Alanine Racemase

D-Alanine ↔ L-Alanine

AlaR

EC 5. 1. 1. 1

Bacillus stearothermophilus

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

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.

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

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

1. Buffer solution ; 200 mM Sodium hydrogencarbonate, pH 10.5
2. D-Alanine solution ;1 M (0.891 g D-alanine/10 mL distilled water)
3. NAD^+ solution ; 100 mM (0.663 g NAD^+/10 mL distilled water)
4. 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 1. 16.50 mL	Solution 3. 0.75 mL	H_2O 8.25 mL
Solution 2. 3.00 mL	Solution 4. 1.50 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.

Alanine Racemase | (AlaR)

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 ($cm^2/\mu mol$)
*Protein concentration ; determined by Bradford's method

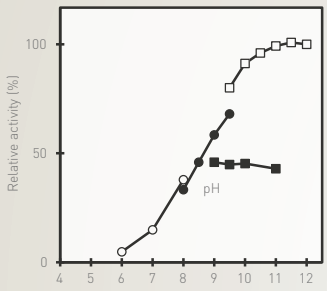

Fig. 1 pH profile

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

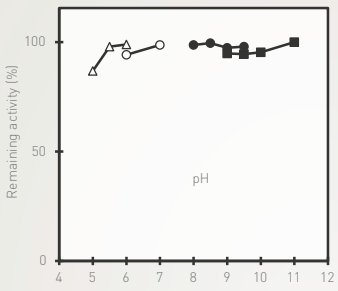

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.2 M);

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

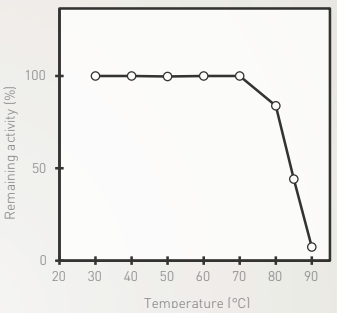

Fig. 3 Thermal stability

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

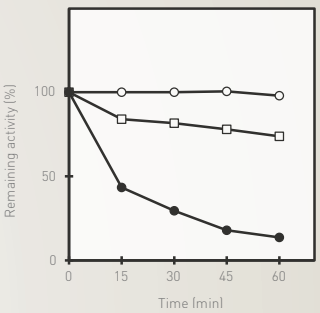

Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 9.0

○ 70 °C, □ 80 °C, ● 85 °C

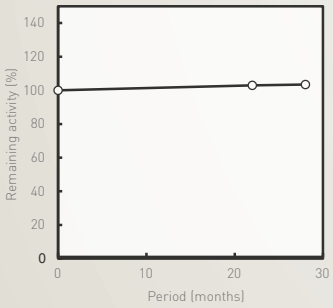

Fig. 5 Stability (Liquid form) at -25 °C

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