UIBC FS* Diagnostic reagent for quantitative in vitro determination of the unsaturated iron binding capacity in serum and plasma on photometric systems

Order Information
Cat. No. Kit size
1 1921 99 10 930 R1 4 x 20 mL + R2 2 x 10 mL
5 9100 99 10 064 6 x 3 mL TruCal U
5 9100 99 10 063 20 x 3 mL TruCal U

Summary [1,2]
The measurement of unsaturated iron binding capacity (UIBC) in combination with serum iron is a useful diagnostic tool in the determination of various iron disorders. The sum of UIBC and serum iron gives a value for the total iron binding capacity (TIBC). TIBC represents the maximum concentration of iron that serum proteins can bind. Serum UIBC levels vary in disorders of iron metabolism where iron capacities are often increased in iron deficiency and decreased in chronic inflammatory disorders or malignancies.

Method
Photometric test using Ferene

Principle
A known ferrous ion concentration incubated with sample, binds specifically with transferrin at unsaturated iron binding sites. Remaining unbound ferrous ions are measured with the ferene reaction.

\[ 2 \text{Fe}^{2+} (\text{known}) + \text{Transferrin} \rightarrow \text{Transferrin (Fe}^{3+}) + \text{Fe}^{2+} (\text{excess}) \]

\[ \text{Fe}^{2+} (\text{excess}) + 3 \text{Ferene} \rightarrow \text{Ferene (blue complex)} \]

Reagents

Components and Concentrations
R1: Buffer pH 8.7 100 mmol/L
Ammonium iron (II) sulfate 13 µmol/L
Thiourea 120 mmol/L
R2: Ascorbic acid 240 mmol/L
Ferene 6 mmol/L
Thiourea 125 mmol/L

Storage Instructions and Reagent Stability
The reagents are 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 reagents! Protect reagents from light!

Waste Management
Please refer to local legal requirements.

Reagent Preparation
The reagents are ready to use.

Materials required but not provided
NaCl solution 9 g/L
General laboratory equipment

Specimen
Serum, heparin plasma
Separate serum/plasma at the latest 2 h after blood collection to avoid hemolysis.

Stability [3]
in serum:
5 days at 20 – 25°C
1 month at 2 – 8°C
1 month at –20°C
in plasma:
1 month at 2 – 8°C
1 month at –20°C
Freeze only once!
Discard contaminated specimens!

Assay Procedure
Application sheets for automated systems are available on request.

Wavelength 600 – 620 nm, Hg 578 nm, 623 nm
Optical path 1 cm
Temperature 37°C
Measurement Against reagent blank

<table>
<thead>
<tr>
<th>Blank</th>
<th>Sample or calibrator</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>75 µL</td>
</tr>
<tr>
<td>Dist. Water</td>
<td>75 µL</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Mix, read absorbance A1 after 5 min., then add:</td>
<td>Mix, read absorbance A2 after exactly 5 min.</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>250 µL</td>
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</table>

\[ \Delta A = (A_2 – 0.81 A_1) \text{Sample/cal} \]

The factor 0.81 compensates the decrease of the absorbance by addition of reagent 2. The factor is calculated as follows: (Sample + R1)/Total volume. This compensation is necessary as a high sample volume is used.

Calculation
With calibrator

\[ \text{UIBC} [\mu g/dL] = \frac{\Delta A \text{Sample}}{\Delta A \text{ Cal}} \times \text{Conc Cal [µg/dL]} \]

\[ \text{UIBC [µg/dL]} \times 0.1791 = \text{UIBC [µmol/L]} \]

\[ \text{TIBC [µg/dL]} = \text{UIBC [µg/dL]} + \text{Iron [µg/dL]} \]

Transferrin [mg/dL] = 0.7 x TIBC [µg/dL]

* fluid stable
Calibrators and Controls

For calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. The assigned values of the calibrator have been made traceable to a measurement of transferrin and iron. Thereby, the transferrin value is traceable to ERM® DA470k/IFCC and the iron value is traceable to NIST SRM 682. DiaSys TruLab N control should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

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

Performance Characteristics

Measuring range

The test has been developed to determine UIBC within a measuring range from 6 – 750 µg/dL (1 – 135 µmol/L). When values exceed the upper limit samples should be diluted 1 + 2 with NaCl solution (9 g/L) and the result multiplied by 3.

Specificity/Interferences

No interference was observed by ascorbate up to 30 mg/dL, conjugated and free bilirubin up to 60 mg/dL, lipemia up to 2000 mg/dL, triglycerides, RF up to 350 IU/mL, copper up to 15 mg/dL and zinc up to 15 mg/dL. No interference was observed in hemolytic samples with hemoglobin <200 mg/dL. With stronger hemolysis interference occurs as destroyed erythrocytes release iron.

For further information on interfering substances refer to Young DS [6].

Sensitivity/Limit of Detection

The lower limit of detection is 6 µg/dL (1 µmol/L).

Precision

<table>
<thead>
<tr>
<th></th>
<th>Mean [µg/dL]</th>
<th>SD [µg/dL]</th>
<th>CV [%]</th>
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</thead>
<tbody>
<tr>
<td>Intra-assay</td>
<td>n = 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>65.8</td>
<td>1.27</td>
<td>1.93</td>
</tr>
<tr>
<td>Sample 2</td>
<td>264</td>
<td>3.62</td>
<td>1.37</td>
</tr>
<tr>
<td>Sample 3</td>
<td>531</td>
<td>8.73</td>
<td>1.64</td>
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</table>

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<td>Inter-assay</td>
<td>n = 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>170</td>
<td>4.43</td>
<td>2.61</td>
</tr>
<tr>
<td>Sample 2</td>
<td>263</td>
<td>3.61</td>
<td>1.37</td>
</tr>
<tr>
<td>Sample 3</td>
<td>475</td>
<td>6.31</td>
<td>1.33</td>
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Method Comparison

A comparison of DiaSys UIBC FS (y) with values calculated from transferrin and iron measurement (x) using 98 samples gave following results:

\[ y = 0.985 \times x - 6.558 \text{ µmol/L} \; \text{r} = 0.993 \]

Reference Range [4,5]

Taking into account reference values for iron and transferrin the following reference range results for UIBC: 120 – 470 µg/dL (21 – 84 µmol/L)

Each laboratory should define its own reference range for the relevant population to take into account all affecting factors.

Literature

3. Data on file at DiaSys Diagnostic Systems GmbH.

Manufacturer

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany