N BIO - Alpha HBDH

(DGKC Kin. method)

KIT NAME	KIT SIZE	CAT. NO
N BIO - Alpha HBDH	2 X 50 ml	DHBD02050M

GENUINE BIOSYSTEM

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 für Klinische Chemie (DGKC).

2-oxybutyrate + NADH + H+ $<\alpha\text{-HBDH}>$ 2-hydroxybutyrate + NAD+

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

REAGENTS

Reagent Name	DHBD02050M
R1 HBDH reagent	2 x 40 ml
R2 HBDH reagent	2 x 10 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package.
The reagents are stable for 4 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 R1 HBDH and R2 HBDH reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of R1 HBDH with 1 part of R2 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

1) Product for in vitro diagnostic use only.
2) The reagents contain 0.09% sodium azide as a preservative.
3) Avoid contact with skin and mucous membranes.
4) 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
- Thermostat at 25°C or 37°C
- General laboratory equipment

SPECIMEN

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

PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analyzers. Applications for them are available on request.

Wavelength	340 nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Test (T)
R1 HBDH reagent	800 µl
R2 HBDH reagent	200 µl
Bring to assay temperature, then add	
Sample	20 µl

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

α-HBDH activity [U/L] = ΔA/ min x 10200

REFERENCE VALUES

72 to 182 U/L
It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls

PERFORMANCE CHARACTERISTICS

Linearity : up to 3000 U/L. Dilute the sample approximately and re-assay if ADA activity exceeds 3000 U/L. Multiply result with dilution factor.

Precision : The CV of the test should be CV ≤ 5%

Interference : The following levels indicated found not to interfere Intralipd 3000 mg/dl, Bilirubin 50 mg/dl, VC 50 mg/dl

WASTE MANAGEMENT

Please refer to local legal requirement

LITERATURE

1. Rosalki, S.B. amd Wilikinson, J.H., Nature 188:1110(1960).
2. Z.Klin. Chem. U. Klin. Biochem. 8:658 (1970).
3. Z.Klin Chem. U. Klin. Biochem. 10:182 (1972).

SYSTEM PARAMETERS

Method	Kinetic
Wavelength	340 mm
Zero Setting	Distilled Water
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	----
Delay Time	60 secs
Read Time	180 secs
No. of Reading	3
Interval Time	60 secs
Sample Volume	0.02 ml (20 ul)
Reagent Volume	1.0 ml (1000 ul)
Standard Concentration	----
Units	U/L
Factor	10200
Reaction Slope	Increasing
Linearity	3000 U/L

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