

**Lactate Dehydrogenase (P-L) - Primary tube****Order information**

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
20767123 322	Lactate Dehydrogenase (P-L) (300 tests)	System-ID 07 6712 3 COBAS INTEGRA 400 plus COBAS INTEGRA 800
10759350 190	Calibrator f.a.s. (12 x 3 mL)	System-ID 07 3718 6
12149435 122	Precinorm U plus (10 x 3 mL)	System-ID 07 7999 7
12149443 122	Precipath U plus (10 x 3 mL)	System-ID 07 8000 6
10171743 122	Precinorm U (20 x 5 mL)	System-ID 07 7997 0
10171735 122	Precinorm U (4 x 5 mL)	System-ID 07 7997 0
10171778 122	Precipath U (20 x 5 mL)	System-ID 07 7998 9
10171760 122	Precipath U (4 x 5 mL)	System-ID 07 7998 9
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	System-ID 07 7469 3
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	System-ID 07 7469 3
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	System-ID 07 7470 7
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	System-ID 07 7470 7

**English****System information**

Test LDHPL, test ID 0-312

The application is intended for customers facing non-valid results due to a lactate dehydrogenase gradient within plasma primary tubes.

**Intended use**

In vitro test for the quantitative determination of the catalytic activity of LDH (EC 1.1.1.27; L-lactate: NAD<sup>+</sup> oxidoreductase) in human serum and plasma on COBAS INTEGRA systems.

**Summary**<sup>1,2,3</sup>

The lactate dehydrogenase (LDH) enzyme is widely distributed in tissue, particularly heart, liver, muscle, and kidney. The 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.

Elevated serum levels of LDH have been observed in a variety of disease states. The highest levels are seen in patients with megaloblastic anemia, myocardial infarction, disseminated carcinoma, leukemia, and trauma. Mild increases in LDH activity have been reported in cases of hemolytic anemias, muscular dystrophy, pulmonary infarction, hepatitis, nephrotic syndrome, and cirrhosis.

**Test principle**

Optimized standard method according to the Deutsche Gesellschaft für Klinische Chemie (DGKC).<sup>4,5</sup>

LDH catalyzes the reaction between pyruvate and NADH to form L-lactate and NAD<sup>+</sup>.



The initial rate of the NADH oxidation is directly proportional to the catalytic LDH activity. It is determined by measuring the decrease in absorbance at 340 nm.

**Reagents - working solutions**

**R1** Phosphate buffer: 68 mmol/L, pH 7.5; pyruvate: 0.73 mmol/L; stabilizers; preservative

**SR** NADH: 1.1 mmol/L; stabilizers; preservative

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

**Precautions and warnings**

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

**Reagent handling**

Ready for use

**Storage and stability**

Shelf life at 2-8 °C

See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C

4 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C

8 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 (free from hemolysis)

Plasma (free from hemolysis): Li-heparin plasma

Do not use other anticoagulants. Plasma may be contaminated with platelets which contain high concentrations of lactate dehydrogenase and should be avoided.<sup>6,7</sup>

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.

Centrifuge samples containing precipitates before performing the assay.

Stability:<sup>8</sup>

7 days at 15-25 °C

The sample may be stored for 4 days at 2-8 °C or 6 weeks at -20 °C. In connection with certain diseases (e.g. hepatopathy, diseases of skeletal muscles, 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.

**Materials provided**

See "Reagents – working solutions" section for reagents.

**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.

**Application for serum and plasma****COBAS INTEGRA 400 plus test definition**

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Decrease
Wavelength A/B	340/659 nm
Calc. first/last	46/57
Predilution factor	10
Unit	U/L

**Pipetting parameters**

		Diluent (H <sub>2</sub> O)
R1	100 µL	
Sample	25 µL	
SR	20 µL	
Total volume	145 µL	

**COBAS INTEGRA 800 test definition**

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Decrease
Wavelength A/B	340/659 nm
Calc. first/last	65/84
Predilution factor	10
Unit	U/L

**Pipetting parameters**

		Diluent (H <sub>2</sub> O)
R1	100 µL	
Sample	25 µL	
SR	20 µL	
Total volume	145 µL	

**Calibration**

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures.

Traceability: This method has been standardized manually against Roche reagent.

**Quality control**

Reference range	Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1
Pathological range	Precipath U, Precipath U plus or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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. 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 INTEGRA analyzers automatically calculate the analyte activity of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

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

**Limitations - interference**

Criterion: Recovery within ± 10 % of initial value.

**Serum/plasma**

Icterus:<sup>9</sup> 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:<sup>9</sup> No significant interference up to an H index of 10 (approximate hemoglobin concentration: 6 µmol/L or 10 mg/dL).

Lipemia: Highly lipemic specimens may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>10,11</sup>

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.<sup>12</sup>

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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

**Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.**

**Limits and ranges****Measuring range**

40-1200 U/L (0.67-20 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

**Lower limits of measurement**

Lower detection limit of the test:

40 U/L (0.67 µkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 30).

**Expected values<sup>13</sup>**

37 °C\* Adults 240-480 U/L (4.00-8.00 µkat/L)

\*Calculation by means of a temperature conversion factor of 2.00 (25 → 37 °C).<sup>14</sup>

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 COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

**Precision**

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 2 runs per day, 6 days). The following results were obtained:

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Repeatability	Level 1	Level 2
Mean	301 U/L (5.02 µkat/L)	497 U/L (8.28 µkat/L)
CV	1.2 %	0.4 %

Intermediate precision	Level 1	Level 2
Mean	303 U/L (5.06 µkat/L)	485 U/L (8.10 µkat/L)
CV	2.0 %	1.2 %

**Method comparison**

LDH values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Lactate Dehydrogenase reagent and the LDHPL application (y) were compared with those determined using the corresponding reagent, but LDHL application on a COBAS INTEGRA 700 analyzer (x). Samples were measured in single replicate. Sample size (n) = 60

	COBAS INTEGRA 700 analyzer
Method	LDHL
Corr. coefficient	$r = 0.999$ $r_s = 0.963$
Linear regression	$y = 0.981x + 4.06 \text{ U/L}$
Passing/Bablok <sup>15</sup>	$y = 0.982x + 3.17 \text{ U/L}$

The sample activities were between 186 and 1146 U/L (3.1 and 19.1 µkat/L).

**References**

- 1 Dito WR. Lactate dehydrogenase: A brief review. In: Griffiths JC, ed. Clinical Enzymology. New York:Masson Publishing USA 1979:1-8.
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- 13 Weishaar D, Gossrau E, Faderl B. Normalbereiche von α-HBDH, LDH, AP und LAP bei Messung mit substrat-optimierten Testansätzen Med Welt 1975;26:387-390.
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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.

**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 after reconstitution or mixing

COBAS, COBAS INTEGRA, COBAS C, PRECICONTROL, PRECINORM and PRECIPATH are trademarks of Roche.

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Significant additions or changes are indicated by a change bar in the margin.

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