α-HBDH FS*

Diagnostic reagent for quantitative in vitro determination of α-HBDH in serum or plasma on photometric systems

Order Information

Cat. No.	Kit size					
1 3201 99 10 021	R1	5 x	20 mL	+ R2	1 x	25 mL

Summary [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.

Method

Optimized UV test according to DGKC (German Society of Clinical Chemistry)

Principle

2-Oxobutyrate + NADH + H^+ <u>< α-HBDH</u> > 2-Hydroxybutyrate + NAD^+

Reagents

Components and Concentrations

R1:	Phosphate	pH 7.4	60 mmol/L
	2-Oxobutyrate		3.8 mmol/L
R2:	NADH		1 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. The reagents contains 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. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
4. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Substrate Start

The reagents are ready to use.

Sample Start

Mix 4 parts of R1 + 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = mono-reagent

Stability:			
	5 days	at	2 – 8 °C
	8 hours	at	15 – 25 °C

The mono-reagent must be protected from light.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma
Stability in serum:

Stability [3]:			
	3 days	at	15 – 25 °C
	20 days	at	2 – 8 °C

Do not freeze the samples!

Discard contaminated specimens.

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	340 nm, Hg 365 nm, Hg 334 nm
Optical path	1 cm
Temperature	25 °C/30 °C/37 °C
Measurement	Against air

Substrate start

Temperature	**25 °C/30 °C**	**37 °C**
Sample	20 µL	10 µL
Reagent 1	1000 µL	1000 µL
Mix, incubate for approx. 1 – 5 min., Then add:		
Reagent 2	250 µL	250 µL
Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.		

Sample start

Temperature	**25 °C/30 °C**	**37 °C**
Sample	20 µL	10 µL
Mono-reagent	1000 µL	1000 µL
Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.		

Calculation

From absorbance readings calculate ΔA/min and multiply by the corresponding factor from table below:

ΔA/min x factor = α-HBDH activity [U/L]

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

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

Conversion factor

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

* fluid stable

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. This method is traceable to the Molar Extinction Coefficient. For internal quality control DiaSys TruLab N and P controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL

Performance Characteristics

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 maximum of ΔA/min of 0.15 at 340 and 334 nm or of 0.07 at 365 nm.
If these values are exceeded the sample should be diluted 1 + 9 with NaCl solution (9 g/L) and results multiplied by 10.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL and lipemia up to 2,000 mg/dL triglycerides. Hemoglobin interferes even at minimal concentrations. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 3 U/L.

Precision (at 25 °C)

Intra-assay precision 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 precision 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

Method Comparison

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

Reference Range [4]

	25 °C [U/L]	25 °C [µkat/L]	37 °C [U/L]	37 °C [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.

Literature

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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.
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Manufacturer

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