NIPRO ENZYMES

GALACTOSE DEHYDROGENASE (GalDH)

[EC 1. 1. 1. 48]

from recombinant *E. coli*

$$D\text{-Galactose} + NAD(P)^+ \leftrightarrow D\text{-Galactono-}\delta\text{-lactone} + NAD(P)H + H^+$$

SPECIFICATION

State	:	Ammonium sulphate suspension	
Specific activity	:	more than 80 U/mg protein	
Contaminants	:	(as GalDH activity = 100 %)	
		NADH oxidase	< 0.10 %
		LDH	< 0.10 %
		ADH	< 0.01 %

PROPERTIES

Subunit molecular weight	:	ca. 33,800	
Optimum pH	:	10.5	(Fig. 1)
pH stability	:	5.0 - 10.0	(Fig. 2)
Thermal stability	:	No significant decrease in activity up to 50 °C with Ammonium sulphate and 40 °C without Ammonium sulphate .(Fig. 3, 4)	
Michaelis constants	:	D-Galactose	0.25 mM
		NAD^+	0.15 mM
Substrate specificity (100mM)	:	D-Galactose	100 %
		D-Glucose	0.2 %
		D-Xylose	8.7 %
		D-Maltose	0.1 %
		D-Sucrose	0.1 %

STORAGE

Store at 2 to 10 °C (Do not freeze)
Stable at 4 °C for at least one year

APPLICATION

This enzyme is useful for determination of galactose.

ASSAY

Principle

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

$$\text{D-Galactose} + \text{NAD}^+ \xrightarrow{\text{GalDH}} \text{D-Galactono-}\delta\text{-lactone} + \text{NADH} + \text{H}^+$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH9.1 (at 30 °C)
Ⅱ NAD^+ solution ; 100 mM
Ⅲ D-Galactose solution ; 1 M
Ⅳ Enzyme diluent ; 20 mM potassium phosphate, 0.1 % bovine serum albumin, pH 7.5

Preparation of Enzyme Solution

Dilute the enzyme suspension to approx. 5 U/mL with the enzyme diluent.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution Ⅰ 27.60 mL
   Solution Ⅱ 0.90 mL
   Solution Ⅲ 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.

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 the Bradford's method

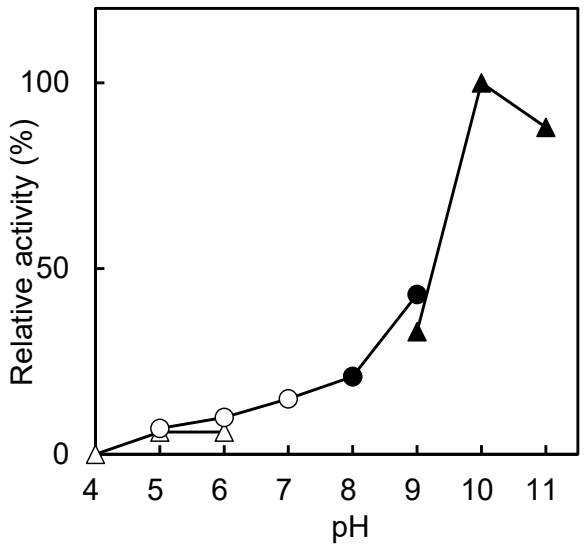

Fig. 1 pH profile

△ acetate, ○ phosphate, ● Tris-HCl, ▲ Glycine-KOH

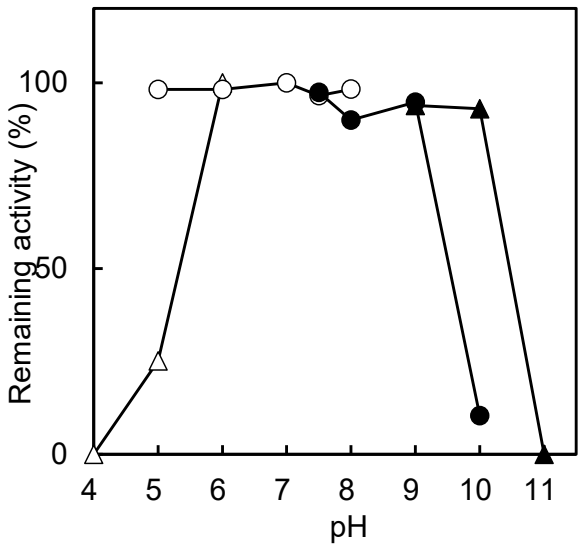

Fig. 2 pH stability

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

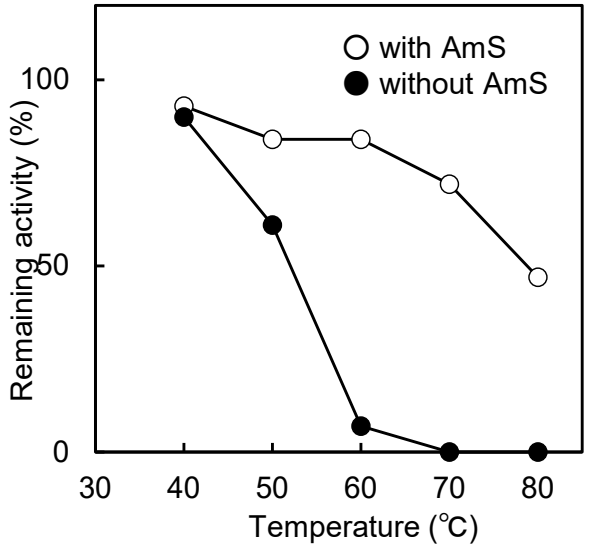

Fig. 3 Thermal stability

treated for 15 min in 25 mM potassium phosphate buffer pH 7.5, with or without 3.2 M ammonium sulphate (AmS).

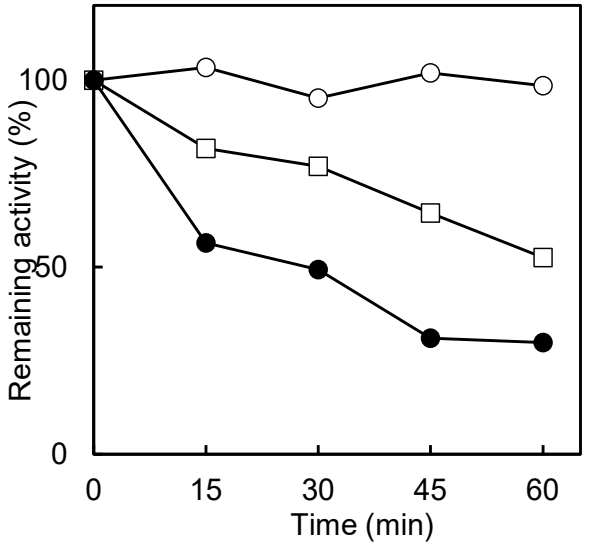

Fig. 4 Thermal stability

treated in 25 mM potassium phosphate buffer pH 7.5 at ○ 40 ℃, □ 50 ℃, ● 60 ℃ without ammonium sulphate.

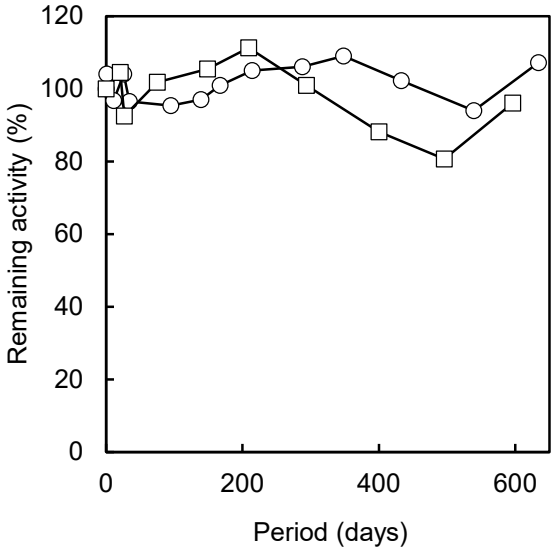

Fig. 5 Storage Stability

(ammonium suiphate suspension (ca. 1300 U/mL) store at 4 ℃(○)