

β-HYDROXYBUTYRATE (β-HBA)

UV DETERMINATION IN SERUM OR PLASMA
 For in vitro diagnostic use only

Kit: 10 x 10 mL

Cod. HB8855

SUMMARY

The β-HBA concentration in biological fluids is of elevated interest because discovered in serum and urine of diabetic patients. Monitoring the level of β-HBA in patients with severe ketoacidosis is a concrete means of evaluating insulin therapy. Diabetes mellitus and alcohol consumption are the common way of ketoacidosis.

PRINCIPLE

The β-hydroxybutyrate dehydrogenase (β-HBA-DH) catalyzes the oxidation of β-hydroxybutyrate to acetoacetate with simultaneous reduction of NAD to NADH. The increase of absorbance of NADH, is directly proportional to the β-hydroxybutyrate in the sample.

REAGENTS

Components of the kit: **Cod. HB8855**
 *REAGENT 1 (liquid) **2 x 50 mL**
 *REAGENT 2 (lyo) **10 x 10 mL**
 *REAGENT 3 (liquid) **1 x 20 mL**
 *REAGENT 4 Standard (liquid) **1 x 5 mL**

β-hydroxybutyrate (50 mg/dL = 500 mg/L = 0,5 g/L = 4,8 mmol/L)

Good Buffer pH >7,5 >250 mmol/L
 β-HBA-DH >50 KU/L
 NAD >2,1 mmol/L

Activators, Stabilizers

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

SAMPLE

• No haemolyzed fresh serum, plasma heparin or Sodium Fluoride.

AUXILIARY REAGENTS

In order to have a Calibration Curve, we suggest the use of the following kit:

– β-HYDROXYBUTYRATE (β-HBA) Standard Set 3x (2x5) mL
Cod. HBST8856 (see the related insert)

AUXILIARY REAGENTS FOR QUALITY CONTROL

The reliability of test results should be monitored by routine use of Controls of known concentrations. We suggest following kits:

– β-HYDROXYBUTYRATE (β-HBA) LOW CONTROL kit 6x1 mL
Cod. HBCL2511 (see the related insert)

– β-HYDROXYBUTYRATE (β-HBA) HIGH CONTROL kit 6x1 mL
Cod. HBCH3511 (see the related insert)

PREPARAT. OF THE WORKING REAGENT (*R1+*R2)

*KIT 10 x 10 ml (Cod. HB8855)

Add 10 ml of *Reagent 1 to one vial of *Reagent 2. Mix gently until dissolution. STABILITY: 7 days at 2-8°C.

Mix kindly and let the working reagent reaches the working temperature before use. Close immediately after handling.

PREPARATION *REAGENT 3 and *REAGENT 4 REAGENTS READY TO USE.

Let the reagents reach the working temperature before use. Mix kindly before use. Close immediately after handling.

The Reagents have to be used properly, to avoid contamination. Incompetent handling will keep us harmless from any responsibility.

ANALYTICAL PROCEDURE

- Wavelength: 340 nm (334-365 nm)
- Pathlength: 1 cm
- Reading: against Water
- Temperature: 37°C
- Method: end-point
- Reaction: 10-15 minutes
- Linearity: up to 80 mg/dL (7,68 mmol/L)
- Sample/Reagent: 1/60/10

Let reagents reach the working temperature before using.

Pipette in 3 test tubes or cuvettes so labelled :

R/B: Blank Reagent, S: Sample, ST: Standard

	R/B	ST	S
Working Reagent	3000 µl	3000 µl	3000 µl
Distilled water	50 µl	----	----
*Reagent 4 Standard	----	50 µl	----
Sample	----	----	50 µl

Mix kindly and incubate at 37°C.

Read for the first time the absorbance of standard (Astd1) and sample (As1) against Blank Reagent. After this one, add:

*Reagent 3	500 µl	500 µl	500 µl
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Mix kindly. Exactly 10-15 min. after the first reading, make the second reading of standard (Astd2) and sample (As2) against Blank Reagent.

The color is stable for at least 30 minutes.

CALCULATION

$[(As2 - As1) / (Astd2 - Astd1)] \times 50 = \text{mg/dL of } \beta\text{-Hydroxybutyrate}$
 $\beta\text{-Hydroxybutyrate in mmol/L} = 0.096 \times \text{mg/dL of } \beta\text{-Hydroxybutyrate}$
 or

Plot each value found on the Calibration Curve (Cod.HBST8856). The Calibration Curve has to be always repeated for each new lot of Reagent.

REFERENCE VALUES

β-Hydroxybutyrate Adults 0 - 4,39 mg/dL (0 - 0,421 mmol/L)
 Children (1-18 years) 0,25 - 3,05 mg/dL (0,024 - 0,293 mmol/L)

It is suitable that every laboratory determine its 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 β-HBA Reagent is linear up to 80 mg/dL (7,68 mmol/L).
 For concentrations ≥ 80 mg/dL, dilute the sample 1:10 with saline sol., repeat the determ. and multiply the result x 10.

Sensitivity: The minimum detectable is 0,2 mg/dL.

Within-run Precision:

	Mean (mg/dL) ± 2s	CV %
Serum 1	3,4 ± 0,46	5,8
Serum 2	71,9 ± 2,3	2,1

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

	Mean (mg/dL) ± 2s	CV %
Serum 1	3,3 ± 0,7	6,8
Serum 2	72,5 ± 3,4	2,6

Interferences: See References point 2.

Correlation: A group of 20 sera from 0,3 to 76 mg/dL was assayed by this procedure and using a similar commercially available β -HBA Reagent. Comparison of the data gave following results:

Linear regression equation $y = 1,0393x - 0,32$
Correlation coefficient $r = 0,9916$

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 concent. of β -Hydroxybutyrate ≥ 80 mg/dL (7,68 mmol/L), dilute the sample 1:10 (1+9) with saline sol., repeat the determination and multiply the result $\times 10$.
4. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of β -Hydroxybutyrate (see References 2.).
5. PAY ATTENTION!
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
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).

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