INTENDED USE
This set of reagents is intended for the quantitative in vitro measurement of iron and iron binding capacity in serum.

CLINICAL SIGNIFICANCE
Iron in the body is a necessary metal required not only for the synthesis of hemoglobin but also for many cellular enzymes and coenzymes. Iron is transported in serum bound to the protein transferrin. Normally, only about one third of the available binding sites on transferrin are occupied by iron. The total iron binding capacity in serum therefore, includes the amount of iron already bound to the transferrin (serum iron) plus the amount of iron required to saturate the unoccupied binding sites of transferrin.

Clinically the determination of serum iron and total iron binding capacity is useful in the differential diagnosis of anemias and other iron disorders.

TEST SUMMARY
The spectrophotometric measurement of serum iron is accomplished by releasing the protein bound iron from its carrier protein transferrin and complexing the released iron with a suitable chromogen.

In our method the serum sample is added to an acidic buffered reagent containing hydroxyamine, thiourea and Ferene®. The acid pH of the buffered reagent releases the iron which is in the ferric form from the transferrin (2). The released ferric iron is then reduced to the ferrous form by hydroxyamine. This ferrous iron reacts with Ferene to produce a colored complex. The absorbance of this colored complex, read at 595 nm, is proportional to the concentration of iron in the sample. The thiourea in the buffered reagent effectively suppresses any interference from copper (3). The total iron binding capacity (TIBC) is the sum of the serum iron concentration and the amount of additional iron required to saturate the unbound iron binding sites of transferrin. In this procedure, the unbound iron binding capacity of the serum is determined by adding a known amount of ferrous iron to a serum sample in a buffered reagent at an alkaline pH.

At an alkaline pH, the unbound sites of the transferrin will bind the ferrous iron (4). The added ferrous iron not bound to the transferrin at this pH, will react with the Ferene forming a colored complex. The absorbance of this iron Ferene measured at 595 nm is inversely proportional to the unbound iron binding capacity of the serum.

REAGENT COMPOSITION

**Serum Iron Buffer**
- Reactive ingredients: Hydroxyamine Hydrochloride 216 mmol/L, Thiourea 26 mmol/L
- Non-reactive ingredients: Stabilizers and preservatives

**TIBC Buffer**
- Reactive ingredients: Thiourea 236 mmol/L
- Non-reactive ingredients: Stabilizers and preservatives

**Chromogen**
- Reactive ingredients: Ferene 33 mmol/L

**Iron Standard**
- Reactive ingredients: Ferrous Iron 500 μg/dL, Hydroxyamine Hydrochloride 719 mmol/L

**REAGENT PREPARATION**

1. Serum Iron Color Reagent:
   - Prepare by mixing 1 mL of Iron/TIBC Chromogen (Cat. No. R85166VI) with 50 mL of Serum Iron Buffer (Cat. No. R85164VI).
   - This color reagent is stable for 6 weeks stored at 2–8 °C. Store in amber container and protect from light. Bring Iron Color Reagent to room temperature prior to use.

2. TIBC Color Reagent:
   - Prepare by mixing 1 mL of Iron/TIBC Chromogen (Cat. No. R85166VI) with 50 mL of TIBC Buffer (Cat. No. R85165VI).
   - This color reagent is stable for 6 weeks stored at 2–8 °C.
   - Store in amber container and protect from light. Bring TIBC Color Reagent to room temperature prior to use.

**REAGENT STORAGE AND STABILITY**

The reagents as supplied are stable at 2–8 °C until the expiration date on the bottle label. The prepared color reagents are stable for 6 weeks at 2–8 °C. Mix and store the combined reagents in amber containers free of iron contamination; protect from light (see: Reagent Preparation, above). Avoid contamination with iron. Use only iron-free glassware and pipettes.

**PRECAUTIONS**

Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water for at least 10 minutes. If swallowed, seek medical advice immediately and show this container or label.

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety, Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

**SPECIMEN COLLECTION AND STORAGE**

Use only serum samples, free of hemolysis, separated from the clot as soon as possible. DO NOT use hemolyzed samples. DO NOT use heparinized plasma. Use only iron-free tubes and syringes to collect blood samples.

**INTERFERING SUBSTANCES**

Drugs and other substances that interfere with the determination of iron and iron binding capacity have been reported by Young (6, 7).

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Spectrophotometer or colorimeter capable of measuring absorbance at 595 nm.
2. Matched cuvettes, preferably with 1 cm light path.
3. Pipettes to measure reagents and samples.
4. Distilled or deionized water.
5. Constant temperature water bath. Temperature is not critical but should be constant.

**MATERIALS PROVIDED**

Reagents for the quantitative measurement of serum iron and total iron binding capacity.

1. Serum Iron Buffer, Cat. No. R85164VI, 2 x 100 mL.
2. TIBC Buffer, Cat. No. R85165VI, 2 x 100 mL.
3. Iron/TIBC Chromogen, Cat. No. R85166VI, 1 x 10 mL.
4. Iron Standard, Cat. No. R85167VI, 1 x 100 mL.

**TEST PROCEDURE**

**Serum Iron Assay:**

Bring Iron Color Reagent to room temperature prior to performing assay.

Set up assay as follows:

<table>
<thead>
<tr>
<th>Water</th>
<th>Sample</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Test</td>
<td>Blank</td>
</tr>
<tr>
<td>Iron Buffer</td>
<td>2 mL</td>
<td>–</td>
</tr>
<tr>
<td>Iron Color Rgt.</td>
<td>–</td>
<td>2 mL</td>
</tr>
<tr>
<td>Water</td>
<td>0.4 mL</td>
<td>0.4 mL</td>
</tr>
<tr>
<td>Standard</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sample</td>
<td>–</td>
<td>0.4 mL</td>
</tr>
</tbody>
</table>

Mix. Incubate tests and blanks at 37 °C for 10 minutes.

Set instrument to 595 nm and adjust to zero absorbance with distilled or deionized water.

Read and record the absorbance of each test and its respective blank at 595 nm. Subtract the absorbance of each blank from the absorbance of its respective test. This is the corrected absorbance (A_r) for each test: water, sample and standard.

\[
A_r = A_{test} - A_{blank}
\]

The corrected absorbances (A_r) for each test of the Serum Iron Assay, water, sample and standard, are used in the calculation of the serum iron concentration as indicated in the Calculations Section.

**Total Iron Binding Capacity Assay (TIBC)**

The TIBC value is a calculated result and is the sum of the concentrations of the serum iron and the unsaturated iron binding capacity (UIBC). The UIBC concentration in this assay procedure is measured using the TIBC Buffer and the TIBC Color Reagent. The results from this assay (UIBC concentration) are used in the calculation of the TIBC value as indicated below.

Bring TIBC Color Reagent to room temperature prior to performing assay.

Set up the assay in the following specific sequence:

<table>
<thead>
<tr>
<th>Water</th>
<th>Sample</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Test</td>
<td>Blank</td>
</tr>
<tr>
<td>TIBC Buffer</td>
<td>2 mL</td>
<td>–</td>
</tr>
<tr>
<td>TIBC Color Rgt.</td>
<td>–</td>
<td>2 mL</td>
</tr>
<tr>
<td>Water</td>
<td>0.8 mL</td>
<td>0.8 mL</td>
</tr>
<tr>
<td>Standard</td>
<td>–</td>
<td>0.4 mL</td>
</tr>
<tr>
<td>Sample</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Mix. Incubate tests and blanks at 37 °C for 10 minutes.

Set instrument to 595 nm and adjust to zero absorbance with distilled or deionized water.

Read and record the absorbance of each test and its respective blank at 595 nm. Subtract the absorbance of each blank from the absorbance of its respective test. This is the corrected absorbance (A_r) for each test: water, sample and standard.

Therefore: 

\[
A_r = A_{test} - A_{blank}
\]
The corrected absorbances (Aₐ) for each test measuring the unsaturated iron binding capacity (UIBC) concentration for water, sample, and standard are used in the calculation of the total iron binding capacity (TIBC) concentration as indicated in the Calculations Section.

CALIBRATION
This assay requires the use of an iron standard which is an integral part of the assay. Use only the iron standard provided with this reagent as a calibrating standard. The use of other iron standards may not produce accurate results.

QUALITY CONTROL
Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Commercially available control material with established values for serum iron and TIBC concentrations may be used. Assayed Control Serum, Level 1 (Cat. No. R83082) and Level 2 (Cat. No. R83083) are recommended for this purpose.

CALCULATIONS
Serum Iron:

\[
\frac{(Aₐ \text{ sample}) - (Aₐ \text{ water})}{(Aₐ \text{ standard}) - (Aₐ \text{ water})} \times C \text{ standard} = \text{serum iron in } \mu \text{g/dL}
\]

where C standard is the concentration of the standard in µg/dL.

Serum Iron Calculation Example:

\[
Aₐ \text{ water} = 0.010
\]
\[
Aₐ \text{ sample} = 0.098
\]
\[
Aₐ \text{ standard} = 0.525 \text{ for } 500 \mu \text{g/dL iron}
\]
\[
0.098 - 0.010 = 0.088
\]
\[
0.088 \times 500 = 44 \mu \text{g/dL serum iron}
\]

Total Iron Binding Capacity (TIBC):

\[
TIBC = \text{Serum Iron} + \text{UIBC}
\]

Calculate the values for the unsaturated iron binding capacity (UIBC) using the corrected absorbances obtained from the TIBC assay above.

\[
\text{UIBC} (\mu \text{g/dL}) = \left( \frac{(Aₐ \text{ sample}) - (Aₐ \text{ water})}{(Aₐ \text{ standard}) - (Aₐ \text{ water})} \right) \times 500
\]

where 500 is the concentration of the iron standard in µg/dL.

UIBC Calculation Example:

\[
Aₐ \text{ water} = 0.010
\]
\[
Aₐ \text{ sample} = 0.028
\]
\[
Aₐ \text{ standard} = 0.425
\]
\[
\left( \frac{0.028 - 0.010}{0.425 - 0.010} \right) \times 500 = 172 \mu \text{g/dL UIBC}
\]

Calculate TIBC: TIBC = Serum Iron + UIBC

Using the values from the above calculation examples:

\[
\text{TIBC} = 85 \mu \text{g/dL} + 172 \mu \text{g/dL} = 257 \mu \text{g/dL}
\]

LIMITATIONS OF THE PROCEDURE
1. Samples with serum iron concentrations higher than 500 µg/dL should be diluted with iron-free water and the assay repeated. Multiply the results by the dilution factor used.

REFERENCE RANGE
The reported (2) reference range for serum iron in adult men is 70 - 180 µg/dL and for adult women 60 - 150 µg/dL. The reported reference range for total iron binding capacity is 250 - 450 µg/dL (2). It is recommended that each laboratory establish its own reference range.

REFERENCES

Reference is a registered trademark of Diagnostic Chemicals, Ltd.