LDHI2

cobas®

Lactate Dehydrogenase acc. to IFCC ver.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057958190	08057958500	Lactate Dehydrogenase acc. to IFCC ver.2 (850 tests)	System-ID 2081 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 × 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

LDHI2: ACN 20810

LDHI2P: ACN 20811 (with automatic sample pre-dilution)

Intended use

In vitro test for the quantitative determination of lactate dehydrogenase in human serum and plasma on **cobas c** systems.

Summary

Lactate dehydrogenase (LDH) measurements, performed with this assay in human serum and plasma are used as an aid for diagnosis and monitoring of various clinical conditions associated with tissue damage (e.g. myocardial infarction, liver disorders such as severe toxic liver injury, malignant tumors such as leukemias), and for the prognosis of certain solid tumors.

LDH is a nicotinamide dinucleotide (NAD^+)-dependent oxidoreductase and catalyzes the reversible transformation of lactate to pyruvate under anaerobic conditions, coupled with the oxidation of NADH to NAD^+.[1,2] LDH is widely distributed in tissue, particularly in the heart, liver, muscles and kidneys. Upon cell injury and/or necrosis, LDH is released into the circulation. LDH in serum can be separated into five different isoenzymes based on their electrophoretic mobility. Each isoenzyme is a tetramer composed of two different subunits. These two subunits have been designated heart and muscle, based on their polypeptide chains. There are two homotetramers, LDH-1 (heart) and LDH-5 (muscle), and three hybrid isoenzymes.[1,2] In disease conditions, the LDH activity measured in serum is dependent on the isoenzymes entering the plasma from the tissues, the elimination rate of the isoenzymes and their subunits.[1,2]

Elevated serum levels of LDH have been observed in a variety of disease states.[1] The highest levels are seen in patients with megaloblastic anemia (up to 50 times the upper reference limit), disseminated carcinoma, sepsis and other causes of shock (because of damage to multiple organs).[1,2] Moderate increases occur in muscular disorders, nephrotic syndrome and cirrhosis. Mild increases in LDH activity have been reported in cases of myocardial or pulmonary infarction, leukemia, hemolytic anemia and non-viral hepatitis. Because of its wide tissue distribution and its lack of tissue specificity for diagnostic use, serum LDH measurement is relevant in broad indications like hematology and oncology.[1,3] LDH is routinely used as a marker of hemolysis in sickle cell disease, along with the elevated reticulocyte count, elevated levels of unconjugated bilirubin concentration and aspartate aminotransferase, and decreased level of serum haptoglobin. While none of those parameters are specific markers of hemolysis, LDH has however been considered the most relevant biomarker of hemolysis and has been proposed as a diagnostic and prognostic marker of acute and chronic complications of sickle cell disease.[4] LDH has demonstrated to have prognostic significance in several tumor types, including pancreatic cancer, lung cancer, advanced thymic carcinoma, osteosarcoma, renal cell carcinoma, colorectal cancer, melanoma, prostate cancer, bladder cancer, and urologic cancer.[1,5,6,7,8,9] As a biochemical marker of tumor burden, LDH has been incorporated into several prognostic scores and staging for several types of cancer (e.g. renal cell carcinoma, melanoma and colorectal cancer).[5,6]

The method described here is derived from the formulation recommended by the IFCC[10,11]and was optimized for performance and stability.

Test principle

UV assay

Lactate dehydrogenase catalyzes the conversion of L-lactate to pyruvate; NAD is reduced to NADH in the process.

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

The initial rate of the NADH formation is directly proportional to the catalytic LDH activity. It is determined by photometrically measuring the increase in absorbance.

Reagents - working solutions

R1 N-methylglucamine: 400 mmol/L, pH 9.4 (37 °C); lithium lactate: 62 mmol/L; stabilizers

R3 NAD: 62 mmol/L; stabilizers; preservatives

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

LDHI2

Lactate Dehydrogenase acc. to IFCC ver.2

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	26 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin plasma. Plasma must be free from cells.

Caution: Plasma from primary tubes handled according to the manufacturer's instructions can still contain cells, leading to implausibly high results. One option for these cases is an application with automatic sample pre-dilution (ACN 20811). Alternatively it is recommended to transfer the plasma from the primary tube to a secondary sample tube.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Separate the serum or plasma from the clot or cells promptly.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Stability:[12]	7 days at 15-25 °C The sample may be stored for 4 days at 2-8 °C or 6 weeks at -20 °C (±5 °C). In connection with certain diseases (e.g. hepatopathy, diseases of skeletal muscle, malignant tumors), the LDH-4 and LDH-5 isoenzyme portions are increased and unstable in cooled and frozen samples; this may lead to an incorrect LDH value in samples collected from patients suffering from such diseases.

Freeze only once.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Test definition

Reporting time	10 min	
Wavelength (sub/main)	700/340 nm	
Reagent pipetting		Diluent (H_2O)
R1	79 µL	–
R3	16 µL	–

Sample volumes LDHI2	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.2 µL	–	–
Decreased	2.8 µL	25.0 µL	56 µL
Increased	2.2 µL	–	–

Sample volumes LDHI2P	*Sample*	*Sample dilution*	
		Sample	Diluent (NaCl)
Normal	11.0 µL	16.0 µL	64 µL
Decreased	4.4 µL	16.0 µL	64 µL
Increased	11.0 µL	16.0 µL	64 µL

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change Full calibration - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the original IFCC[11] formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

Quality control

For quality control, use control materials as listed in the “Order information” section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory’s individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

cobas c systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 20 U/L of initial values of samples ≤ 200 U/L and within ± 10 % for samples > 200 U/L

Icterus:[13] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[13] No significant interference up to an H index of 15 (approximate hemoglobin concentration: 9.6 µmol/L or 15 mg/dL).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[13] No significant interference up to an L index of 900. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

10-1000 U/L (0.17-16.7 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:2.5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 10 U/L (0.17 µkat/L)
Limit of Detection	= 10 U/L (0.17 µkat/L)
Limit of Quantitation	= 10 U/L (0.17 µkat/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity lactate dehydrogenase samples.

Expected values

U/L

Acc. to IFCC measured at 37 °C:[17]

Females	135-214 U/L
Males	135-225 U/L
Children (2-15 y)	120-300 U/L
Newborns (4-20 d)	225-600 U/L

Consensus values:[18]

Males & Females	up to 250 U/L

µkat/L

Acc. to IFCC measured at 37 °C:[17]

Females	2.25-3.55 µkat/L
Males	2.25-3.75 µkat/L
Children (2-15 y)	2.00-5.00 µkat/L
Newborns (4-20 d)	3.75-10.0 µkat/L

Consensus values:[18]

Males & Females	up to 4.2 µkat/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

LDHI2

Repeatability	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[a)]	172	1.06	0.6
PCCC2[b)]	294	1.35	0.5
Human serum 1	22.4	0.646	2.9
Human serum 2	164	1.29	0.8
Human serum 3	265	1.56	0.6
Human serum 4	520	2.09	0.4
Human serum 5	943	3.31	0.4

Intermediate precision	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[a)]	166	1.43	0.9
PCCC2[b)]	287	2.20	0.8
Human serum 1	22.4	0.779	3.5
Human serum 2	164	2.38	1.4
Human serum 3	265	2.32	0.9
Human serum 4	520	4.30	0.8
Human serum 5	943	5.65	0.6

LDHI2P

Repeatability	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[a)]	165	1.01	0.6
PCCC2[b)]	292	1.26	0.4
Human serum 1	21.5	0.555	2.6
Human serum 2	164	1.47	0.9
Human serum 3	262	1.78	0.7
Human serum 4	519	1.80	0.3
Human serum 5	941	2.92	0.3

Intermediate precision	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[a)]	168	1.25	0.7
PCCC2[b)]	292	2.08	0.7
Human serum 1	21.5	0.783	3.6
Human serum 2	164	2.48	1.5
Human serum 3	262	2.39	0.9
Human serum 4	520	4.43	0.9

Human serum 5	940	5.58	0.6

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Lactate dehydrogenase values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

LDHI2

Sample size (n) = 66

Passing/Bablok[19]	Linear regression
y = 0.999x - 2.72 U/L	y = 1.001x - 3.32 U/L
τ = 0.992	r = 1.000

The sample activities were between 19.8 and 973 U/L.

LDHI2P

Sample size (n) = 66

Passing/Bablok[19]	Linear regression
y = 0.997x - 2.26 U/L	y = 1.003x - 3.70 U/L
τ = 0.982	r = 1.000

The sample activities were between 19.8 and 973 U/L.

Lactate dehydrogenase values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

LDHI2

Sample size (n) = 60

Passing/Bablok[19]	Linear regression
y = 1.007x - 0.451 U/L	y = 1.016x - 3.51 U/L
τ = 0.982	r = 1.000

The sample activities were between 60.1 and 960 U/L.

LDHI2P

Sample size (n) = 60

Passing/Bablok[19]	Linear regression
y = 0.998x - 0.521 U/L	y = 0.999x - 1.75 U/L
τ = 0.983	r = 1.000

The sample activities were between 62.1 and 973 U/L.

Lactate dehydrogenase values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

LDHI2

Sample size (n) = 65

Passing/Bablok[19]	Linear regression
y = 1.002x + 0.668 U/L	y = 1.008x - 0.274 U/L
τ = 0.963	r = 1.000

The sample concentrations were between 14.1 and 955 U/L.

LDHI2P

Sample size (n) = 65

Passing/Bablok[19]	Linear regression
y = 1.002x + 1.55 U/L	y = 1.001x + 1.91 U/L
τ = 0.974	r = 1.000

The sample concentrations were between 13.1 and 952 U/L.

References

1 Pincus MR, Carty RP. Clinical Enzymology. In: McPherson RA, Pincus MR, editors. Henry's Clinical Diagnosis and Management by Laboratory Methods, Elsevier, 24th edition, 2022, chapter 21, p. 291-313.e3.

2 Panteghini M. Serum Enzymes. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 32, p. 350-350.e36.

3 Huijgen HJ, Sanders GT, Koster RW, et al. The clinical value of lactate dehydrogenase in serum: a quantitative review. Eur J Clin Chem Clin Biochem 1997 Aug;35(8):569-579.

4 Stankovic Stojanovic K, Lionnet F. Lactate dehydrogenase in sickle cell disease. Clin Chim Acta 2016 Jul 1;458:99-102.

5 Petrelli F, Cabiddu M, Coinu A, et al. Prognostic role of lactate dehydrogenase in solid tumors: a systematic review and meta-analysis of 76 studies. Acta Oncol 2015 Jul;54(7):961-970.

6 Zhang J, Yao YH, Li BG, et al. Prognostic value of pretreatment serum lactate dehydrogenase level in patients with solid tumors: a systematic review and meta-analysis. Sci Rep 2015 Apr 22;5:9800.

7 Gao D, Ma X. Serum lactate dehydrogenase is a predictor of poor survival in malignant melanoma. Panminerva Med 2017 Dec;59(4):332-337.

8 Zhang Y, Xu T, Wang Y, et al. Prognostic Role of Lactate Dehydrogenase Expression in Urologic Cancers: A Systematic Review and Meta-Analysis. Oncol Res Treat 2016;39(10):592-604.

9 Faloppi L, Bianconi M, Memeo R, et al. Lactate Dehydrogenase in Hepatocellular Carcinoma: Something Old, Something New. Biomed Res Int 2016;2016:7196280.

10 van der Heiden C, Bais R, Gerhardt W, et al. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 8. IFCC method for lactate dehydrogenase. Eur J Clin Chem Clin Biochem 1994;32:639-655.

11 Schumann G, Bonora R, Ceriotti F, et al. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37 °C – Part 3. Reference Procedures for the Measurement of Catalytic Concentrations of Lactate Dehydrogenase. Clin Chem Lab Med 2002;40(6):643-648.

12 Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB/99.1 Rev. 2: Jan 2002.

13 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

14 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

15 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

16 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

17 Lorentz K, Röhle G. Einführung der neuen Standardmethoden 1994 zur Bestimmung der katalytischen Enzymkonzentration bei 37 °C. Klin Chem Mitt 1995;26:290-293.

18 Thomas L, Müller M, Schumann G, et al. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005; 29(5):301-308.

19 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

LDHI2

Lactate Dehydrogenase acc. to IFCC ver.2

cobas®

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606

Roche