**Intended use**

For the quantitative determination of Human Ferritin concentration in human serum.

**Introduction**

One of the most prevalent disorders of man is the dietary deficiency of iron and the resulting anemia. Therefore, the assays of iron, total iron binding capacity and other assessments of iron compounds in the body are clinically significant. Iron-storage compounds in the body include hemoglobin, hemosiderin, myoglobin and the cytochromes. In most tissues, ferritin is a major iron-storage protein. Human ferritin has a molecular weight of approximately 450,000 daltons, and consists of a protein shell around an iron core; each molecule of ferritin may contain as many as 4,000 iron atoms. Under normal conditions, this may represent 25% of the total iron found in the body. In addition, ferritin can be found in several isomers. High concentrations of ferritin are found in the cytoplasm of the reticuloendothelial system, the liver, spleen and bone marrow. Methods previously used to measure iron in such tissues are invasive, cause patient trauma and lack adequate sensitivity. The measurement of ferritin in serum is useful in determining changes in body iron storage, and is non-invasive with relatively little patient discomfort. Serum ferritin levels can be measured routinely and are particularly useful in the early detection of iron-deficiency anemia in apparently healthy people.

Serum ferritin measurements are also clinically significant in the monitoring of the iron status of pregnant women, blood donors, and renal dialysis patients. High ferritin levels may indicate iron overload without apparent liver damage, as may be noted in the early stages of idiopathic hemochromatosis. Ferritin levels in serum have also been used to evaluate clinical conditions not related to iron storage, including inflammation, chronic liver disease, and malignancy. The Ferritin Enzyme Immunoassay Test Kit provides a rapid, sensitive and reliable assay. The antibodies developed for the test will determine a minimal concentration of human ferritin of 5 ng/mL. There is minimal cross-reactivity with human serum albumin, alpha-fetoprotein, human hemoglobin, human transferrin, and ferric chloride.

**Materials and components**

- Antibody-coated microtiter plate with 96 wells.
- Reference standard set, contains 0, 10, 50, 100, 400, and 800 ng/mL (liquid, ready to use) or lyophilized form.
- Enzyme conjugate reagent, 12 mL.
- TMB Substrate, 12 mL.
- Stop Solution, 12 mL.
- Wash Buffer Concentrate (50X), 15 mL.

**Materials required but not provided:**
- Precision pipettes: 0.05–0.2 mL and 1.0 mL.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter well reader.

**Specimen collection and preparation**

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

**Storage of test kits and instrumentation**

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10mm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

**Reagent preparation**

1. All reagent should be brought to room temperature (18-22°C) before use.
2. If reference standards are lyophilized, reconstitute each Standard with 0.5 mL distilled water. Allow the reconstituted Material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.

**Example of standard curve**

Results of typical standard run with optical density reading at 450nm shown in the Y-axis against Ferritin concentrations shown in the X-axis.

<table>
<thead>
<tr>
<th>Ferritin (ng/mL)</th>
<th>Absorbance (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.003</td>
</tr>
<tr>
<td>10</td>
<td>0.093</td>
</tr>
<tr>
<td>50</td>
<td>0.401</td>
</tr>
<tr>
<td>100</td>
<td>0.714</td>
</tr>
<tr>
<td>400</td>
<td>1.995</td>
</tr>
<tr>
<td>800</td>
<td>2.963</td>
</tr>
</tbody>
</table>

**Calculation of results**

Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples. Constructed a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/mL on a graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of Ferritin in ng/mL from the standard curve.
### Concentrations

<table>
<thead>
<tr>
<th>Level</th>
<th>Replicates</th>
<th>Mean (ng/mL)</th>
<th>S.D.</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level I</td>
<td>20</td>
<td>326.28</td>
<td>15.20</td>
<td>4.66</td>
</tr>
<tr>
<td>Level II</td>
<td>32</td>
<td>20</td>
<td>189.08</td>
<td>26.52</td>
</tr>
</tbody>
</table>

### Male and Female Numbers

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number</th>
<th>Mean (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>80</td>
<td>170.0</td>
</tr>
<tr>
<td>Female</td>
<td>90</td>
<td>71.0</td>
</tr>
</tbody>
</table>

### Performance characteristics

1. **Accuracy:** Comparison between Our kits and commercial Available Kits provide the following data

   - N = 115
   - Correlation Coefficient = 0.990
   - Slope = 0.817
   - Intercept = -3.74
   - Mean (Our) = 243.4

2. **Precision:**

   - **Intra-Assay:**
     - Sample A
       - Undiluted: 186.94
       - 2x: 93.47
       - 4x: 46.74
       - 8x: 23.37
       - 16x: 11.69
       - 32x: 5.85
     - Average Recovery: 103.2%
   - **Inter-Assay:**
     - Sample B
       - Undiluted: 146.25
       - 2x: 73.13
       - 4x: 36.57
       - 8x: 18.29
       - 16x: 9.15
       - 32x: 4.58
     - Average Recovery: 103.2%

4. **Recovery**

   Various patient serum samples of known ferritin levels were mixed and assayed in duplicate. The average recovery was 98.1%.

   - **Expected Concentration**
   - **Observed Concentration**
   - **% Recovery**
     - 5.07: 5.05
     - 9.30: 8.79
     - 21.27: 20.11
     - 43.13: 48.03
     - 85.34: 90.00
     - 168.60: 174.06

   - Average Recovery: 98.1%

5. **Sensitivity**

   The minimum detectable concentration of this assay is Estimated to be 5.0 ng/mL.

6. **Cross-reactivity**

   - The following human materials were tested for crossreactivity Of the assay:
     - **Antigens**
     - **Concentration**
     - **Equivalent Ferritin**
       - Human Serum Albumin: 10.0 g/dL, 0.0 ng/mL
       - Human AFP: 8.000 ng/mL, 0.0 ng/mL
       - Ferric Chloride: 100.0 mg/dL, 0.0 ng/mL
       - Human Transferrin: 100.0 mg/dL, 0.0 ng/mL
       - Human Hemoglobin: 500.0 mg/dL, 0.0 ng/mL

7. **Hook Effect**

   No hook effect was observed up to 12,000 ng/mL ferritin in This assay.

### Limitations of the Procedure

There are some limitation of the assay. We should let our customers know about that.

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee to eliminate all the effects of that.

3. The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

### References

2. Valberg, L. CMAJ. 122:1240; 1980
5. Smimes, M.A.; Addiego, Jr.J.E. and Dallman, P.R. Blood. 43:581; 1974

7/2014