D-Lactate Dehydrogenase

D-Lactate + NAD^+ ↔ Pyruvate + NADH + H^+

D-LDH

EC 1. 1. 1. 28

Microorganism

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	: (NH4)2 SO4, 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.

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

1. Buffer solution ; 100 mM Potassium phosphate buffer, pH 7.5
2. Sodium pyruvate solution ; 100 mM (100 mg sodium pyruvate/10 mL distilled water)
3. 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 1. 28.00 mL Solution 2. 1.20 mL Solution 3. 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 (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 ($cm^2/\mu mol$)
*Protein concentration ; determined by Bradford's method

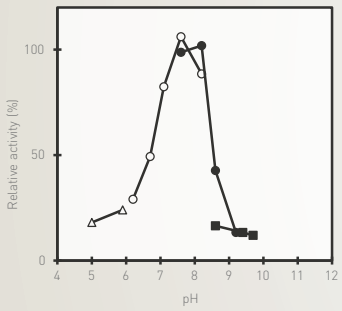

Fig. 1 pH profile

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

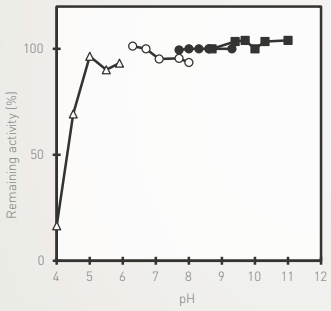

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);

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

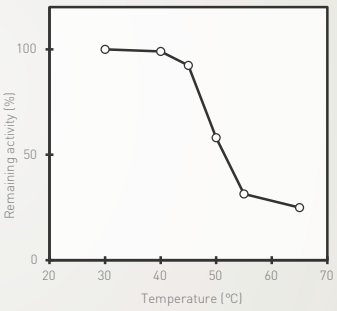

Fig. 3 Thermal stability

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

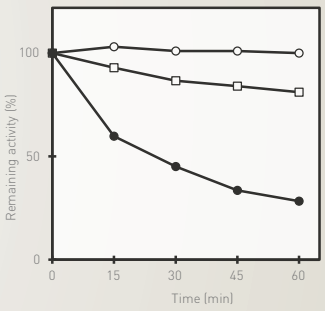

Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 7.0

○ 40 °C, □ 45 °C, ● 50 °C