NIPRO

NIPRO ENZYMES

ALCOHOL DEHYDROGENASE (ZM-ADH)

[EC 1 .1 .1 .1]

from *Zymomonas mobilis*

SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 400 U/mg protein	
Contaminants	: (as ZM-ADH activity = 100 %)	
	Glucose-6-phosphate dehydrogenase	< 0.10 %
	Glucokinase	< 0.02 %
	Pyruvate kinase	< 0.02 %
	NADH oxidase	< 0.01 %
	Lactate dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	: ca. 148,000	
Subunit molecular weight	: ca. 37,000	
Optimum pH	: 9.5 - 10.0	(Fig. 1)
pH stability	: 7.0 - 9.0	(Fig. 2)
Thermal stability	: No detectable decrease in activity up to 40 °C.	(Fig. 3, 4)
Michaelis constants	: (100 mM Glycine-KOH buffer, pH 9.0, at 30 °C)	
	Ethanol	110 mM
	Methanol	350 mM
	NAD^+	0.12 mM
	Acetaldehyde	1.66 mM
	NADH	0.03 mM
Substrate specificity	: Ethanol	100 %
	Methanol	0.05 %
	n-Propanol	42.3 %
	n-Butanol	0.28 %

STORAGE

Stable at -20 °C for at least six months

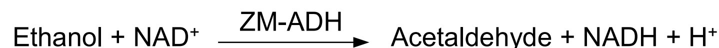
APPLICATION

The enzyme is useful for determination of alcohols or aldehydes.

ASSAY

Principle

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



$$\text{Ethanol} + \text{NAD}^+ \xrightarrow{\text{ZM-ADH}} \text{Acetaldehyde} + \text{NADH} + \text{H}^+$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 80 mM Glycine-KOH, pH 9.5
Ⅱ NAD^+ solution ; 10 mM (0.0663 g NAD^+ free acid/10 mL distilled water)
Ⅲ Ethanol solution ; Ethanol (96 %)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50 mM Tris succinate buffer containing 1mg/mL BSA and 0.2 mM $CoCl_2$, pH 7.0

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution Ⅰ 22.90 mL
   Solution Ⅱ 6.00 mL
   Solution Ⅲ 1.10 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. Neale, A.D., Scopes. R.K., Kelly, J.M., and Wettenhall, R.E.H.; *Eur. J. Biochem.*, **154**, 119 (1986)

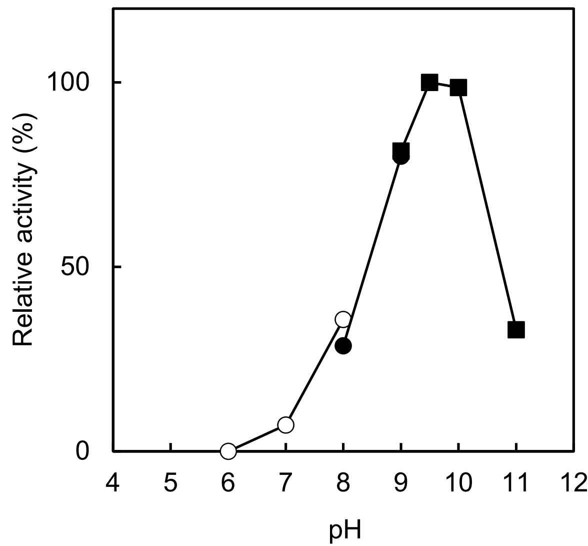


Fig. 1 pH profile

(○ phosphate, ● Tris-HCl, ■ Gly-KOH)

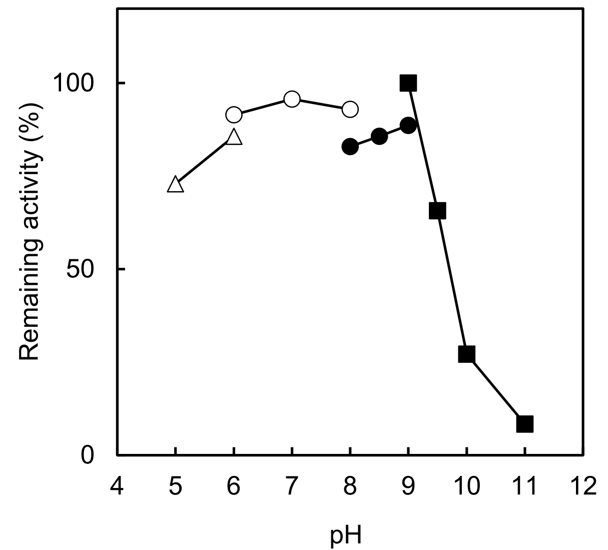


Fig. 2 pH stability

(treated for 24 hr at 4 ℃ in the following buffer solution (0.1 M), containing 0.5 mM $CoCl_2$;
△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH)

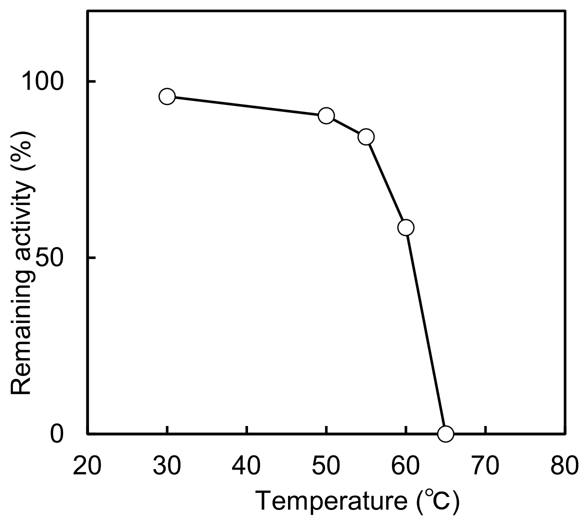


Fig. 3 Thermal stability

(treated for 15 min in 0.1 M phosphate buffer containing 0.2 mM $CoCl_2$, pH 6.5)

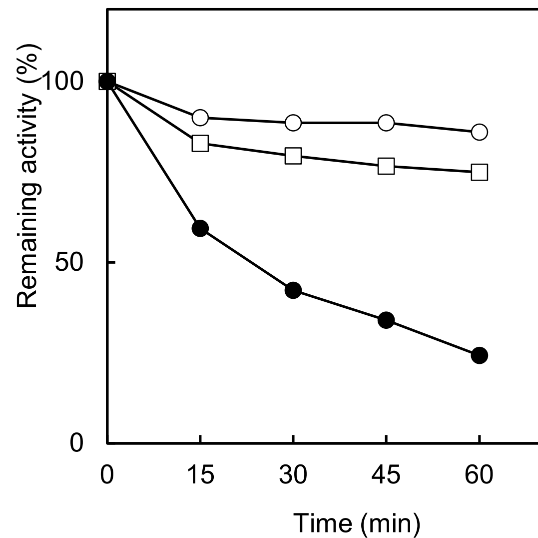


Fig. 4 Thermal stability

(treated in 0.1 M phosphate buffer containing 0.2 mM $CoCl_2$, pH 6.5
○ 50 ℃, □ 55 ℃, ● 60 ℃)