Bicarbonate FS *

Diagnostic reagent for quantitative in vitro determination of bicarbonate/total CO₂ in serum or plasma on photometric systems

Order Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0950 99 10 021 R</td>
<td>5 x 25 mL</td>
<td>1 x 3 mL Standard</td>
</tr>
<tr>
<td>1 0950 99 10 026 R</td>
<td>6 x 100 mL</td>
<td>60 mL</td>
</tr>
<tr>
<td>1 0950 99 10 917 R</td>
<td>10 x 60 mL</td>
<td></td>
</tr>
<tr>
<td>1 0950 99 10 930 R</td>
<td>6 x 20 mL</td>
<td></td>
</tr>
<tr>
<td>1 0950 99 10 030 R</td>
<td>6 x 3 mL</td>
<td>Standard</td>
</tr>
</tbody>
</table>

Summary [1]

Measurement of bicarbonate is used in the diagnosis of the acid-base-balance in the blood. Elevated and decreased values indicate disorders associated with disturbances of the metabolic and respiratory systems.

Method

Enzymatic test using phosphoenolpyruvate carboxylase (PEPC) and a stable NADH analog. This method has been standardized against a primary standard on basis of sodium carbonate.

Phosphoenolpyruvate + HCO₃⁻ PEPC + Mg²⁺ → Oxaloacetate + H₂PO₄⁻

Oxaloacetate + Cofactor red. → Malate + Cofactor

The reaction disturbs following equilibrium.

CO₂ + H₂O ⇌ H₂CO₃ ⇌ H⁺ + HCO₃⁻

This results in a conversion of CO₂ to bicarbonate (HCO₃⁻) which then is included in the reaction. Therefore, the total CO₂ concentration is measured.

The decrease of reduced cofactor concentration is measured at 405 or 415 nm and is proportional to the concentration of total carbon dioxide in the sample.

Reagents

Components and Concentrations

Reagent:

- Buffer pH 7.5
- Phosphoenolpyruvate (PEP) 12.5 mmol/L
- Phosphoenolpyruvate carboxylase (PEPC) > 400 U/L
- Malate dehydrogenase (MDH) > 4100 U/L
- NADH analog 0.6 mmol/L
- Standard: 30 mmol/L

Storage Instructions and Reagent Stability

The reagent is stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagent! The standard is stable up to the end of the indicated month of expiry, if stored at 2 – 8°C. Once opened, the standard is stable for 12 months, if recapped immediately after use. Protect reagent and standard from light!

Warnings and Precautions

1. The reagent contains sodium azide (0.8 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. The reagent contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. Please refer to the safety data sheet 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 reagent and the standard are ready to use.

Materials required but not provided

- NaCl solution 9 g/L
- General laboratory equipment

Specimen

Serum or heparin plasma

Serum or plasma should be separated from cells immediately and stored at 2 – 8°C. Exposure of samples to air should be avoided. Samples should be stored tightly sealed to prevent loss of carbon dioxide and assayed as soon as possible after collection.

Stability [4]:

- 1 day at 20 – 25°C
- 7 days at 4 – 8°C
- 2 weeks at −20°C

Freeze only once! Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

- Wavelength 405 nm, 415 nm
- Optical path 1 cm
- Temperature 37°C
- Measurement Against reagent blank

AA = (A₂ – A₁) sample or standard

Sample or standard

<table>
<thead>
<tr>
<th>Sample or standard</th>
<th>10 µL</th>
<th>Reagent 1000 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mix, incubate and read absorbance A1 after exactly 2 min. and absorbance A2 after exactly 10 min. against reagent blank.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calculation

With standard

Bicarbonate [mmol/L] = \( \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Std.}}} \times \text{Conc. Std [mmol/L]} \)

Conversion factor

Bicarbonate [mmol/L] = Bicarbonate [mEq/L]
**Controls**
DiaSys TruLab Bicarbonate control should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

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<td>TruLab Bicarbonate</td>
<td>5 9700 99 10 065</td>
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</table>

**Performance Characteristics**

**Measuring Range**
The test has been developed to determine CO$_2$ concentrations within a measuring range from 4 – 50 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, conjugated bilirubin up to 50 mg/dL, free bilirubin up to 40 mg/dL, hemoglobin up to 500 mg/dL and lipemia up to 1400 mg/dL triglycerides. For further information on interfering substances refer to Young DS [5].

**Sensitivity/Limit of Detection**
The lower limit of detection is 1 mmol/L.

**Precision**

<table>
<thead>
<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>17.6</td>
<td>0.14</td>
<td>0.80</td>
</tr>
<tr>
<td>Sample 2</td>
<td>19.9</td>
<td>0.16</td>
<td>0.80</td>
</tr>
<tr>
<td>Sample 3</td>
<td>30.1</td>
<td>0.28</td>
<td>0.93</td>
</tr>
</tbody>
</table>

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<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>16.8</td>
<td>0.53</td>
<td>3.16</td>
</tr>
<tr>
<td>Sample 2</td>
<td>20.3</td>
<td>0.49</td>
<td>2.40</td>
</tr>
<tr>
<td>Sample 3</td>
<td>30.0</td>
<td>0.68</td>
<td>2.26</td>
</tr>
</tbody>
</table>

**Method Comparison**
A comparison of DiaSys Bicarbonate FS (y) with a commercially available assay (x) using 107 samples gave following results:

\[ y = 0.989 \times x + 0.354 \text{ mmol/L}; r = 0.998 \]

**Reference Range**

**Adults:** 22 – 29 mmol/L (mEq/L)

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

**Literature**
3. US patent #5,801,006

**Manufacturer**
DiaSys Diagnostic Systems GmbH
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