

SPECIFICATION SHEET

Product Name: D-Lactate Dehydrogenase
Catalog # LD01D-E311H

Product Description: Recombinant research grade D-lactate dehydrogenase from a microorganism. This enzyme is sold as a lyophilized white powder.

EC 1.1.1.28

Storage and Stability: Product can be stored at 2~8°C for transportation process up to ten days but long-term storage should be at -20°C.

Alternative Names	: D-LDH, D-specific 2-hydroxyacid dehydrogenase, (R)-Lactate: NAD ⁺ oxidoreductase
Unit Definition	: One unit causes the oxidation of one micromole of NADH per minute at 37°C pH 7.5
Appearance	: White amorphous powder, lyophilized
Molecular Weight	: 39.1 kDa
Activity	: ≥500U / ≥2500U
Pack Size	: 500U / 2500U
Scientific Background	: D-Lactate dehydrogenase catalyzes the conversion of pyruvate into D-lactate, via the oxidation of NADH to NAD ⁺ . It can also catalyze the reverse reaction, conversion of D-lactate into pyruvate through the reduction of NAD ⁺ to NADH ¹ . This reaction is a principal step in the production of d-lactate in lactic acid bacteria ² . Because D-LDH is elevated in a variety of disorders, analysis of total enzyme activity and predominantly its isoenzyme pattern can significantly contribute to the diagnosis of diseases which are linked to tissue damage ³ . Applications of this enzyme include enzymatic determination of metabolites such as ATP, ADP, glucose, creatinine, pyruvate, lactate and glycerol, and of enzyme activities, e.g., GPT, PK, and CPK.

Reference	1. Farhana A, et al. Biochemistry, Lactate Dehydrogenase. [Updated 2022 May 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557536/ 2. Baolei Jia, et al. Catalytic, Computational, and Evolutionary Analysis of the d-Lactate Dehydrogenases Responsible for d-Lactic Acid Production in Lactic Acid Bacteria. <i>Journal of Agricultural and Food Chemistry</i> 2018 66(31), 8371-8381 DOI: 10.1021/acs.jafc.8b02454 3. Klein R, et al. Clinical and Diagnostic Significance of Lactate Dehydrogenase and Its Isoenzymes in Animals. <i>Vet Med Int.</i> 2020 Jun 15;2020:5346483. PMID: 32607139
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D-Lactate Dehydrogenase Activity Assay Protocol

Reaction Components

- 25 mM Pyruvate Solution, Sigma P2256 (prepared fresh)
- 0.1 M K-phosphate buffer, pH 7.5
- 2 mM NADH solution, Carbosynth NN12622 (prepared fresh)
- 50 mM Enzyme Dilution Buffer (50 mM K-phosphate buffer, pH 7.5 containing 0.1% BSA)

Step 1	Reconstitute lyophilized enzyme powder with water to desired concentration.
Step 2	Dilute the reconstituted enzyme preparation with ice-cold Enzyme Dilution Buffer to 0.2-1 U/mL immediately before the assay.
Step 3	Prepare reaction mixture by mixing 4 mL pyruvate solution (A), 5 mL K-phosphate buffer (B), and 1 mL NADH solution (C) in an opaque (light proof) bottle.

- Step 4** Pipette 3 mL of the reaction mixture into a test tube and equilibrate at 37°C for about 5 minutes. Keep reaction protected from light.
- Step 5** Add 0.05 mL enzyme solution to the reaction mixture and mix gently.
- Step 6** Record the decrease of optical density at 340 nm for 5 minutes in a spectrophotometer. Keep the reaction protected from light and at a temperature of 37°C.
- Note: Blank the spectrophotometer with water
- Step 7** Calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test).
- Step 8** Measure the blank rate (ΔOD blank) by the same method as the test except that the enzyme diluent (D) is added instead of the enzyme solution.

Unit Definition: One unit causes the oxidation of one micromole of NADH per minute under the conditions described above.

Calculation: Activity can be calculated by using the following formula:

$$\text{Volume Activity (U/mL)} = \frac{\Delta OD/\text{min} (\Delta OD \text{ test} - \Delta OD \text{ blank}) \times V_t \times df}{(6.22 \times 1.0 \times V_s)} = \Delta OD/\text{min} \times 9.81 \times df$$

$$\text{Weight Activity (U/mg)} = (\text{U/mL}) \times 1/C$$

V_t : Total volume (3.05 mL)

V_s : Sample volume (0.05 mL)

6.22 : Millimolar extinction coefficient of NADH ($\text{cm}^2/\text{micromole}$)

1.0: Light path length (cm)

df: Dilution factor

C: Enzyme concentration (mg/mL)

Version: 1.0