Lactate Dehydrogenase (LDH-P)

from porcine heart

(*S*)-Lactate : NAD^+ oxidoreductase, EC 1.1.1.27

Pyruvate + NADH + H^+ $\rightleftharpoons$ L-Lactate + NAD^+

SPECIFICATION

Appearance	white lyophilizate	
Activity	≧150 U/mg lyophilizate	
Contaminants	GPT	≦1.0×10^{-2}%
	malate dehydrogenase	≦5.0×10^{-2}%
	pyruvate kinase	≦1.0×10^{-2}%
Storage	at −20°C	

PROPERTIES

Molecular weight	*ca.* 115 kDa (gel filtration)	
Michaelis constants	2.5×10^{-2} M (lactate)	
	1.0×10^{-4} M (pyruvate)	
pH Optimum	7.4–8.0	(Fig. 1)
pH Stability	7.0–7.5	(Fig. 2)
Optimum temperature	50–55°C	(Fig. 3)
Thermal stability	below 45°C	(Fig. 4)

ASSAY PROCEDURE

Principle

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{lactate dehydrogenase}} \text{L-Lactate} + \text{NAD}^+$$

The disappearance of NADH is measured spectrophotometrically at 340 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of NAD^+ per min at 25°C and pH 7.4 under the conditions described below.

Reagents

A. Potassium phosphate buffer, 50 mM; pH 7.4: mix 50 mM KH_2PO_4 solution and 50 mM K_2HPO_4 solution to make a pH 7.4 solution.
B. Buffered water, 5 mM; pH 7.4: 10 ml of potassium phosphate buffer (Reagent A)/100 ml of distilled water.
C. NADH solution, 6 mM: 21.3 mg of NADH · Na_2/5 ml of buffered water (Reagent B).
D. Sodium pyruvate solution, 23 mM: 12.5 mg of sodium pyruvate/5 ml of buffered water (Reagent B).
E. Enzyme dilution buffer: potassium phosphate buffer (Reagent A) containing 0.1% bovine serum albumin (BSA).

Sample: dissolve the enzyme preparation to a volume activity of 0.2–0.5 U/ml with ice-cold enzyme dilution buffer (Reagent E) immediately before measurement.

Procedure

1. Pipette the following reagents into a cuvette (light path: 1 cm).

2.7 ml	Potassium phosphate buffer	(Reagent A)
0.1 ml	NADH solution	(Reagent C)
0.1 ml	Sodium pyruvate solution	(Reagent D)

2. Equilibrate at 25°C for about 5 min.
3. Add 0.1 ml of sample and mix.
4. Record the decrease of absorbance at 340 nm in a spectrophotometer thermostated at 25°C, and calculate the ΔA per min using the linear portion of the curve (ΔA_S).
   The blank solution is prepared by adding enzyme dilution buffer (Reagent E) instead of sample (ΔA_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_S - \Delta A_0) \times 3.0(\text{ml}) \times df}{6.2 \times 0.1(\text{ml})} = \Delta A \times 4.84 \times df$$

Weight activity (U/mg) = (U/ml) × 1/*C*

6.2 : Millimolar extinction coefficient of NADH ($cm^2/\mu mol$)
df : Dilution factor
C : Content of lactate dehydrogenase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of numerous metabolites (ATP, ADP, glucose, creatinine, pyruvate, lactate and glycerol) and of enzyme activities (GPT, PK and CPK) when coupled with the related enzymes.

$$\text{L-Alanine} + \text{2-Oxoglutarate} \xrightarrow{\text{GPT}} \text{Pyruvate} + \text{L-Glutamate}$$

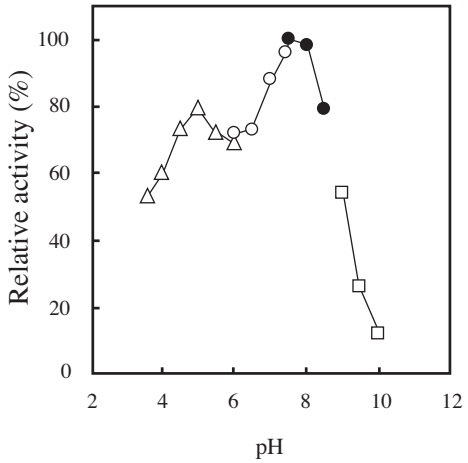
$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{lactate dehydrogenase}} \text{L-Lactate} + \text{NAD}^+$$

REFERENCES

Jaenicke, R. and Pfleiderer, G., *Biochim. Biophys. Acta*, **60**, 615 (1962).
Everse, J. and Kaplan, N. O., *Adv. in Enzymol.*, **37**, 61 (1973).
Holbrook, J. J. *et al.*, "The Enzyme," Vol. 11 (3rd ed.), Academic Press, New York and London, 1975, pp. 191–292.
Loshon, C. A. *et al.*, *Clin.Chem.*, **23**, 1579 (1977).

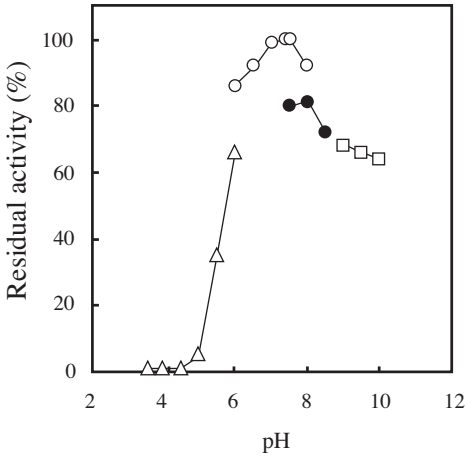
EXPERIMENTAL DATA

Fig. 1 pH Optimum



△ : 0.1 M acetate buffer
○ : 0.1 M phosphate buffer
● : 0.1 M Tris-HCl buffer
□ : 0.1 M CHES buffer

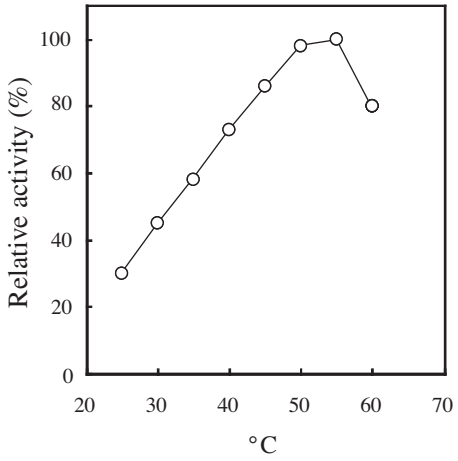
Fig. 2 pH Stability



Treatment: 37°C, 60 min

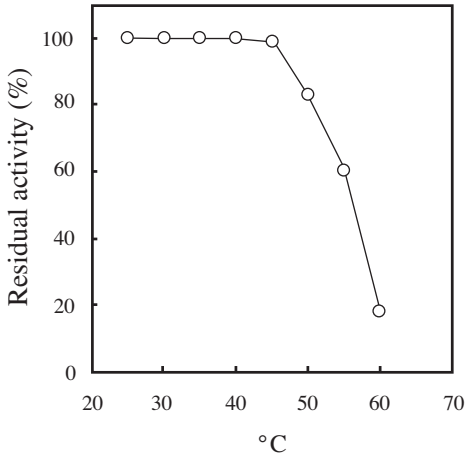
△ : 0.1 M acetate buffer
○ : 0.1 M phosphate buffer
● : 0.1 M Tris-HCl buffer
□ : 0.1 M CHES buffer

Fig. 3 Optimum temperature



Buffer: 50 mM phosphate buffer, pH 7.4

Fig. 4 Thermal stability



Treatment: 0.1 M phosphate buffer, pH 7.4, containing 0.1% BSA, 15 min