Product Information

Ferritin ELISA Kit

Catalog Number SE120155
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
Human ferritin has a molecular mass of ~450,000 Da 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 were invasive, caused patient trauma, and lacked 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 ELISA Kit is intended for the quantitative measurement of ferritin in human serum and plasma.

The Ferritin ELISA Kit is a solid phase sandwich assay method based on a streptavidin-biotin principle. The standards, samples, and the biotinylated anti-ferritin antibody reagent are added into designated wells, coated with streptavidin. Endogenous ferritin in the serum binds to the antigenic site of the biotinylated anti-ferritin antibody. Simultaneously, the biotinylated antibody is immobilized onto the wells through the high affinity streptavidin-biotin interaction. Unbound protein and excess biotin conjugated antibody are washed off by the wash buffer.

Upon the addition of the peroxidase (HRP) conjugated anti-ferritin antibody reagent, a sandwich complex is formed, the analyte of interest being in between the two highly specific antibodies, labeled with biotin and HRP. Unbound excess enzyme conjugated antibody reagent is washed off by wash buffer. Upon the addition of the substrate, the intensity of color developed is directly proportional to the concentration of ferritin in the samples. A standard curve is prepared relating color intensity to the concentration of the ferritin.

Components

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with Streptavidin</td>
<td>12 × 8 × 1</td>
</tr>
<tr>
<td>Ferritin Standards: 6 vials (ready to use)</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Anti-Ferritin Biotin Reagent (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Anti-Ferritin Enzyme Reagent (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>20× Wash concentrate: 1 bottle</td>
<td>25 ml</td>
</tr>
</tbody>
</table>

Reagents and Equipment Required but Not Provided.
- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.
Preparation Instructions
Sample Preparation
1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at 2–8 °C for 5 days. If storage time exceeds 5 days, store frozen at –20 °C for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.
   Note: Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

20x Wash Buffer Concentrate
Prepare 1x Wash buffer by adding the contents of the bottle (25 ml, 20x) to 475 ml of distilled or deionized water. Store at room temperature (18–26 °C).

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, controls, and serum samples be run in duplicate.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Storage/Stability
Store the kit at 2–8 °C.

Procedure
1. Place the desired number of coated strips into the holder.
2. Pipette 25 µl of Ferritin standards, control, and samples into appropriate wells.
3. Add 100 µl of Biotin reagent into each well. Shake the plate 10–30 seconds to mix the reagents.
4. Cover the plate and incubate for 30 minutes at room temperature (18–26 °C).
5. Remove liquid from all wells. Wash wells 3 times with 300 µl of 1x wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of Enzyme Reagent into each well.
7. Cover the plate and incubate for 30 minutes at room temperature (18–26 °C).
8. Remove liquid from all wells. Wash wells 3 times with 300 µl of 1x wash buffer. Blot on absorbent paper towels.
9. Add 100 µl of TMB Substrate to all wells.
10. Incubate for 15 minutes at room temperature (18–26 °C).
11. Add 50 µl of Stop Solution to all wells. Shake the plate gently to mix the solution.
12. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the Stop Solution.
Results
Calculations
The standard curve is constructed as follows:
1. Check Ferritin standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for the Ferritin standards (vertical axis) versus the Ferritin standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

Example of a Standard Data

<table>
<thead>
<tr>
<th>Std</th>
<th>OD 450 nm</th>
<th>Concentration ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1</td>
<td>0.019</td>
<td>0</td>
</tr>
<tr>
<td>Std 2</td>
<td>0.144</td>
<td>10</td>
</tr>
<tr>
<td>Std 3</td>
<td>0.531</td>
<td>50</td>
</tr>
<tr>
<td>Std 4</td>
<td>1.197</td>
<td>150</td>
</tr>
<tr>
<td>Std 5</td>
<td>1.987</td>
<td>400</td>
</tr>
<tr>
<td>Std 6</td>
<td>2.543</td>
<td>800</td>
</tr>
</tbody>
</table>

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Expected Values
It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:

Men: 20–350 ng/ml
Women: 10–200 ng/ml

References

GA,KK,MAM 11/15-1