Lactate Dehydrogenase“Amano”3

((*R*)-Lactate : NAD^+ oxidoreductase, EC 1.1.1.28)

Lactate Dehydrogenase“Amano”3 is a lactate dehydrogenase preparation manufactured by a unique fermentation process using a selected strain of bacteria.

Catalysis

D-Lactate + NAD^+ + H_2O ⇄ Pyruvate + NADH + H^+

Specification and Preparation

Activity:	Lactate dehydrogenase activity	≧ 100 u/mg (Amano method)
Contaminants:	NADH oxidase activity	≦ 1×10^{-4}%
	α-KGDH activity	≦ 3×10^{-4}%
	GOT activity	≦ 5×10^{-4}%
	GPT activity	≦ 5×10^{-4}%
Appearance:	White powder, lyophilized	
Additive:	Dextran	

Characteristics

1. Molecular weight: 64,000 (Gel filtration)
2. Isoelectric point: 4.3
3. Km: 6.7×10^{-4} M (Pyruvate)
   7.6×10^{-4} M (NADH)
4. Optimum pH: 5.0
5. pH stability: 5.5-8.5 (30°C, 1hr)
6. Optimum temperature: 40°C
7. Thermal stability: up to 35°C (pH 7.0, 1hr)
8. Application: Used for the enzymatic determination of numerous metabolites, e.g. ATP, ADP, glucose, creatinine, pyruvate, lactate and glycerol, and enzyme activities, e.g. GPT, PK and CPK by coupling with the related enzymes in clinical diagnosis.

Expiration (Storage)

24 months from the date of analysis when stored at -20°C or below in a dry place under sealed conditions.

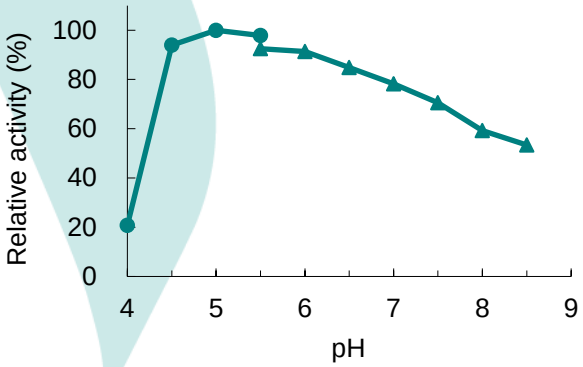
Safe Handling

1. Do not inhale.
2. In case of direct contact with skin or eyes, immediately wash or rinse with plenty of water.
3. Please refer to SDS for more details.

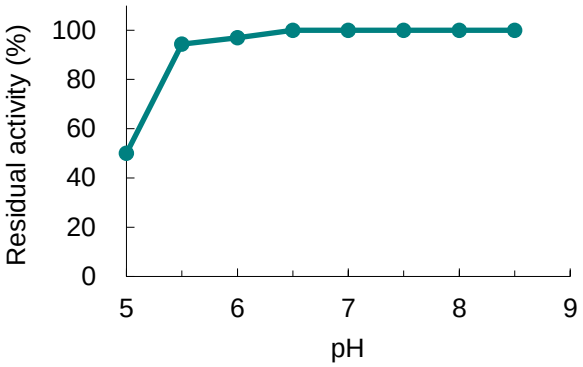
General properties

The following results demonstrate the activities of enzyme solution prepared in various buffers. Enzyme activity may vary under different experimental conditions.

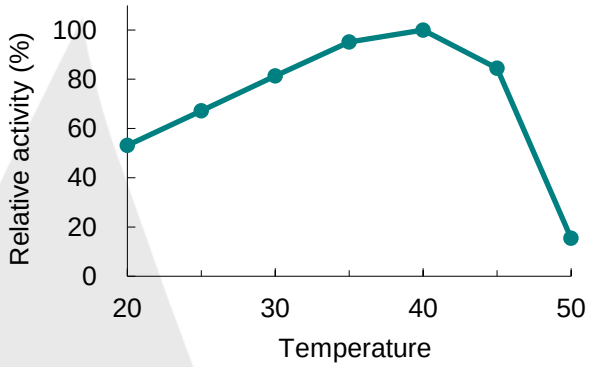
pH and Activity



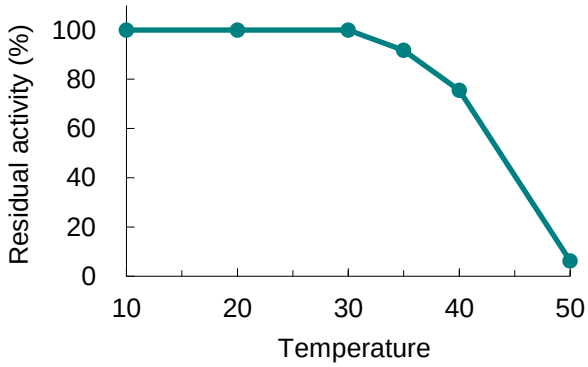
pH stability



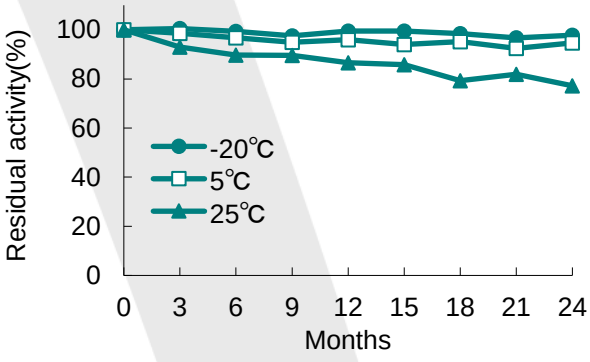
Temperature and Activity



Thermostability



Stability (powder form)



Assay method of Lactate dehydrogenase activity

Principle

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

The consumption of NADH is measured at 340 nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which oxidizes 1 μmol of NADH per minute under the conditions described below.

Reagents

A. 0.1M Tris-HCl buffer (pH 7.8)

B. 0.01 M Phosphate buffer (KH_2PO_4-NaOH (pH 7.0))

C. Substrate solution
Weigh 12.4 mg of β-NADH (Oriental Yeast Co., Ltd), add 20.5 mg of sodium pyruvate (Sigma-Aldrich), then dissolve in 0.1M Tris-HCl buffer (A). Fill up to 100 ml with 0.1M Tris-HCl buffer (A). (Can be used for 5 days in light-shielded and refrigerated storage)

D. Enzyme solution
Weigh out Lactate Dehydrogenase“Amano”3 and dissolve in chilled 0.01M Phosphate buffer (B). Enzyme solution should be prepared so that the value of ΔOD/minute becomes in the range of 0.015±0.005min.

Procedure

Pipette 2.9 mL of Substrate solution (C) into a quartz cell (d=10 m) and keep at 25±0.5 ℃ for 5 minutes. Then, pipette 0.1 mL of Enzyme solution (D) into the quartz cell and mix well immediately. Keep the reaction mixture at 25±0.5 ℃. Exactly at 2 minutes and 5 minutes after the addition of Enzyme Solution (D), measure the absorbances of the reaction mixture at 340 nm. (A2 and A5). As a blank, pipette 0.01M Phosphate buffer (B) into another quartz cell (d=10 mm) instead of Enzyme Solution (D), and take the same procedure described above. (Ab2 and Ab5)

Calculation

$$\text{Lactate dehydrogenase activity (u/mg)} = \frac{(A2-A5)-(Ab2-Ab5)}{3} \times \frac{1}{6.22} \times 3.0 \times \frac{n}{0.1}$$

3: Reaction time

6.22: Millimolar absorption coefficient of NADH at 340 nm

3.0: Volume of the reaction mixture

0.1: Volume of Enzyme solution

n: Dilution factor of Enzyme solution

Contact

Area	Branch	Location	E-mail
North, Central, South America	Amano Enzyme U.S.A. Co., Ltd.	Illinois,U.S.A.	aeu.sales@amano-enzyme.com
Europe, the Middle East and Africa	Amano Enzyme Europe Limited	Oxfordshire, U.K.	aee.sales@amano-enzyme.com
Asia Pacific	Amano Enzyme Asia Pacific Co., Ltd.	Pathum Thani, THAILAND	aeap.sales@amano-enzyme.com
China	Amano Enzyme Manufacturing (China), Ltd. Shanghai Branch	Shanghai, P.R.CHINA	shanghai@amano-enzyme.com.cn
Japan, Headquarters	Amano Enzyme Inc.	Nagoya, JAPAN	