For life science research only.
Not for use in diagnostic procedures.

L-Lactate Dehydrogenase (L-LDH)

from rabbit muscle

L-Lactate: NAD oxidoreductase

Version: 09

Content Version: November 2021

Cat. No. 10 127 230 001	10 mg 2 ml
Cat. No. 10 127 876 001	25 mg 5 ml
Cat. No. 10 127 884 001	100 mg 10 ml

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	L-Lactate Dehydrogenase (L-LDH)	Suspension in 3.2 M ammonium sulfate solution, pH approximately 7.	10 127 230 001	1 vial, 10 mg, 2 ml
			10 127 876 001	1 vial, 25 mg, 5 ml
			10 127 884 001	1 vial, 100 mg, 10 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	L-Lactate Dehydrogenase (L-LDH)	Store at +2 to +8°C.

Storage Conditions (Working Solution)

⚠ ***LDH-4 and LDH-5 are cold labile, therefore preparations which contain these isozymes must not be stored at −15 to −25°C. LDH-1 and LDH-2 may be stored at −15 to −25°C.***

2. How to Use this Product

2.1. Before you Begin

General Considerations

Additional information

- Skeletal muscle LDH requires a higher optimum pyruvate concentration than does heart LDH. Heart LDH is inhibited at concentrations of pyruvate (approximately 1.6 mM) that are near optimum for the muscle preparation. To minimize these differences in an assay of total LDH (as in a mixture of muscle and heart enzyme), the assay may be performed with the higher level of pyruvate in the presence of 200 mM NaCl.
- In 20 mM Tris, pH 7.4, containing 2 mM EDTA (+20°C), LDH-1 and LDH-2 are preferentially adsorbed onto DEAE agarose, while LDH-3, -4 and -5 will pass through.
- Competitive inhibitors of LDH can form with time in phosphate solutions of NADH. To prevent inhibitor formation, make phosphate solutions of NADH immediately before the assay or make stock solutions of NADH in Tris buffer (no inhibitor formation in Tris after 5 days at +2 to +8°C or several months at −15 to −25°C).
- The maximum velocity of the reduction reaction is approximately threefold higher than the maximum velocity of the oxidation reaction with LDH.

2.2. Parameters

Absorbance

The purified enzyme from beef heart has an absorbance of 14.9 (10 mg/ml, 280 nm).

Activator

2-amino-2-methyl-1-propanol, diethanolamine, fluoride, and heparin (oxidation reaction).
- Sodium sulfite (Na_2SO_3) will protect the enzyme from the effects of thiol-attacking reagents.
- Mercaptans can reverse the inhibitory effects of thiol-attacking reagents.

Contaminants

- <0.001% PK and aldolase, each.
- <0.01% GOT, GPT, MDH, and myokinase, each.

EC-Number

EC 1.1.1.27

Inhibition

The oxidation and reduction reaction is not inhibited by cyanide and EDTA.

Oxidation reaction (lactate → pyruvate)

- Pyrophosphate
- Phosphate buffers
- Non-competitive inhibitors (K_i approximately 0.003 mM) formed from NAD (especially at alkaline pH and in the presence of inorganic phosphate, carbonate, or oxalate ions).

Reduction reaction (pyruvate → lactate)

- Excess lactate (K_i = 130 to 210 mM for muscle LDH).
- Non-competitive inhibitors formed from NADH (especially in phosphate buffer).

Oxidation and reduction reactions

Both of these reactions are inhibited by excess concentrations of:
- Pyruvate (K_i 0.3 to 2.0 mM for muscle LDH, oxidation reaction; pyruvate concentrations >2 mM inhibit the reduction reaction slightly).
- Excess NAD
- Oxalic acid (0.2 mM) inhibits LDH-1 more than it inhibits LDH-5.
- Oxamic acid (oxalic acid monoamide)
- Malonic acid
- Tartronic acid (hydroxymalonic acid)
- Urea (2 M) inhibits muscle LDH (LDH-4 and LDH-5) to a greater degree than it inhibits heart LDH (LDH-1 and LDH-2).
- Cu^{2+}
- Thiol-attacking reagents (iodine, Ag^{+}, Hg^{2+}, 4-chloromercuribenzoate).

Molecular Weight

LDH is a tetramer (MW approximately 140,000 Da, rabbit muscle or pig heart LDH). In mammals, there are two types of LDH subunits, M and H, with similar molecular weight (subunit MW approximately 36,500 Da but differing amino acid composition. Therefore, 5 electrophoretically distinguishable LDH isoenzymes, LDH-1 through LDH-5, of differing subunit composition are found in mammals.
The molecular weight of all LDH isoenzymes is approximately the same.

- LDH-1 (H_4), LDH-2 (H_3M): predominant components of heart LDH.
- LDH-3 (H_2M_2): principal component of LDH from lymphatic tissue.
- LDH-4 (HM_3), LDH-5 (M_4): predominate in skeletal muscle or liver LDH.

The M subunit of LDH used to be designated the A subunit; the H subunit was the B subunit. Under the old designation, LDH-1 isoenzyme was LDH-B4.

pH Optimum

Pyruvate reduction

- pH 6.0 for rabbit muscle LDH (activity at pH 7.0 ≈84% of maximum; activity at pH 8.0 ≈50% of maximum).
- pH 7.0 for pig heart LDH (activity at pH 6.0 ≈59% of maximum; activity at pH 8.0 ≈69% of maximum).

Specific Activity

Approximately 550 U/mg at +25°C (1,100 U/mg at +37°C) with pyruvate as the substrate.

Specificity

Specificity

L(+)-Lactate dehydrogenase (LDH) is specific for L(+)-lactate (relative rate = 100). It does not react with D(-)-lactate.

- LDH will also slowly oxidize glyoxylate (oxoacetate), glycerate, and 3-halogen derivatives of L-lactate (rate with each, ≤1); 3-phosphoglycerate does not react.
- LDH will reduce pyruvate (2-oxopropanate; relative rate = 100) and other 2-oxoacids, such as 2-oxobutyrate, with rates decreasing rapidly with increasing chain length of the acid.
- Heart LDH reacts with 2-oxobutyrate at a greater rate than does skeletal muscle LDH.
- LDH will also oxidize 2,4-diketoacids (relative rate = 10).
- The enzyme is relatively specific for NAD(H) (relative rate = 100); NADP(H) is utilized much less efficiently (relative rate <1).

Representative K_ms for (hog) skeletal muscle LDH (pH 7.5)

- Lactate, 8.3 mM
- Pyruvate, 0.12 mM
- NAD, 0.10 mM
- NADH, 0.012 mM

Representative K_ms for (pig) heart LDH (pH 7.5)

- Lactate, 3.3 mM
- Pyruvate, 0.15 mM
- NAD, 0.07 mM
- NADH, 0.011 mM

In general, the concentration of pyruvate (reduction reaction) or of L-lactate (oxidation reaction) necessary to obtain a maximal rate with LDH is lower for preparations from heart tissue than for the preparations from skeletal muscle.

Unit Conversion

Reduction reaction, pyruvate as substrate

- 1 U (+25°C) ≈ 1.5 U (+37°C), hog muscle LDH in glycerol.
- 1 U (+25°C) ≈ 1.8 U (+37°C), hog muscle LDH in ammonium sulfate.
- 1 U (+25°C) ≈ 1.1 U (+30°C) ≈ 2.0 U (+37°C), rabbit muscle LDH.
- 1 U (+25°C) ≈ 1.4 U (+30°C) ≈ 2.5 U (+37°C), pig heart LDH.
- 1 U (+25°C) ≈ 2.5 U (+37°C), beef heart LDH.

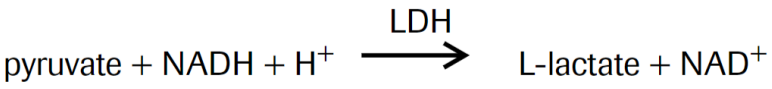
Unit Definition

One unit Lactate Dehydrogenase will reduce 1 µmol of pyruvate to L-lactate in 1 minute at +25°C and pH 7.0.
For preparations H and I, the activity is defined at +30°C, pH 7.8.

3. Additional Information on this Product

3.1. Test Principle

Control assay



The control assay consumes 1 µmol of NADH per µmol of pyruvate reduced.

Equilibrium

The formation of L-lactate is greatly favored.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Trademarks

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4.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

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