**Iron Assay Kit**

**Ferrozine Chromogenic method**

**Biochemical Significance and Test Summary**

Iron is one of the most important elements, which function as enzyme cofactor. Iron in the blood is bounded with transferrin and transported throughout the body to synthesize globin proteins such as myoglobin and hemoglobin. Iron is crucial for synthesis of oxygen-transport protein. Its deficiency causes iron deficiency anemia, chronic blood loss anemia and infectious anemia.

This product is a colorimetric assay kit without sample deproteinization. Iron bounded with transferrin is dissociated from proteins in weakly acidic region. Fe(III) is reduced to Fe(II), and blue colored complex with Frrozine [Fe(II)–ferrozine complex] is formed. The concentration of iron in sample is determined by the absorbance of Fe(II)–ferrozine complex at 560 nm.

**1. Kit contents**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1 Buffer</td>
<td>1 x 20 mL</td>
<td>Ready to use</td>
</tr>
<tr>
<td>R-2 Chelate color</td>
<td>1 x 0.8 mL</td>
<td>Ready to use</td>
</tr>
<tr>
<td>STD 200 µg/dL Fe Standard</td>
<td>1 x 4.0 mL</td>
<td>Ready to use</td>
</tr>
</tbody>
</table>

*Storage conditions: Store at 2-8°C. Don’t freeze.*

*Expiration: 1 year. After the vials are opened, the kit should be used in one month.

*Measuring range: 5-1000 µg/dL*

**2. Materials required but not provided**

(1) Distilled water
(2) Micropipettors and pipette tips
(3) Clear flat-bottom 96-well plate
(4) Microplate reader with 560 nm capability

**3. Assay preparation**

(1) Bring all reagents to room temperature before use.
(2) Wash test tube and glassware by 1M HNO3 or 1M HCl, and rinse with distilled water.

**4. Sample preparation**

**Serum/Plasma:** Insoluble substances in serum/plasma samples should be removed by filtration or centrifugation. EDTA-plasma cannot be used.

**Urine (24 hour pooled urine)/Tissue homogenate/Cell lysate/Saliva:** Add 6M HCl to the sample and adjust pH 1.5-3.0 (e.g. 5-10 µL of 6M HCl/1 mL of sample). Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.

**Tissue:** Add 5% TCA solution, voltex for 1 minute and incubate for 30 minutes at 4-8°C. Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.

**5. Assay protocol**

(1) Add 40 µL of distilled water (Blank)/STD (Standards)/samples to each well.
(2) Add 200 µL of R-1 to each well and incubate for 5 minutes at room temperature.
(3) Read the absorbance at 560 nm (540-580 nm). --&gt; OD₁
(4) Add 8 µL of R-2 to each well and incubate for 5 minutes at room temperature.
(5) Read the absorbance at 560 nm (540-580 nm). --&gt; OD₂
6. Calculation

\[
\Delta OD_{\text{Standard}} = OD_{\text{Standard}} - OD_{\text{Blank}}, \quad \Delta OD_{\text{Sample}} = OD_{\text{Sample}} - OD_{\text{Blank}}
\]

Iron (µg/dL) = \( \frac{\Delta OD_{\text{Sample}}}{\Delta OD_{\text{Standard}} \times 200} \)

Iron (µM) = \( \frac{\Delta OD_{\text{Sample}}}{\Delta OD_{\text{Standard}} \times 35.8} \)

(Assay example)

<table>
<thead>
<tr>
<th></th>
<th>OD₁</th>
<th>OD₂</th>
<th>( \Delta OD (OD₂ - OD₁) )</th>
<th>Iron (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW (Blank)</td>
<td>0.030</td>
<td>0.030</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>0.029</td>
<td>0.133</td>
<td>0.104</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>0.044</td>
<td>0.099</td>
<td>0.055</td>
<td>106</td>
</tr>
</tbody>
</table>

\[
\Delta OD_{\text{Standard}} = (OD_{2\text{Standard}} - OD_{1\text{Standard}}) - (OD_{2\text{Blank}} - OD_{1\text{Blank}}) = (0.133 - 0.029) - (0.030 - 0.030) = 0.104
\]

\[
\Delta OD_{\text{Sample}} = (OD_{2\text{Sample}} - OD_{1\text{Sample}}) - (OD_{2\text{Blank}} - OD_{1\text{Blank}}) = (0.099 - 0.044) - (0.030 - 0.030) = 0.055
\]

\[
\text{Iron}_{\text{Sample}} = \frac{\Delta OD_{\text{Sample}}}{\Delta OD_{\text{Standard}} \times 200} = 0.055 / 0.104 \times 200 = 106 \text{ (µg/dL)}
\]

\[
\text{Iron}_{\text{µM}} = \frac{\Delta OD_{\text{Sample}}}{\Delta OD_{\text{Standard}} \times 35.8} = 0.055 / 0.104 \times 35.8 = 19 \text{ (µM)}
\]

7. Interferences

EDTA inhibits iron to chromogenic system. The test is not affected by presence of bilirubin-F and bilirubin-C up to 40 mg/dL, hemoglobin up to 0.2 g/dL and chyle up to 1,000 FTU.

8. Quality Control

Use of control sera is recommended to monitor the assay quality.

9. References


10. Technical support & troubleshooting

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(Supported languages: Japanese and English)

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