HBDH2

α-Hydroxybutyrate Dehydrogenase Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
11876937 216	α-HBDH optimized 6 x 100 tests	System-ID 07 6790 5	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171735 122	Precinorm U (4 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
10171760 122	Precipath U (4 x 5 mL)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
04593138 190	**cobas c** pack MULTI		
On request	Open/Close tool		

English

System information

For **cobas c** 311/501 analyzers:

HBDH2: ACN 567

For **cobas c** 502 analyzer:

HBDH2: ACN 8567

Intended use

In vitro test for the quantitative determination of α-hydroxybutyrate dehydrogenase (α-HBDH) in serum and plasma on Roche/Hitachi **cobas c** systems.

Summary

Lactate dehydrogenase in serum is composed of 5 isoenzymes. These enzymes are present in a tetrameric structure. There are two types of subunits, namely the M-subunit predominantly found in skeletal muscle, and the H-subunit, found predominantly in the myocardium. Due to their electrophoretic migration characteristics to the anode, the isoenzymes are termed LDH1, LDH2, LDH3, LDH4 and LDH5. LDH1 migrates most rapidly to the anode. The subunits are composed accordingly: H4, H3M, H2M2, HM3 and M4. By using various substrates (e.g. α-ketobutyrate is used for α-HBDH), lactate dehydrogenases from the liver and the heart can be differentiated from each other.

Each organ is associated with a characteristic enzyme pattern which can contribute to the identification of organ damage.[1] Recent studies have shown that changes in the proportion of heart-specific LDH isoenzyme activities to the total LDH activity yield a reliable indication of the severity and progress of a recent myocardial infarction.[2,3] Rudolph et al.[4] report that the combination of CK-MB- and heart-specific LDH isoenzyme determinations can predict with 99 % certainty the classification of a myocardial infarction as being acute or non-acute. Rotenberg et al.[5,6] report also that the measurement of heart-specific LDH isoenzymes 24 to 48 hours after heart surgery is a meaningful test for the diagnosis of perioperative myocardial infarction.

This method is in accordance with the optimized standard method as recommended by the German Society for Clinical Chemistry in 1972.[7]

Test principle

UV test according to a standardized method.

α-hydroxybutyrate dehydrogenase catalyzes the conversion of α-ketobutyrate to α-hydroxybutyrate in a reaction where NADH is oxidized to NAD.

$$\alpha\text{-ketobutyrate} + NADH + H^+ \xrightarrow{\alpha\text{-HBDH}} \alpha\text{-hydroxybutyrate} + NAD^+$$

The rate of the NADH decrease is directly proportional to the α-HBDH activity and is measured photometrically.

Reagents - working solutions

R1 Phosphate buffer: 68 mmol/L, pH 7.5 (25 °C); α-ketobutyrate: 3.7 mmol/L; preservative

R2 NADH: ≥ 1.1 mmol/L; preservative

Reagent preparation and cobas c pack assembly

Reagent handling

Ready for use

Labeling the **cobas c** *pack MULTI*

Turn the barcode labeled side of a new **cobas c** pack MULTI toward you. Affix the supplied HBDH2 barcode label directly over the existing barcode label.

Filling the **cobas c** *pack MULTI*

1. Turn the **cobas c** pack MULTI toward you as shown above.
2. Position A of the **cobas c** pack is now in the center, position B on the left side, position C on the right side of the **cobas c** pack.
3. Unscrew the screw cap of the bottle in position A in the center of the **cobas c** pack MULTI using the open/close tool.
4. Pour the content of bottle 1 (19 mL) into the opened bottle of the **cobas c** pack (position A).
5. Close the bottle tightly using the open/close tool.
6. Unscrew the screw cap of the bottle in position C on the right side of the **cobas c** pack MULTI using the open/close tool.
7. Pour the content of bottle 2 (5 mL) into the opened bottle of the **cobas c** pack (position C).
8. Close the bottle tightly using the open/close tool.
9. Leave position B empty.

The HBDH2 **cobas c** pack is now ready for use.

HBDH2

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Note

Use only the **cobas c** *pack MULTI. Always use a new* **cobas c** *pack MULTI when preparing fresh reagent. Never reuse accessories designed for single use, as this may result in reagent contamination and could affect test results. If the* **cobas c** *pack MULTI bottles are not filled correctly, this may result in faulty reagent pipetting and could cause erroneous results.*

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Storage and stability

HBDH2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 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, K_2-EDTA plasma.

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:[8]	3 days at 15-25 °C
	7 days at 2-8 °C (activity decrease 5 %)

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

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 17-24		
Wavelength (sub/main)	546/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.8 µL	–	–
Decreased	1.1 µL	–	–
Increased	2.8 µL	–	–

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 24-34		
Wavelength (sub/main)	546/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.8 µL	–	–
Decreased	1.1 µL	–	–
Increased	2.8 µL	–	–

cobas c 502 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 24-34		
Wavelength (sub/main)	546/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.8 µL	–	–
Decreased	1.1 µL	–	–
Increased	5.6 µL	–	–

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • as required following quality control procedures

Traceability: This method has been standardized against the Roche system reagent 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. 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.

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cobas®

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte activity of each sample.

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

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at an α-hydroxybutyrate dehydrogenase activity of 180 U/L (3.00 µkat/L).

Icterus:[9] 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:[9] No significant interference up to an H index of 10 (approximate hemoglobin concentration: 6.2 µmol/L or 10 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):[9] No significant interference up to an L index of 600. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[12]

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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

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

Limits and ranges

Measuring range

6-700 U/L (0.1-11.7 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

Lower detection limit of the test

6 U/L (0.1 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 the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values[13]

72-182 U/L[a)] (1.20-3.03 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.

a) Calculated with a temperature conversion factor of 1.30 (25 →37 °C).14

Specific performance data

Representative performance data on the 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, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	148 (2.47)	2 (0.03)	1.0
Precipath U	260 (4.35)	2 (0.03)	0.8
Human serum 1	132 (2.21)	3 (0.05)	2.2
Human serum 2	323 (5.39)	4 (0.07)	1.2

Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	157 (2.62)	3 (0.05)	1.9
Precipath U	259 (4.32)	4 (0.07)	1.4
Human serum 3	109 (1.82)	4 (0.07)	3.8
Human serum 4	333 (5.56)	5 (0.08)	1.5

Method comparison

α-hydroxybutyrate dehydrogenase values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 82

Passing/Bablok[15]	Linear regression
y = 0.994x + 1.42 U/L	y = 0.995x + 2.24 U/L
τ = 0.957	r = 0.998

The sample activities were between 93.7 and 645 U/L (1.56 and 10.8 µkat/L).

References

1 Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995.

2 Wang TY, Godfrey JH, Graham LG, et al. Clinical evaluation of immunochemical assay of lactate dehydrogenase isoenzyme 1 in early detection of acute myocardial infarction. Clin Chem 1982;28(10):2152-2154.

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7 German Society for Clinical Chemistry: Recommendations of the German Society for Clinical Chemistry (DGKC). J Clin Chem Clin Biochem 1972;10:182-193.

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

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

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14 Zawta B, Klein G, Bablok W. Temperature Conversion in Clinical Enzymology? Klin Lab 1994;40:33-42.

15 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.

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
GTIN	Global Trade Item Number

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com