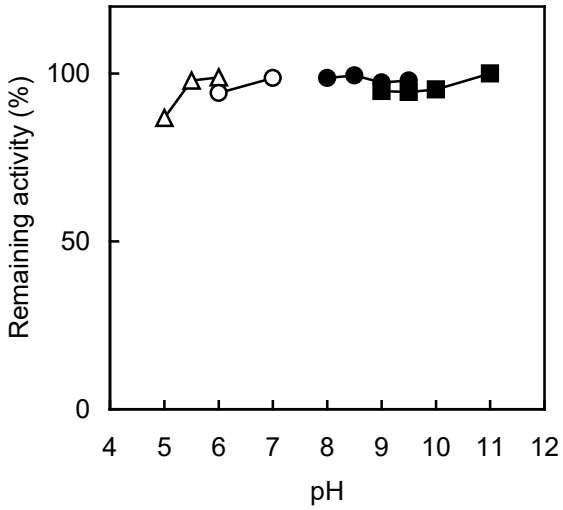

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.2 M);
△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

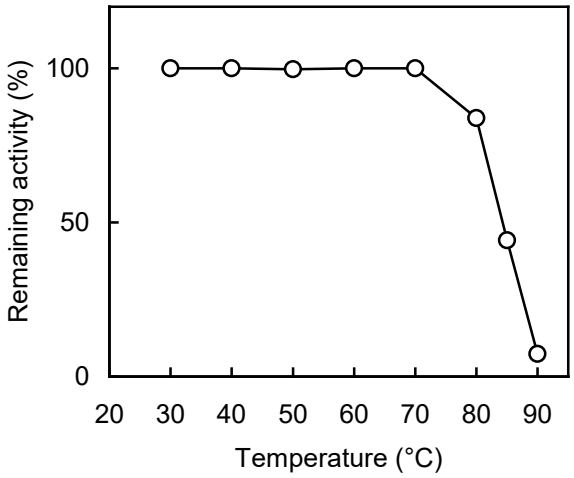

Fig. 3 Thermal stability

treated for 15 min in 50 mM Tris-HCl buffer, pH 9.0

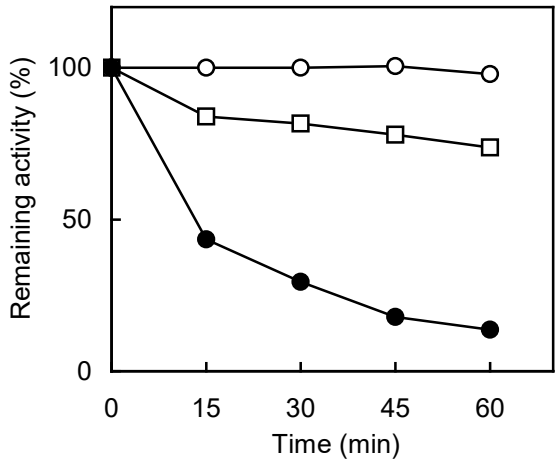

Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 9.0
○ 70 °C, □ 80 °C, ● 85 °C

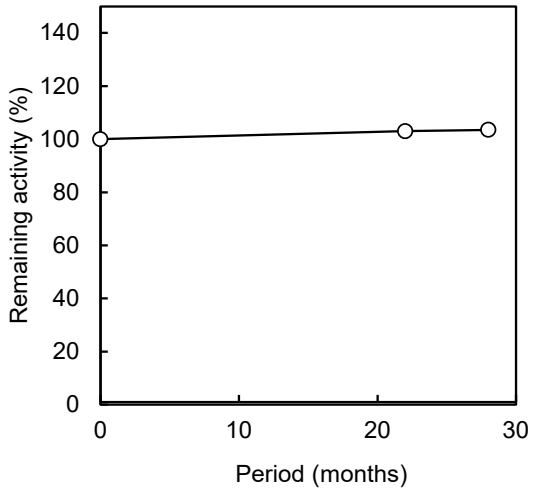

Fig. 5 Stability (Liquid form) at -25 °C