Ferritin ELISA

Catalog Number: FER31-K01
1 x 96 Wells
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
**Intended Use:**

The Eagle Biosciences Ferritin ELISA assay kit is intended for the quantitative determination of ferritin in serum or plasma by enzyme linked immunoassay (ELISA). The Ferritin ELISA assay kit is for research use only and not to be used in diagnostic procedures.

**Assay Background:**

The serum ferritin concentration is proportional to the amount of iron in stores in the human body. The measurement of serum ferritin can help differentiate between the anemia caused by iron deficiency and other forms of anemia\(^1,2,3\). Studies have demonstrated that serum ferritin concentration is a useful, noninvasive screening test for iron overload, which may allow the detection of idiopathic hemochromatosis in the precirrhotic stages\(^4\).

**Principle of Procedure:**

The Ferritin ELISA assay kit for the quantitative determination of serum ferritin is basically a two stage reaction:

**STAGE 1:** The binding of human serum ferritin to a solid phase antihuman ferritin, and the simultaneous binding of the purified antihuman ferritin conjugated with alkaline phosphatase to the insoluble immune-complex.

**STAGE 2:** Reaction of alkaline phosphatase with a substrate solution consisting of phenylphosphate disodium and 4-amino-antipyrine. Following the addition of potassium ferricyanide a color develops, the optical density (490 - 510nm) of which is directly proportional to the ferritin concentration in the sample.

The intensity of the color developed during the 2nd Stage incubation is directly proportional to both time and temperature; therefore, an increase in the 2nd Stage incubation time will increase the intensity of the color, while a decrease in the 2nd Stage incubation time will decrease the intensity of the color. The same applies for an increase or decrease in the incubation temperature.
**Materials Provided:**

The expiration date for the Ferritin ELISA assay kit and each component is stated on the label(s). Store all components at 2-8°C.

**Prediluted Ferritin Calibrator Solutions:** 6 vials containing 0.3ml human spleen ferritin calibrated to concentrations of 6, 20, 60, 200, 600, and 2000 ng/ml, against WHO reference material (94/572), in phosphate buffered saline with rabbit serum and sodium azide as a preservative. Store at 2 - 8C. Do Not Use After Expiration Date on Vial.

**Solid Phase Antihuman Ferritin:** 96 microwells ("wells"), in the form of eight 1 X 12 strips, coated with rabbit antihuman spleen ferritin. Stored in a bottle containing borate buffer with bovine serum albumin, rabbit serum, and sodium chloride with sodium azide as a preservative. Store at 2 - 8C. Do Not Use After Expiration Date on Bottle.

**Sample Diluting Buffer:** 1 bottle containing 20 ml of phosphate buffered saline with rabbit serum and sodium azide as a preservative. Store at 2 - 8C. Do Not Use After Expiration Date on Bottle.

**Conjugated Antihuman Ferritin:** 1 bottle containing 23 ml of alkaline phosphatase conjugated rabbit antihuman spleen ferritin dissolved in 0.15 M phosphate buffered saline with 5% normal rabbit serum, and sodium azide as a preservative. Store at 2 - 8C. Do Not Use After Expiration Date on Bottle.

**Substrate Solution:** 1 bottle containing 23 ml of phenylphosphate disodium, 4-amino-antipyrine in 10% diethanolamine with sodium azide as a preservative. Store at 2 - 8C. Do Not Use After Expiration Date on Bottle.

**Potassium Ferricyanide:** 1 bottle containing 15 ml of potassium ferricyanide (0.24%) in water. Store at 2 - 8C. Do Not Use After Expiration Date on Bottle.

**Well Holder:** 1 micro well holder.

*NOTE: The Prediluted Ferritin Calibrator Solutions contained in this kit were calibrated against the 3rd International Standard for recombinant human ferritin established by the Expert Committee on Biological Standardization of the World Health Organization (coded 94/572).*
Materials required but not provided

- Deionized or distilled water.
- Precision pipettes capable of delivering volumes of 10, 100, and 200 µl.
- Microplate Reader capable of reading a red color at 500 ± 10nm (Optional: a background correction filter set at 600 to 630 nm).
- Clinical rotator table or vibrator table.

Precautions:

- The Ferritin ELISA Kit is for research use only and not to be used in clinical, therapeutic or diagnostic procedures.
- Reagents in this Ferritin ELISA kit contain sodium azide. Contact with copper or lead drain pipes may result in the formation of explosive azide deposits. It is important during disposal to flush drains with copious amounts of water to prevent azide accumulation. Plumbing that may be contaminated with azides can be flushed with 10 percent sodium hydroxide solution.
- Avoid splashing or generating aerosols.
- Follow Ferritin ELISA kit recommendations for incubation times and temperatures to avoid possibly erroneous results.
- Microbial contamination of reagents may cause erroneous results.
- Do not use reagents of the Ferritin ELISA kit with those from other lots or manufacturers.
- Do not use Ferritin ELISA kit reagents after the expiration date.

Sample Preparation:

Collect 5 ml of venous blood aseptically. Allow the blood to coagulate and separate the serum from the clot by centrifugation. Plasma may also be used for ferritin analysis. Moderate hemolysis will not interfere with the assay. If the assay will be performed within 7 days, store the serum refrigerated. If more than 7 days will elapse before the test is performed, the serum specimen should be frozen. Serum specimens may be stored frozen for 4 months without change in the ferritin content.
Assay Procedure:

Determine the number of Solid Phase Antihuman Ferritin micro wells needed for the assay (each strip contains 12 wells) and remove any unnecessary wells before proceeding with step 1. Replace unused wells in the bottle of buffer and store at 2-8°C. Allow all reagents, sera, and samples to reach room temperature before performing assay.

1. Remove the appropriate number of micro wells from the Solid Phase Antihuman Ferritin bottle, place in the Well Holder, and shake dry.
2. Beginning with micro well C1 (skip wells A1 and B1), pipette 10ul of each Prediluted Ferritin Calibrator Solution and sample, in duplicate, into separate wells. Micro wells A1 and B1 measure non-specific binding (NSB) and will contain only the Conjugated Antihuman Ferritin.
3. Pipette 200 µl of Conjugated Antihuman Ferritin into all micro wells.
4. Incubate on a vibrator or clinical rotator table, set at 180 -200 rpm, for 2 hours at room temperature.
5. Wash with deionized water by filling each micro well with water and shaking to decant. Repeat 3 times. After the final wash, tap the tops of the micro wells on absorbent material for about 30 seconds to drain.
6. Pipette 200 µl of the Substrate Solution into each well.
7. Incubate for 30 minutes at room temperature.
8. Develop the color by adding 100µl of the 0.24% Potassium Ferricyanide to each micro well and mix thoroughly (eg 1 minute at 180 - 200 rpm).
9. Zero the Microplate Reader with a blank prepared with 200 µl of the Substrate Solution and 100 µl of the Potassium Ferricyanide.
10. Read the absorbance of all samples at 500 ± 10nm. If possible, use a correction wavelength of 600 -630nm and record the net values. Readings should be taken within one hour of the completion of the assay.
11. If automatic background subtraction is not available, it is suggested that the plate be read a second time at 600 - 630nm and these values be manually subtracted from the initial 500nm readings. Failure to
compensate for the background absorbance may increase the variability of the assay and result in potentially erroneous values.

12. Calculate the results. (See CALCULATIONS section below.)

Calculations:

The serum concentration of serum ferritin can be calculated using a logit log data reduction program. Construct a calibration curve by plotting the net absorbance values obtained for each Prediluted Ferritin Calibrator Solution (mean O.D. of Calibrator minus NSB) on the vertical axis (Y) and the corresponding ferritin concentration in ng/ml on the horizontal axis (X). Calculate the average absorbance value for each control and sample. Determine the location of the average absorbance value on the Y-axis. Follow this point horizontally until it intersects the calibration curve. Follow this point of intersection with the curve vertically until it intersects the X-axis. This X-axis value will be the serum ferritin concentration of the control or sample.

For maximum precision, samples in excess of 1000 ng/ml should be diluted as follows:

1. Dilute the sample 1:10 with Sample Diluting Buffer.
2. Further dilutions may be made if necessary.
3. Re-assay these dilutions using the standard assay procedure and multiply the results by the dilution factor.

Typical Data:

<table>
<thead>
<tr>
<th>Description</th>
<th>Mean O.D.</th>
<th>Minus NSB</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSB</td>
<td>0.0085</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Standard 1</td>
<td>0.0375</td>
<td>0.0295</td>
<td>6</td>
</tr>
<tr>
<td>Standard 2</td>
<td>0.1000</td>
<td>0.0915</td>
<td>20</td>
</tr>
<tr>
<td>Standard 3</td>
<td>0.2635</td>
<td>0.2550</td>
<td>60</td>
</tr>
<tr>
<td>Standard 4</td>
<td>0.6690</td>
<td>0.6605</td>
<td>200</td>
</tr>
<tr>
<td>Standard 5</td>
<td>1.242</td>
<td>1.2335</td>
<td>600</td>
</tr>
</tbody>
</table>
### Expected Values

Normal values of ferritin are age and sex dependent. Studies have shown that serum ferritin concentrations greater than 300 ng/ml may indicate increased iron stores as seen in idiopathic hemochromatosis. The serum ferritin concentration reflects the amount of iron in stores. In iron deficiency, the stores are gone and the serum ferritin is very low (less than 20 ng/ml).

In other kinds of anemia, iron stores are higher than normal and serum ferritin values are usually over 100 ng/ml. Values between 20 and 100 ng/ml in anemic samples may suggest a combination of iron deficiency with some cause of anemia.

### Sensitivity:

Sensitivity is defined as the smallest value of ferritin which can be distinguished from the zero standard with a 95% confidence limit (± two standard deviations). Using the 10µl sample size specified in the assay procedures, the smallest concentration of serum ferritin that can be distinguished from zero is 0.59 ng/ml.

### Intra-Assay Variability:

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th># of RUNS</th>
<th>MEAN ng/ml</th>
<th>+/- 1 SD</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>10.4</td>
<td>0.6</td>
<td>5.80%</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>54.5</td>
<td>2.87</td>
<td>9.60%</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>208</td>
<td>9.69</td>
<td>4.70%</td>
</tr>
</tbody>
</table>
Inter-Assay Variability:

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th># of RUNS</th>
<th>MEAN ng/ml</th>
<th>+/- 1 SD</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>11</td>
<td>0.74</td>
<td>6.80%</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>55.7</td>
<td>4.3</td>
<td>7.70%</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>219</td>
<td>9.2</td>
<td>8.70%</td>
</tr>
</tbody>
</table>

Limitations of the Procedure:

- Moderate hemolysis has no effect upon the reproducibility or accuracy of the Ferritin ELISA assay kit.
- Iron administration causes the serum ferritin to increase.
- Anticoagulants have not been shown to influence the test so long as they do not result in dilution of the plasma.
- Strict adherence to precise laboratory procedure is essential for maximum accuracy of the final results.

Bibliography:


6. Alfrey, C.P.: Serum Ferritin Assay. CRC Critical Reviews in Laboratory Sciences, 179, November 1978


Warranty Information

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.