

LACTATE DEHYDROGENASE (LDH)

IFCC Method

Diagnostic reagent for the determination of LDH concentration.

Liquid. Dual reagent. Store at +2/+8°C. For In Vitro Diagnostic use. **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
N2260	220 mL	MD260	250 mL	D2260	660 mL	HT2260	675 mL
N2261	110 mL	M4260	500 mL	D2261	350 mL	HT2261	450 mL
T2260	660 mL	M4261	250 mL	A2260N	500 mL	L2260	675 mL
T2261	350 mL	M2260	500 mL	A2261N	250 mL	L2261	300 mL
HN260	300 mL	M2261	250 mL	A2262N	125 mL	L2262	250 mL
HN261	225 mL	S2260	125 mL	8A2260	675 mL	LB260	250 mL
K2260	600 mL	S2261	288 mL	8A2261	450 mL	PL2260	150 mL
K2261	300 mL	TB2260	250 mL	BY2260	675 mL	RD2260	300 mL
M3260	250 mL	TB2261	150 mL	BY2261	450 mL	RD2261	150 mL
M3261	200 mL	BB125	200 mL	LM188	250 mL	S2262	125 mL
M3262	75 mL	DM2260	277.5 mL	BZ2135	375 mL	MS-105	150 mL

Changes made in the instructions for use are marked as gray.

INTENDED USE

The test is applied for the quantitative determination of LDH in serum and plasma.

GENERAL INFORMATION

Lactate dehydrogenase (EC 1.1.1.27; L-lactate: NAD⁺ oxido-reductase; LDH) is a hydrogen transfer enzyme that catalyzes the oxidation of L-lactate to pyruvate via NAD⁺ as a hydrogen acceptor. The reaction is reversible and the reaction equilibrium strongly favors the reduction of pyruvate to lactate (P→L) (reverse reaction). The optimum pH for the reaction of pyruvate formation from lactate substrate (L→P) is pH: 8.8-9.8 (Figure 1).¹

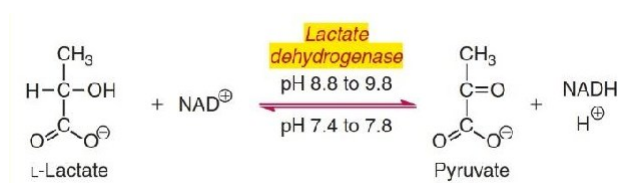


Figure 1. Reversible oxidation-reduction reaction catalyzed by the enzyme lactate dehydrogenase ¹

The enzyme has a molecular weight of 134,000 D and consists of four peptide chains of two types, M (or A) and H (or B), each under separate genetic control. As a result, there are 5 isoenzymes: H4 (named LDH1 because of its electrophoretic mobility towards the anode), H3M1 (LDH2), H2M2 (LDH3), H1M3 (LDH4) and M4 (LDH5). A distinct 6th LDH isoenzyme, LD-X (also called LDc), composed of four X (or C) subunits, is present in human testes after puberty. A seventh LDH, called LDH6, has been detected in the serum of severely ill patients. The most common method used to separate LDH isoenzymes is electrophoresis.²

LDH activity is present in all cells of the body and is always found only in the cytoplasm of the cell. Concentrations of the enzyme in various tissues are approximately 1500 to 5000 times higher than those physiologically present in serum.

Therefore, leakage of the enzyme from even a small mass of damaged tissue significantly increases the observed serum activity of LDH. Different tissues show different isoenzyme composition. LDH1 and LDH2 predominate in the heart, kidneys (cortex) and erythrocytes, whereas LDH4 and LDH5 isoenzymes predominate in liver and skeletal muscle.³

Because of its wide spread in all tissues, increases in serum LDH activity occur in a variety of clinical conditions, including myocardial infarction, hepatitis, hemolysis, and renal, pulmonary and muscular disorders. A systematic review of the literature shows that the serum LDH analyte is only relevant in hematology and oncology.⁴

Hemolytic anemias significantly increase LDH concentrations in serum. Significant increases in LDH activity up to 50 times the upper reference limit (URL) have been observed in megaloblastic anemias. These anemias cause the breakdown of erythrocyte precursor cells in the bone marrow (ineffective erythropoiesis), resulting in the release of large amounts of LDH1 and LDH2 isoenzymes. These elevations rapidly return to normal after appropriate treatment. For monitoring purposes, LDH levels are relevant for estimating the survival rate (probability of survival) and duration of Hodgkin's disease and non-Hodgkin's lymphoma.²

Patients with malignant disease often show increased LDH activity in serum. Up to 70% of patients with liver metastases and 20% to 60% of patients with other non-hepatic metastases have increased total LDH activity. Significantly increased LDH is observed in germ cell tumors (60% of cases) such as teratoma, testicular

seminoma and ovarian dysgerminoma.⁵ LDH appears to be a useful predictor of outcome in patients with testicular germ cell tumors, melanoma and small cell lung cancer.² Increases in LDH activity (predominant LDH-4 and LDH-5 isoenzymes) are observed in liver disease, but their clinical utility in liver profiling appears to be limited and they do not appear to contribute significantly to the investigation of aminotransferase activity.¹

LDH measured in pleural fluid (in combination with serum LDH) helps differentiate between exudative effusions and transudative effusions.⁶

Macro-LDH, which results from the formation of an autoantibody-enzyme complex leading to a persistent increase in the amount of circulating enzyme up to eight times the URL, is present in less than 1/10,000 individuals. Documentation of a macro-LDH in suspected individuals (e.g. the presence of an abnormally displaced band on electrophoresis) should be provided to avoid additional follow-up, investigations or unnecessary treatment.¹

TEST PRINCIPLE

IFCC method

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme that catalyzes the conversion of L-lactate to pyruvate. During this reaction, NAD⁺, which acts as a hydrogen acceptor, is reduced to NADH and H⁺. The amount of NADH produced is determined by monitoring the increase in absorbance at a wavelength of 340 nm. The rate of change in absorbance is directly proportional to the LDH enzyme activity in the sample.

Annotation:

- An optimized L→P reference method for LDH1 has been developed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).⁷ This method has recently formed the basis for the development of an IFCC primary reference procedure for LDH at 37°C.⁸ Electrophoretic separation using agarose gel or cellulose acetate membranes is the most widely used procedure to demonstrate LDH isoenzymes.⁹

REAGENT COMPONENTS

Phosphate buffer	: ≤60 mmol/L
Sodium pyruvate	: ≤70 mmol/L
NADH	: ≤20 mmol/L

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related

to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.

SAMPLE REQUIREMENTS

Serum or li-heparin plasma collected by standard procedure must be used. Repeated freeze-thaw cycles of samples should be avoided.

LDH activity stability in serum and plasma^{24,25,27}:

7 days at +20/+25°C
4 days at +2/+8°C
6 weeks at -20°C

Annotation:

- Serum is the preferred sample for measuring LDH activity. Plasma samples may be contaminated with platelets containing high concentrations of LDH.¹¹
- Different isoenzymes differ in their sensitivity to cold, in particular LDH4 and LDH5 are characterized as labile in cold. The activity of LDH4 and LDH5 is lost if samples are stored at -20°C. Therefore, when necessary, serum samples can be stored at room temperature where no loss of activity is likely to occur for at least up to 3 days.^{1,2}
- It is possible that EDTA inhibits the enzyme by binding Zn⁺², which has been suggested to be an activator of LDH.¹
- After serum or plasma samples are collected, they must be separated from clots and cellular components without delay.
- Samples in which a precipitate is observed prior to analysis should be centrifuged.

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a Arcal Auto Calibrator.

Arcal Auto Calibrator

Ref.No: A39052

Ref.No: A39054

Ref.No: Ref.No: A39055 (For Olympus AU series.)

Calibration stability is 30 days. Changes in the reagent lot number require recalibration. Calibration is necessary if quality control results are not within acceptable limits.

Control: Commercially available control materials with values assigned by this method may be used. We recommend:

Arcon N Level 1 Control- Lyophilized

Ref.No: A3910

Ref.No: Ref.No: A3912 (For Olympus AU series.)

Ref.No: A3913 (For BS series.)

Ref.No: A3914 (For Erba.)

Arcon P Level 2 Control- Lyophilized

Ref.No: A3920

Ref.No: A3922 (For Olympus AU series.)

Ref.No: A3923 (For BS series.)

Ref.No: A3924 (For Erba.)

At least two levels of control should be run every 24 hours. Each laboratory should establish its own quality control procedures. If more stringent control monitoring is required, refer to the quality control procedures established for your laboratory.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Adults²⁸: 230 - 460 U/L

Values of LDH activity in serum vary considerably depending on the direction of the enzyme reaction and the method used.²

The reference interval in white adult subjects determined by a traceable assay according to the IFCC reference procedure at 37°C has been found to be 125 to 220 U/L.¹² LDH reference limits are higher in children (180 to 360 U/L).¹³

Each laboratory should investigate the transferability of expected values for its own patient population and, if necessary, establish its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.¹⁴

Unit Conversion:

U/L × 0,0167 = µkat/L

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).¹⁵

The determined analytic measuring interval for LDH is 10 – 1500 U/L.

Detection Capability

Limit of Detection (LoD): 7,2 U/L

Limit of Quantitation (LoQ): 10,0 U/L

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.¹⁶

Linearity

This method shows measurement linearity in the activities up to 1500 U/L. For samples with results above this value, the automatic dilution procedure should be applied. For additional information, please refer to the instrument's user manual.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity studies data have been verified by using CLSI EP06-A:2003 protocol.¹⁷

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.¹⁸

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of LDH have been given in the table 1 and 2 respectively.

Table 1. LDH Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
132,8 U/L	1.29	0.97	80
402,6 U/L	2.56	0.63	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.¹⁹

Table 2. LDH Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
132,8 U/L	2.25	1.69	80
402,6 U/L	5.88	1.46	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.¹⁹

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablok equation:²⁰

$y = 6,28 + 0,95 \times x$

$r = 0,993$

Interference

Endogenous interferant and analyte concentrations that have been used in the LDH scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.^{21,22}

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from LDH interference scanning test is appropriate, is determined as $\pm 10\%$.²³

In LDH test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	LDH Target (U/L)	N*	Observed Recovery %
Triglyceride 2978 mg/dL	171.5	3	102
Bilirubin Total 60 mg/dL	228.4	3	102
Hemoglobin 1350 mg/dL	169.8	3	109

**In calculating how many times the control and test samples (containing interferant), prepared as a serum pool, would be run repetitively, the within-run SD values previously determined for the relevant method and the total allowable error rate defined as the interference limit were used. In the calculations, a type 1 error (α error) rate of 5% and a type 2 error (β error) rate of 10% (90% power) were accepted.²²*

Annotation:

- The P→L reaction is more dependent on NAD⁺ and lactate concentrations than the L→P reaction, and there may be more contamination of NAD⁺ with inhibitory products.^{1,7,8}
- Since the level of the analyte in red blood cells is higher than in normal serum, contamination with red blood cells will elevate the results. The level of interaction may vary depending on the analyte content in the lysed red blood cells.

It should be noted that, in addition to endogenous interferents, various drugs and metabolites; anticoagulants (e.g., heparin, EDTA, citrate, oxalate) and preservatives (e.g., sodium fluoride, iodoacetate, hydrochloric acid); substances that may come into contact with samples during collection and handling (such as serum separation devices, sample collection containers and their contents, catheters, catheter flushing solutions, skin disinfectants, hand sanitizers and lotions, glassware detergents, powdered gloves, etc.); dietary substances known to affect certain tests (e.g., caffeine, beta-carotene, poppy seeds); or certain substances present in a sample that may cause interference due to foreign proteins (e.g., heterophilic antibodies), autoimmune responses (e.g., autoantibodies), or malignancy (e.g., paraproteins causing interference in phosphate tests and indirect ion-selective electrode methods) may lead to various interferences and erroneous evaluations for many different reasons.²²

These performance characteristics were obtained using an autoanalyzer. Results may vary when different equipment or manual procedures are used.

WARNINGS AND PRECAUTIONS

IVD: For In Vitro Diagnostic use.

Do not use expired reagents.

Reagents with two different lot numbers should not be interchanged.

For professional use.

For professional use.

Follow Good Laboratory Practice (GLP) guidelines.

Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032 : Releases a very toxic gas if contacts with acid.

H317 : May cause allergic skin reaction.

Precaution

P280 : Use protective gloves / clothes / glasses / mask.

P264 : Wash your hands properly after using.

P272 : Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 : Wash with plenty of water and soap if it contacts with skin.

P333+P313 : Seek medical help if it irritates your skin or develops rash.

P362+P364 : Remove contaminated clothes and wash properly before using.

Disposal

P501 : Dispose the vials and contents according to the local regulations.

REFERENCES

- Rifai, N., Chiu, R. W., & Young, I., et al., (2023) Tietz Textbook of Laboratory Medicine (7th ed.), Chapter 47: Serum Enzymes, p.350-350.e36, Elsevier, St. Louis, Missouri 63043
- Pesce, A. J., & Kaplan, L. D. (2009). Methods in Clinical Chemistry: Kaplan and Pesce's: Clinical Chemistry: Theory, Analysis, Correlation: Vol. I (5th ed.), Chapter: Lactate Dehydrogenase and Lactate Dehydrogenase Isoenzymes, p.793-96. Elseviers.
- Panteghini M, Bais R, van Solinge WW. Enzymes. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. Philadelphia, PA: Elsevier Saunders, 2006:597-643.

4. Huijgen HJ, Sanders GTB, Koster RW, Vreeken J, Bossuyt PMM. The clinical value of lactate dehydrogenase in serum: a quantitative review. *Eur J Clin Chem Clin Biochem* 1997; 35: 569-575.
5. Von Eyben FE. A systematic review of lactate dehydrogenase isoenzyme 1 and germ cell tumors. *Clin Biochem* 2001; 34: 441-54.
6. Wilcox ME, Chong CA, Stanbrook MB, Tricco AC, Wong C, Straus SE. Does this patient have an exudative pleural effusion? The Rational Clinical Examination systematic review. *JAMA* 2014;311:2422-31.
7. Bais R, Philcox M. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 8. IFCC method for lactate dehydrogenase (LLactate: NAD⁺ oxidoreductase, EC 1.1.1.27). International Federation of Clinical Chemistry (IFCC). *Eur J Clin Chem Clin Biochem* 1994; 32: 639-655.
8. Schumann G, Bonora R, Ceriotti F, Clerc-Renaud P, Ferrero CA, Férard G, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 3. Reference procedure for the measurement of catalytic concentration of lactate dehydrogenase. *Clin Chem Lab Med* 2002;40:643-648.
9. Moses GC, Ross ML, Henderson AR. Ten electrophoretic methods compared with a selected method for quantifying lactate dehydrogenase isoenzymes in serum. *Clin Chem* 1988; 34: 1885-1890.
10. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009.
11. Bais R, Edwards JB. Plasma lactate dehydrogenase activity will be increased if detergent and platelets are present. *Clin Chem* 1977; 23:1056-1058.
12. Pagani F, Bonora R, Panteghini M. Reference interval for lactate dehydrogenase catalytic activity in serum measured according to the new IFCC recommendation. *Clin Chem Lab Med* 2003; 41: 970-971.
13. Freer DE, Statland BE, Johnson M, Felton H. Reference values for selected enzyme activities and protein concentrations in serum and plasma derived from cord-blood specimens. *Clin Chem* 1979; 25: 565-569.
14. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI Document EP28-A3c. Wayne, PA: CLSI; 2010.
15. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – 1st Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018.
16. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
17. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach - 1st Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
18. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
19. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
20. Bablok W et al. A General Regression Procedure for Method Transformation. *J Clin Chem Clin Biochem* 1988;26:783-790.
21. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry - First Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.
22. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry - Third Edition. CLSI Document EP07. Wayne, PA: CLSI; 2018.
23. CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register July 11, 2022;87(131:41194-242.
24. Cuhadar S, Atay A, Koseoglu M, et al. Stability studies of common biochemical analytes in serum separator tubes with or without gel barrier subjected to various storage conditions. *Biochem Med* 2012;22(2):202-214.
25. Shimizu Y, Ichihara K. Elucidation of stability profiles of common chemistry analytes in serum stored at six graded temperatures. *Clin Chem Lab Med* 2019;57(9):1388-1396
26. Pesce A. Lactate dehydrogenase. Kaplan A et al. *Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton* 1984; 1124-117, 438.
27. Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB/99.1 Rev. 2: Jan 2002.
28. Rifai N, Horvath AR, Wittwer CT, editors. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. 6th ed. St. Louis, MO: Elsevier; 2018.



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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests