Iron
FerroZine, Colorimetric
Quantitative determination of iron
Store at 2-8°C
For In-Vitro and professional use only

INTENDED USE
For the quantitative determination of iron in human serum or heparenized plasma.

CLINICAL SIGNIFICANCE
The iron is the component of a great number of enzymes. The myoglobin, muscular protein, contains iron, as well as the liver.
Iron is necessary for the hemoglobin production, molecule that transports oxygen inside red globules. Their deficit in the last causes the ferropenic anemia.
High levels of iron are found in hemochromatosis, cirrhosis, hepatitis and in increased transferrin levels.
The variation day to day is quite marked in healthy people.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE
The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with FerroZine a coloured complex:

$$\text{Transferrin(Fe}^{3+}\text{)}_2 + e^- + \text{Ascorbic acid} \rightarrow 2 \text{Fe}^{2+} + \text{Transferrin}$$

$$\text{Fe}^{2+} \rightarrow \text{FerroZine} \rightarrow \text{Coloured complex}$$
The intensity of the color formed is proportional to the iron concentration in the sample.

EQUIPMENTS NEEDED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 562 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment

PREPARATION
- Working reagent (WR): Dissolve the content of one vial R2 Reductant in one bottle of R1 Buffer
- Cap and mix gently to dissolve contents.
- Stability: 3 months at 2-8°C or 1 month at 15-25°C.

STORAGE AND STABILITY
- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
- Do not use reagents over the expiration date.
- IRON STD: Store at 2-8°C protected from light and contamination.

SAMPLES
Serum or heparinized plasma.
Free of hemolysis and separated from cells as rapidly as possible.
Stability of the sample: 2-8°C for 7 days.

PROCEDURE
1. Adjust the instrument to zero with distilled water.
2. Pipette into a cuvette:

<table>
<thead>
<tr>
<th></th>
<th>WR Blank</th>
<th>Standard</th>
<th>Sample Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>WR (mL)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>R 3 (drops)</td>
<td>1</td>
<td>1</td>
<td>--</td>
<td>1</td>
</tr>
<tr>
<td>Distilled water (µL)</td>
<td>200</td>
<td>--</td>
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<td>--</td>
</tr>
<tr>
<td>Standard (µL)</td>
<td>--</td>
<td>200</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Sample (µL)</td>
<td>--</td>
<td>--</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

4. Mix and incubate 5 min at 37°C or 10 min at room temperature.
5. Measure the absorbance (A) of Standard and sample against WR Blank. The colour is stable for at least 30 minutes.

CALCULATIONS
$$\text{Conversion factor: } \frac{\mu g/dL \times 0.179}{1} = \mu mol/L.$$
QUALITY CONTROL

- Control sera are recommended to monitor the performance of assay procedures.
- If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
- Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Male

65 - 175 µg/dL = 11.6 – 31.3 µmol/L

Female

40 - 150 µg/dL = 7.16 - 26.85 µmol/L

NOTE

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

**Measuring range:**
From detection limit of 1.85 µg/dL to linearity limit of 1000 µg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

**Precision:**

<table>
<thead>
<tr>
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<th>Intra-assay (n=20)</th>
<th>Inter-assay (n=20)</th>
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</thead>
<tbody>
<tr>
<td>Mean (µg/dL)</td>
<td>102</td>
<td>190</td>
</tr>
<tr>
<td>SD</td>
<td>0.88</td>
<td>1.31</td>
</tr>
<tr>
<td>CV (%)</td>
<td>0.86</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Sensitivity:
1 µg/dL = 0.0009 A.

**Accuracy:**
Results obtained using ATLAS reagents did not show systematic differences when compared with other commercial reagents. The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.987
Regression equation : y= 1.0052x – 2.3159
The results of the performance characteristics depend on the analyzer used.

**INTERFERENCES**
Hemolyzed samples are rejected, since erythrocytes contain iron and therefore falsely elevate the serum results.
A list of drugs and other interfering substances with iron determination has been reported by Young et.

NOTES

1. It is recommended to use disposable material. If glassware is used the material should be soaking for 6 h in diluted HCl (20% v/v) and then thoroughly rinsed with distilled water and dried before use.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. Strongly method dependent.
5. Iron STD : proceed carefully with this product because due its nature it can get contaminated easily.

REFERENCES


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