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Liquick Cor - HBDH

Kit name	**(EN) Cat. No**
Liquick Cor-HBDH mini	1-297
Liquick Cor-HBDH 30	1-241

INTENDED USE

Diagnostic kit for determination of α- hydroxybutyrate dehydrogenase activity intended to use both for manual assay (Sample Start and Reagent Start method) and in several automatic analyzers.
The reagents must be used only for *in vitro* diagnostic, by suitably qualified laboratory personnel, only for the intended purpose, under appropriate laboratory conditions.

INTRODUCTION

Lactate dehydrogenase (LDH, LD) is a tetrameric molecule containing two possible forms of subunits (H and M). The result is five isoenzymes, one of which is hydroxybutyrate dehydrogenase (HBDH, LD-1) formed by four H subunits. HBDH is present mainly in heart muscle but occur also in kidney and erythrocytes. Normal serum contains mostly LD-2 with lesser amount of LD-1. Changes in the ratio of LD-1 to LD-2 indicate myocardial infarction or hemolysis.

METHOD PRINCIPLE

Kinetic method of Deutsche Gesselschaft for Klinische Chemie (DGKC).

$$\text{2-oxybutyrate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\alpha\text{-HBDH}} \text{2-hydroxybutyrate} + \text{NAD}^+$$

The rate of absorbance changing at λ=340 nm is directly proportional to α-hydroxybutyrate dehydrogenase activity.

REAGENTS

Package

	Liquick Cor-HBDH mini	**Liquick Cor-HBDH 30**
1-HBDH	2 x 24 ml	5 x 24 ml
2-HBDH	1 x 12 ml	1 x 30 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination!

Working reagent preparation and stability

Assay can be performed with use of separate 1-HBDH and 2-HBDH reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of 1-HBDH with 1 part of 2-HBDH. Avoid foaming.
Stability of working reagent: 5 days at 2-8°C
24 hours at 15-25°C
Protect from light and avoid contamination!

Concentrations in the test

phosphate buffer (pH 7.5)	50 mmol/l
2-oxybutyrate	3 mmol/l
NADH	0.25 mmol/l

Warnings and notes

- The reagents contain < 0.1% sodium azide as a preservative. Avoid contact with skin and mucous membranes.
- The reagents are usable when absorbance of working reagent is higher than 1.000 (read against distilled water, wavelength λ=340 nm, cuvette l=1 cm, at temp. 25°C).

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 340 nm (Hg 334 nm, 365 nm);
- thermostat at 25ºC or 37ºC;
- general laboratory equipment;

SPECIMEN

Serum.
Do not use hemolyzed blood because erythrocytes contain very high HBDH activity. Do not chill or freeze samples.
HBDH activity is unstable and is rapidly lost during storage. Specimens can be stored up to 6 hours at 15-25ºC.
Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

Applications for them are available on request.

Manual procedure

wavelength	340 nm (Hg 334 nm, 365 nm)
temperature	25ºC/37°C
cuvette	1 cm

Sample Start method

Pipette into the cuvette:

working reagent	1000 µl

Bring up to the temperature of determination. Then add:

sample	20 µl (temp. 25ºC) or 10 µl (temp. 37ºC)

Mix and incubate at adequate temperature. After about 1 min. read the absorbance against air or water. Repeat the reading after exactly 1, 2 and 3 minutes. Calculate the mean absorbance change per minute (ΔA/min.).

Calculation

HBDH activity [U/l] = ΔA/min. x F
F value depends on the used wavelength:

λ	25ºC	37ºC
340 nm	8095	16030
334 nm	8250	16345
365 nm	15000	29705

Reagent Start method

The determination can be also performed with use of separate 1-HBDH and 2-HBDH reagents.

Pipette into the cuvette:

1-HBDH	1000 µl

Bring up to the temperature of determination. Then add:

sample	20 µl (temp. 25ºC) or 10 µl (temp. 37ºC)

Mix well, incubate for 1-5 min. Then add:

2-HBDH	250 µl

Mix well, perform measurement as described for Sample Start method.

Calculation

HBDH activity [U/l] = ΔA/min. x F
F value depends on the used wavelength:

λ	25ºC	37ºC
340 nm	10080	20000
334 nm	10275	20390
365 nm	18675	37060

REFERENCE VALUES [1, 7]

	25ºC	37ºC
serum		
adults	55 – 140 U/l (0.917 – 2.33 µkat/l)	< 182 U/l (< 3.04 µkat/l)

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.
For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) or LEVEL 2 (Cat. No 5-175; 5-177) is recommended.
The calibration curve should be prepared every 8 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

The following results have been obtained using automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity**

9.2 U/l (0.153 µkat/l)

- **Linearity**

up to 500 U/l (8.33 µkat/l)

If HBDH activity in tested sample exceeds 500 U/l dilute the sample 10-fold with 0.9% NaCl and repeat the assay. Multiply the result by 10.

- **Specificity / Interferences**

Haemoglobin up to 2.5 g/dl, ascorbate up to 62 mg/l, bilirubin up to 20 mg/dl and triglycerides up to 1000 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 20	Mean [U/l]	SD [U/l]	CV [%]
level 1	141.63	2.12	1.50
level 2	362.68	4.52	1.25
Reproducibility (day to day) n = 20	Mean [U/l]	SD [U/l]	CV [%]
level 1	148.94	2.03	1.37
level 2	380.33	3.28	0.86

- **Method comparison**

A comparison between HBDH values determined at Biolis 24i Premium (y) and at Prestige 24i (x) using 101 samples gave following results:
y = 1.0432 x - 7.6087 U/l;
R = 0.9945 (R – correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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