**β-Hydroxybutyrate FS**

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

**Order Information**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
<th>1 3701 99 10 930</th>
<th>R1 4 x 20 mL + R2 2 x 10 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 3700 99 10 030</td>
<td>3 x 3 mL Standard</td>
</tr>
</tbody>
</table>

**Summary** [1,2]

β-Hydroxybutyrate belongs to the group of ketone bodies and is formed during fat metabolism by reduction of acetoacetate in the liver. Ketone bodies serve as energy suppliers for various tissues (heart, kidney, and in skeletal muscles), especially in case of insulin deficiency, insulin resistance, and with low glucose concentrations. After the release of ketone bodies into the blood, they are quickly absorbed by the tissue which leads to a low concentration of ketone bodies in the blood. Metabolic acidoses due to increased β-hydroxybutyrate concentrations are related to diabetes mellitus, congenital metabolic diseases, alcoholism and fasting.

**Method**

Enzymatic endpoint determination. β-Hydroxybutyrate Standard FS values have been made traceable to the weighing of purest β-hydroxybutyrate.

**Principle**

\[ \beta\text{-Hydroxybutyrate} + \text{NAD} \rightarrow \text{Acetoacetate} + \text{NADH} + \text{H}^+ \]

NADH + NBT (oxidized) → NAD + NBT (reduced)

The absorbance of the blue dye at 546 nm is proportional to the β-hydroxybutyrate concentration in the sample.

**Reagents**

**Components and Concentrations**

**R1:** Buffer pH 8.4 115 mmol/L

β-Hydroxybutyrate dehydrogenase ≥ 2 kU/L

Diaphorase 2.1 kU/L

**R2:** NAD 21 mmol/L

Oxalic acid 66 mmol/L

Nitroblue tetrazolium (NBT) 1.7 mmol/L

**Standard:** 1 mmol/L

**Storage Instructions and Reagent Stability**

The reagents and the standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents.

**Warnings and Precautions**

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.

2. Reagent 1 contains biological material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.

3. In very rare cases, samples of patients with gammapathy might give falsified results [5].

4. Please refer to the safety data sheets 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.

5. For professional use only!

**Waste Management**

Please refer to local legal requirements.

**Reagent Preparation**

The reagents are ready to use.

**Note:** After a long cool storage, slightly violet colored sediment in reagent R2 can accrue which does not influence the measurement; however, it should be re-dissolved into solution by shaking the bottle gently before further measurement.

**Materials required but not provided**

NaCl solution 9 g/L

General laboratory equipment

**Specimen**

Serum and plasma

Effect the measurement immediately after blood collection.

Stability [3]:

1 month at 20 – 25°C

1 month at 2 – 8°C

1 month at –20°C

Discard contaminated specimens! Freeze only once!

**Assay Procedure**

**Application sheets for automated systems are available on request.**

| Wavelength | 546 nm |
| Optical path | 1 cm |
| Temperature | 37°C |
| Measurement | Against reagent blank |

**Blank**

<table>
<thead>
<tr>
<th>Sample/Standard</th>
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<th>Sample/Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>25 µL</td>
<td></td>
</tr>
<tr>
<td>Dist. Water</td>
<td>25 µL</td>
<td></td>
</tr>
<tr>
<td>Reagent 1 1000 µL</td>
<td>1000 µL</td>
<td></td>
</tr>
<tr>
<td>Reagent 2 250 µL</td>
<td>250 µL</td>
<td></td>
</tr>
</tbody>
</table>

Mix and incubate exactly 5 min. at 37°C. Read absorbance A2.

\[ \Delta A = (A2 – A1) \text{Sample/Standard} \]

**Calculation**

**With standard**

\[ \beta\text{-Hydroxybutyrate [mmol/L]} = \frac{\Delta E_{\text{Sample}}}{\Delta E_{\text{Std.}}} \times \text{Conc. Std. [mmol/L]} \]

**Conversion factor**

\[ \beta\text{-Hydroxybutyrate [mg/dL]} \times 0.096 = \beta\text{-Hydroxybutyrate [mmol/L]} \]

**Controls**

For internal quality control, DiaSys TruLab N and P controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

<table>
<thead>
<tr>
<th>Cat.-No.</th>
<th>Kit size</th>
<th>TruLab P</th>
<th>5 9050 99 10 061</th>
<th>6 x 5 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TruLab N</td>
<td>5 9000 99 10 062</td>
<td>20 x 5 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 9000 99 10 061</td>
<td>6 x 5 mL</td>
</tr>
</tbody>
</table>

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Performance Characteristics

Measuring range
The test has been developed to determine β-Hydroxybutyrate concentrations within a measuring range from 0.01 mmol/L – 6.9 mmol/L. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences
No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 60 mg/dL, hemoglobin up to 500 mg/dL, and lipemia up to 2400 mg/dL triglycerides. The addition of oxalic acid to the reagent eliminates interferences with lactate and lactate dehydrogenase. For further information on interfering substances refer to Young DS [4].

Sensitivity/Limit of Detection
The lower limit of detection is 0.01 mmol/L.

Precision

<table>
<thead>
<tr>
<th>Intra-assay precision</th>
<th>Mean [mmol/L]</th>
<th>SD [mmol/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.30</td>
<td>0.004</td>
<td>1.31</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.75</td>
<td>0.008</td>
<td>1.02</td>
</tr>
<tr>
<td>Sample 3</td>
<td>1.13</td>
<td>0.006</td>
<td>0.53</td>
</tr>
</tbody>
</table>

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<tr>
<th>Inter-assay precision</th>
<th>Mean [mmol/L]</th>
<th>SD [mmol/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.26</td>
<td>0.01</td>
<td>3.78</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1.02</td>
<td>0.02</td>
<td>2.33</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2.14</td>
<td>0.05</td>
<td>2.47</td>
</tr>
</tbody>
</table>

Method Comparison
A comparison of DiaSys β-Hydroxybutyrate FS (y) with a commercially available test (x) using 120 samples gave following results:

\[
y = 1.00 \times x + 0.000 \text{ mmol/L}; \quad r = 0.999.
\]

Reference Range [1]

<table>
<thead>
<tr>
<th>Fasting</th>
<th>[mmol/L]</th>
<th>[mg/dL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02 – 0.27</td>
<td>0.21 – 2.81</td>
<td></td>
</tr>
</tbody>
</table>

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

Literature
3. Data on file at DiaSys Diagnostic Systems GmbH.

Manufacturer
DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany