NIPRO ENZYMES

# D-LACTATE DEHYDROGENASE (D-LDH)

[EC 1. 1. 1. 28]

from *Microorganism*

$$\text{D-Lactate} + NAD^+ \leftrightarrow \text{Pyruvate} + NADH + H^+$$

*FOR PYRUVATE → LACTATE REACTION*

**SPECIFICATION**

| | | | |
|---|---|---|---|
| State | : | Lyophilized | |
| Specific activity | : | more than 2,500 U/mg protein | |
| Contaminants | : | (as D-LDH activity = 100 %) | |
| | | NADH oxidase | < 0.01 % |
| | | GOT | < 0.01 % |
| | | GPT | < 0.01 % |

**PROPERTIES**

| | | | |
|---|---|---|---|
| Molecular weight | : | ca. 80,000 | |
| Subunit molecular weight | : | ca. 40,000 | |
| Optimum pH | : | 7.5 | (Fig. 1) |
| pH stability | : | 5.5 - 10.0 | (Fig. 2) |
| Isoelectric point | : | 4.1 | |
| Thermal stability | : | No detectable decrease in activity up to 40 °C. | (Fig. 3, 4) |
| Michaelis constants | : | (94 mM Potassium phosphate buffer, pH 7.5, at 30 °C) | |
| | | Pyruvate | 0.80 mM |
| | | NADH | 0.18 mM |
| Stabilizers | : | $(NH_4)_2 SO_4$, BSA | |
| Inhibitors | : | $Zn_2^+$, $Cu_2^+$ | |

**STORAGE**

Stable at -20 °C at least one year

## ASSAY

### Principle

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

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{D-LDH}} \text{D-Lactate} + \text{NAD}^+$$

### Unit Definition

One unit is defined as the amount of D-LDH that forms 1 μmol of $NAD^+$ per minute at 30 °C.

### Solutions

Ⅰ Buffer solution ; 100 mM Potassium phosphate buffer, pH 7.5
Ⅱ Sodium pyruvate solution ; 100 mM (100 mg sodium pyruvate/10 mL distilled water)
Ⅲ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)

### Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 3 to 5 U/mL with 50 mM potassium phosphate buffer containing 1 mg/mL BSA, pH 7.0.

### Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution Ⅰ 28.00 mL
   Solution Ⅱ 1.20 mL
   Solution Ⅲ 0.80 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 ($\Delta Abs_{340}$) 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

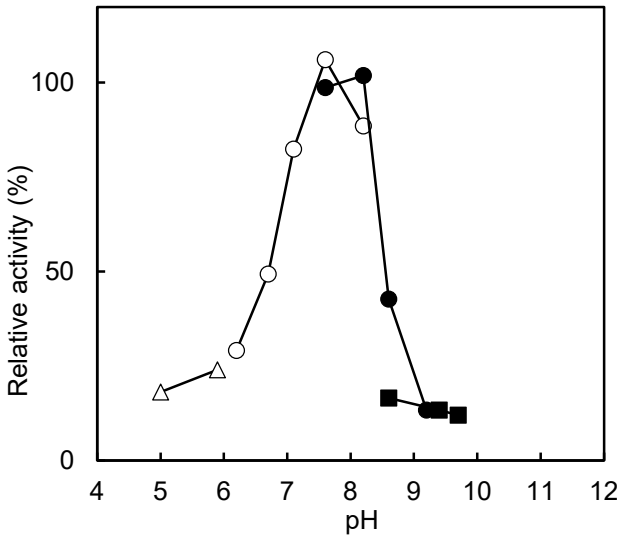


Fig. 1 pH profile

△ acetate, ○ phosphate,

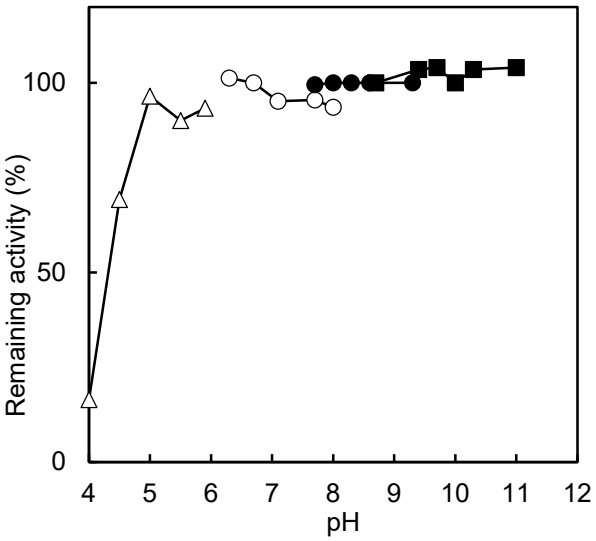


Fig. 2 pH stability

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

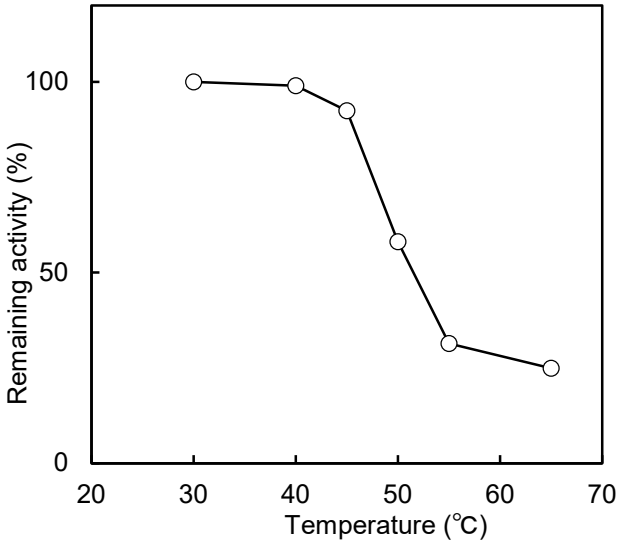


Fig. 3 Thermal stability

treated for 15 min in 0.1 M potassium phosphate buffer, pH 7.0

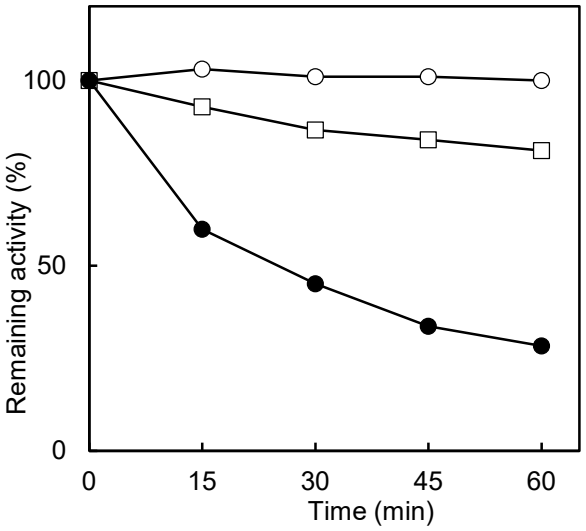


Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 7.0
○ 40 ℃, □ 45 ℃, ● 50 ℃