

HBDH LYOPHILIZED

KINETIC DETERMINATION
 ACCORDING WITH DGKC RECOMMENDATION
 IN SERUM OR PLASMA
 For in vitro diagnostic use only

Kit: 10 x 20 ml
 20 x 3 ml

Cod. AH060
 Cod. AH063

SUMMARY

The Hydroxybutyrate dehydrogenase (HBDH), an enzyme present in myocardium, muscles and liver is mainly involved in the hearth metabolism. Elevated concentrations of this enzyme are associated with myocardial infarction, reaching highest values 30-72 hours after the event and tending to normalize after 12-20 days. Elevated values of enzyme are also connected with myocarditis, polymyositis, or in case of abdominal surgery.

PRINCIPLE

The hydroxybutyrate dehydrogenase (HBDH) catalyses the reaction of alfa-ketobutyrate and NADH forming alfa hydroxybutirrate and NAD.

The decrease of absorbance of NADH, is proportional at the activity of HBDH on the sample.

REAGENTS

Components of the kit:	Cod. AH063	Cod. AH060
*REAGENT 1 (liquid)	1 x 65 ml	3 x 70 ml
Phosphate buffer 50 mmol/L		
EDTA Na ₂ 5 mmol/L		
*REAGENT 2 (liophilised)	20 x 3 ml	10 x 18 ml
Alfa-ketobutyrate 3 mmol/L (only in AH063)		
NADH 0.15 mmol/L		
*REAGENT 3 (liquid)		1 x 20.5 ml
Alfa-ketobutyrate 3 mmol/L (only in AH060)		

STABILITY: the reagents, at 2-8°C, are stable up to the expiry date shown on the package **if not contaminate during handling.**

AUXILIARY REAGENTS FOR CALIBRATION and for QUALITY CONTROL (Not supplied with the kit)

We suggest strongly to calibrate always on the instruments.

To grant a good calibration we suggest to use following kit:

- CALIBRATOR **Cod. CALFAS3**

To grant the correct test performances we suggest to use following kits:

- NORMAL CONTROL **Cod. CNU**
 - PATHOLOGICAL CONTROL **Cod. CPU**

PREPARATION OF WORKING REAGENT

*KIT 10 x 20 ml (Cod. AH060)

Add 18 ml of *Reagent 1 to one vial of *Reagent 2. Mix gently until dissolution.

Add 2 ml of *Reagent 3 to one vial of **reconstitute** *Reagent 2. Mix until complete dissolution.

*KIT 20 x 3 ml (Cod. AH063)

Add 3 ml of *Reagent 1 at one vial of *Reagent 2. Mix gently until complete dissolution.

STABILITY: 2 days at 2-8°C in the dark, **if not contaminated during handling.**

Close immediately after handling.

Incompetent handling will release us from any responsibility.

SAMPLE

- Serum, plasma non haemolyzed with heparin or EDTA.

PROCEDURE

- Wavelength: 340 nm (334-365 nm)
- Pathlength: 1 cm
- Reading: against air or distilled water
- Temperature: 37°C
- Method: kinetic
- Reaction: 3 minutes
- Linearity: up to 1500 U/L
- Sample/Reagent: 1/100

Let reagents reach the working temperature before using.

Pipette in a test tube or cuvette so labelled:

Working Reagent	1000 µl
Sample	10 µl

Mix gently and incubate at 37°C for 1 minute.

Read the absorbance initially against air or distilled water.

Read again after 1, 2 e 3 minutes exactly.

Calculate the mean of (ΔA/min).

CALCULATION

Replace the value of ΔA/min calculate on the following formula:

$$\text{HBDH (U/L)} = \Delta A/\text{min} \times 16032$$

REFERENCE VALUES

	37°C	30°C	25°C
ADULTS	Up to 182 U/L	Up to 165 U/L	Up to 140 U/L

It is suitable that every laboratory determines the reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: The HBDH activity is linear up to 1603,20 U/L. For HBDH activity higher than 1603,20 U/L, dilute the sample 1:5 with saline sol., repeat the determination and multiply the result x 5

Sensitivity: The minimum detectable is 16 U/L.

Within-run Precision:

	Mean (U/L) ± 2s	CV %
Umano 1	263,8 ± 10,6	2,02
Umano 2	458,6 ± 12,3	1,35

Run-to-run (Day-to-day) Precision:

	Mean (U/L) ± 2s	CV %
Umano 1	263,4 ± 8,8	1,7
Umano 2	458,2 ± 12	1,32

Accuracy: with commercially available Control(s)

	Waited (U/L)	Found (U/L)
Normal	143 (116 – 170)	133 – 138 – 150
Pathological	251 (206 – 296)	233 – 241 – 261

Interferences: See References point 2.

Correlation: A group of 20 sera was assayed by this procedure and using a similar commercially available HBDH Reagent. Comparison of the data gave following results:

linear regression: $Y = 0,9965X - 1,77$
 $r = 0,9995n = 20$

NOTE

1. A proportional variation of the reaction volumes does not change the result .
2. We suggest do not mix Reagents from different Production lots.
3. For HBDH activity higher than 1603,20 U/L, dilute the sample 1:5 with saline sol., repeat the determination and multiply the result x 5
4. PAY ATTENTION!
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
5. Very deep attention must be given to interfering substances:certain drugs and other substances are able to influence levels of HBDH (see References 2.).
6. The reagent must be used only for the intended destinations. by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975)
3. Z. Klin. Chem. Klin. Bioch., 10, 182 (1972).
4. Bergmeyer H.U., J. Clin. Chem. Clin. Bioch., 13, 507 (1975).

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