β-Hydroxybutyrate 21 FS*

Order Information

Cat. No.	Kit size				
1 3711 99 10 930	R1	4 x 20 mL	+	R2	2 x 10 mL

Kit for use in conjunction with DiaSys CE applications.

Intended Use

Diagnostic reagent for quantitative in vitro determination of β-hydroxybutyrate in human serum or heparin plasma on automated photometric systems.

Summary

β-Hydroxybutyrate belongs to the group of ketone bodies [1]. Ketone bodies are derived from lipid molecules that serve as energy source, whenever the demand of energy cannot be covered by the glucose resources [2]. During fat metabolism, acetoacetate is produced by ketogenesis, which is mostly converted into β-hydroxybutyrate by β-hydroxybutyrate dehydrogenase, while only a small portion is converted to acetone by spontaneous decarboxylation. In healthy individuals, acetoacetate and β-hydroxybutyrate are equimolar, whereas acetone accounts only 5% of total ketone bodies. This state is physiologically well regulated. In pathological conditions such as alcoholic ketoacidosis or diabetic ketoacidosis within diabetes mellitus type 1, the concentration of β-hydroxybutyrate exceeds the limit the body is able to compensate and blood pH turns sour leading to life-threatening situations [1].

Method

Enzymatic determination with β-hydroxybutyrate-dehydrogenase

β-Hydroxybutyrate in presence of NAD^+ is converted to acetoacetate and NADH + H^+ by β-hydroxybutyrate-dehydrogenase. The absorbance at 340 nm is proportional to the β-hydroxybutyrate concentration in the sample.

$$\beta\text{-Hydroxybutyrate} + NAD \xrightarrow{\beta\text{-Hydroxybutyrate-dehydrogenase}} \text{Acetoacetate} + NADH + H^+$$

Reagents

Components and Concentrations

R1:	Buffer	pH 8.5	< 150 mmol/L
	β-Hydroxybutyrate-dehydrogenase		≥ 1 kU/L
R2:	Buffer	pH 4.3	< 70 mmol/L
	NAD		< 25 mmol/L

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze and protect from light.

The open-vial stability of the reagent is 24 months until expiry date.

Warnings and Precautions

1. Components contained in β-Hydroxybutyrate 21 FS are classified according to EC regulation 1272/2008 (CLP) as follows:

⚠ Reagent 1: Warning. H319 Causes serious eye irritation. P264 Wash hands and face thoroughly after handling. P280 Wear protective gloves/protective clothing/eye protection. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 If eye irritation persists: Get medical advice/attention.

2. Reagent 1 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Reagent 1 contains material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
4. In very rare cases, samples of patients with gammopathy might give falsified results [3].
5. To avoid contamination and carryover, special care should be taken in combination with Magnesium XL FS reagent (1 4610..).
6. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
7. Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.
8. Please refer to the safety data sheets (SDS) 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.
9. For professional use only.

Waste Management

Refer to local legal requirements for chemical disposal regulations as stated in the relevant SDS to determine the safe disposal.

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Reagent Preparation

The reagents are ready to use.

Materials Required

General laboratory equipment

Specimen

Human serum or heparin plasma

Only use suitable tubes or collection containers for specimen collection and preparation.

When using primary tubes, follow the manufacturer's instructions.

Stability [4]:

1 month	at	20 – 25°C
1 month	at	2 – 8°C
1 month	at	-20°C

Only freeze once. Discard contaminated specimens.

Assay Procedure

Basic settings for BioMajesty® JCA-BM6010/C

Wavelength	340/694 nm
Temperature	37°C
Measurement	Endpoint
Sample/Standard	6.0 µL
Reagent 1	80 µL
Reagent 2	20 µL
Addition reagent 2	Cycle 19 (286 s)
Absorbance 1	Cycle 23/24 (340 s/354 s)
Absorbance 2	Cycle 41/42 (586 s/600 s)
Calibration	Linear

Calculation

With Standard

$$\text{HBUT [mmol/L]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std.}} \times \text{Conc. Std. [mmol/L]}$$

Conversion Factor

β-Hydroxybutyrate [mg/dL] x 0.0961 = β-Hydroxybutyrate [mmol/L]

Standard and Controls

DiaSys β-Hydroxybutyrate Standard FS is recommended for calibration. Standard values have been made traceable to the weighing of purest β-hydroxybutyrate. Use DiaSys TruLab N and P for internal quality control. Quality control must be performed after calibration. Control intervals and limits have to be adapted to the individual requirements of each laboratory. Results must be within the defined ranges. Follow the relevant legal requirements and guidelines. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
β-Hydroxybutyrate Standard FS	1 3700 99 10 030	3 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

Data evaluated on BioMajesty® JCA-BM6010/C

Measuring range from 0.05 mmol/L up to 6 mmol/L, linearity is given within ± 5%.
When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Limit of detection**	0.05 mmol/L
Limit of quantitation**	0.05 mmol/L

Interference by	Interferences ≤ 10% up to	Analyte concentration [mmol/L]
Acetaminophen	1.50 mmol/L	0.276
	1.50 mmol/L	4.25
Acetoacetate	5.00 mmol/L	0.267
	5.00 mmol/L	4.24
Acetylsalicylic acid	60 mg/dL	0.274
	60 mg/dL	4.27
Ascorbic acid	50 mg/dL	0.113
	50 mg/dL	2.77
Bilirubin (conjugated)	50 mg/dL	0.234
	50 mg/dL	2.76
Bilirubin (unconjugated)	50 mg/dL	0.213
	50 mg/dL	2.64
Hemolysis	500 mg/dL	0.258
	500 mg/dL	3.04
α-Hydroxybutyrate	7.00 mmol/L	0.270
	7.00 mmol/L	1.26
Lipemia (triglycerides)	1000 mg/dL	0.256
	2000 mg/dL	2.82
N-acetylcysteine (NAC)	1000 mg/L	0.112
	1000 mg/L	2.76

No interference by **lactate and lactate dehydrogenase**.

For further information on interfering substances, refer to the literature [5,6].

Precision			
Repeatability (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mmol/L]	0.262	0.412	3.09
CV [%]	0.557	0.365	0.323
Within-laboratory (n=80)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mmol/L]	0.271	0.554	3.19
CV [%]	2.15	1.39	1.93

Method comparison (n=102)	
Test x	Competitor β-Hydroxybutyrate (Hitachi 917)
Test y	DiaSys β-Hydroxybutyrate 21 FS (BioMajesty® JCA-BM6010/C)
Slope	1.01
Intercept	-0.014 mmol/L
Coefficient of correlation	0.999

** according to CLSI document EP17-A2, Vol. 32, No. 8

Reference Range [1]

	[mmol/L]	[mg/dL]
After overnight fast	< 0.34	< 3.5

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Thomas L. Clinical Laboratory Diagnostics [Internet]. Prof. Lothar Thomas; 2023 [cited 2024 Mar 06]. Available from: https://www.clinical-laboratory-diagnostics.com/
2. Newman JC, Verdin E. β-Hydroxybutyrate: A Signaling Metabolite. Annu Rev Nutr. 2017 Aug 21;37:51-76. doi: 10.1146/annurev-nutr-071816-064916
3. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem Lab Med 2007; 45(9): 1240-1243.
4. Data on file at DiaSys Diagnostic Systems GmbH.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Young DS. Effects on Clinical Laboratory Tests - Drugs Disease, Herbs & Natural Products, https://clinfx.wiley.com/aaccweb/aacc/, accessed in February 2024. Published by AACC Press and John Wiley and Sons, Inc.

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