DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
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alpha-HBDH
opt. DGKC

Diagnostic reagent for quantitative in vitro determination of α-Hydroxybutyrate dehydrogenase (α-HBDH) in human serum or plasma on photometric systems

REF	Kit Size	Configuration
D96635	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
D00642	5 x 25 mL	4 x 25 mL R1 + 1 x 25 mL R2
D00643	5 x 10 mL	4 x 10 mL R1 + 1 x 10 mL R2
D74911	5 x 50 mL	4 x 50 mL R1 + 2 x 25 mL R2
D0429917	5 x 62.5 mL	4 x 62.5 mL R1 + 1 x 62.5 mL R2
DA0831	5 x 20 mL	4 x 20 mL R1 + 1 x 20 mL R2
DT1031	5 x 20 mL	4 x 20 mL R1 + 1 x 20 mL R2
DK0730	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
DE1831	1 x 62.5 mL	1 x 50 mL R1 + 1 x 12.5 mL R2
DB20305	4 x 62.5 mL	4 x 50 mL R1 + 4 x 12.5 mL R2

Additionally available:

D98485	5 x 3 mL	Calibrator	Diacal Auto
D98485SV	1 x 3 mL	Calibrator	Diacal Auto
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98482SV	1 x 5 mL	Control abnormal	Diacon P

For professional in vitro diagnostic use only.

GENERAL INFORMATION

Method	UV, kinetic, decreasing reaction, opt. DGKC
Shelf life	24 months from production date
Storage	2 – 8°C
Wavelength	340 nm, Hg 334 nm, Hg 365 nm
Temperature	25°C, 30 °C, 37 °C
Sample	Serum, EDTA-plasma, heparin plasma

INTENDED USE

Diagnostic reagent for quantitative in vitro determination of α-Hydroxybutyrate dehydrogenase (α-HBDH) in human serum or plasma on photometric systems.

DIAGNOSTIC SIGNIFICANCE [1, 2]

α-hydroxybutyrate dehydrogenase (α-HBDH) is an isoenzyme of lactate dehydrogenase (LDH), which uses α-hydroxybutyrate as an additional substrate. Compared to other isoenzymes of LDH it occurs in higher levels in heart muscle tissue and therefore is somewhat more sensitive and more specific in the diagnosis of myocardial infarction. For differentiation between liver and heart diseases, the HBDH/LDH ratio can be calculated. A decreased HBDH/LDH ratio indicates parenchymal liver diseases, while an increased ratio can be measured in myocardial infarction.

TEST PRINCIPLE

$$\text{2-Oxobutyrate + NADH + H}^+ \xleftrightarrow{\alpha-HBDH} \text{2-Hydroxybutyrate + NAD}^+$$

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Reagent 1:		
2-Oxobutyrate	3.8	mmol/L
Phosphate, pH 7.4	60	mmol/L
Reagent 2		
NADH	1	mmol/L

MATERIAL REQUIRED BUT NOT PROVIDED

- NaCl solution (9 g/L).
- Clinical chemistry analyser.

REAGENT PREPARATION

Substrate Start:
Reagents are ready to use.
Sample Start:
Mix 4 parts of Reagent 1 with 1 part of Reagent 2.
(= working reagent)

STORAGE AND STABILITY

Conditions: Protect from light!
Close immediately after use
Avoid contamination
Do not freeze the reagents!

Substrate Start:
Storage: at 2 – 8 °C
Stability: up to the expiration date indicated on labels
Sample Start (Working Reagent):
Stability: at 2 – 8 °C 5 days
at 15 – 25 °C 8 hours
The working reagent must be protected from light!

WARNINGS AND PRECAUTIONS

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. In very rare cases, samples of patients with gammopathy might give falsified results [6].
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
4. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

SPECIMEN COLLECTION AND STORAGE

Use serum, heparin plasma or EDTA plasma.

Stability [3]: at 15 – 25 °C 3 days
at 2 – 8 °C 20 days
Discard contaminated specimens. Do not freeze the samples!

TEST PROCEDURE

Substrate start:

Pipette into test tubes	25°C	30°C	37°C
Sample	20 µl	20 µl	10 µl
Reagent 1	1000 µl	1000 µl	1000 µl
Mix, Incubate for approx. 1 – 5 min. Then add:			
Reagent 2	250 µl	250 µl	250 µl
Mix, read absorbance against air after 1 min. and start a timer. Read absorbance exactly after 1,2, and 3 minutes. Determine the ΔA/min. during the linear part of the assay.			

Sample start:

Pipette into test tubes	25°C	30°C	37°C
Sample	20 µl	20 µl	10 µl
Working reagent	1000 µl	1000 µl	1000 µl
Mix, read absorbance against air after 1 min. and start a timer. Read absorbance again after 1,2, and 3 minutes. Determine the ΔA/min during the linear part of the assay.			

Automation

Special adaptations for automated analysers can be made on request.

INTERPRETATION OF RESULTS

Calculation

With factor (light path 1 cm)**:**
From absorbance readings calculate ΔA/min and multiply by the corresponding factor:
α-HBDH [U/L] = ΔA/min x Factor

Factors:

Substrate start	25 °C / 30 °C	37 °C
Factor at 340 nm	10080	20000
Factor at 334 nm	10275	20390
Factor at 365 nm	18675	37060

Sample start	25 °C / 30 °C	37 °C
Factor at 340 nm	8095	16030
Factor at 334 nm	8250	16345
Factor at 365 nm	15000	29705

Unit Conversion
α-HBDH [U/L] x 0.0167 = α-HBDH [µkat/L]

QUALITY CONTROL AND CALIBRATION

All control sera with alpha-HBDH values determined by this method can be used.
We recommend the Dialab serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range).
Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration
The use of an alpha-HBDH calibrator is optional.
We recommend the Dialab multi calibration serum **Diacal Auto**.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE
On automated systems, the test is suitable for the determination of α-HBDH activities up to 1200 U/L.
In case of a manual procedure, the test is suitable for α-HBDH activities which correspond to a maximal ΔA/min of 0.15 at 340 and 334 nm or of 0.07 at 365 nm.
If these values are exceeded the samples should be diluted 1 + 9 with NaCl solution (9 g/L) and results multiplied by 10.

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 3 U/L.

PRECISION (at 25°C)

Intra-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	100	2.21	2.20
Sample 2	174	2.97	1.71
Sample 3	388	4.20	1.08

Inter-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	97.8	2.20	2.25
Sample 2	177	2.01	1.14
Sample 3	386	6.96	1.80

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SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Bilirubin	40 mg/dL
Triglycerides	2000 mg/dL

Hemoglobin interferes even at minimal concentrations.
For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

A comparison between Dialab α-HBDH (y) and a commercially available test (x) using 64 samples gave following results: y = 1.00 x – 1.00 U/L; r = 0.999.

TRACEABILITY

This method is traceable to the molar extinction coefficient.

EXPECTED VALUES [4]*

	25 °C		37 °C	
	[U/L]	[µkat/L]	[U/L]	[µkat/L]
Adults	< 140	< 2.33	< 182	< 3.03

HBDH/LDH = 0.63 – 0.81

If HBDH and LDH are increased:

Myocardiac lesion:	HBDH/LDH > 0.9
Liver damage:	HBDH/LDH < 0.6

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

LIMITATIONS

NA

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 617-721.
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4. Elliot BA, Wilkinson JH. The serum α-hydroxybutyrate dehydrogenase in diseases other than myocardial infarction. Clin Sci 1963; 24:343-55.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
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