HUMAN FERRITIN

ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 10601

Enzyme Immunoassay for the Quantitative Determination of Human FerritinConcentration in Human Serum

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, myoglobulin and the cytochromes. In most tissues, ferrrtin 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, ferrritin 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-reativity with human serum albumin, alpha-fetoprotein, human hemoglobin, human transferrin, and ferric chloride.

Principle of the test

The Ferritin Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-ferritin antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minute incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of ferritin is directly proportional to the color intensity of the test sample.

Materials and components

Materials provided with the test kits:

- 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. (NIBSC-WHO 80/602, human liver standard)
- Enzyme conjugate reagent, 12 mL.
- TMB Substrate, 12 mL.
- Stop Solution, 12 mL.
- Wash Buffer Concentrate(50X),15mL

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 expiring date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm 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 0.5mL distilled water. Allow the reconstituted Material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.

Assay procedures

1. Secure the desired number of coated wells in the holder.
2. Dispense 20µL of standard, specimens, and controls into appropriate wells.
3. Dispense 100µL of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this step.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with washing Buffer(1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µL of TMB substrate into each well. Gently mix for 5 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 100µL of Stop Solution to each Well.
12. Gently mix for 30 seconds. It is important to make sure that All the blue color changes to yellow color completely.
13. Read optical density at 450nm with a microtiter reader within 15 minutes.

Important Note

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances readings.

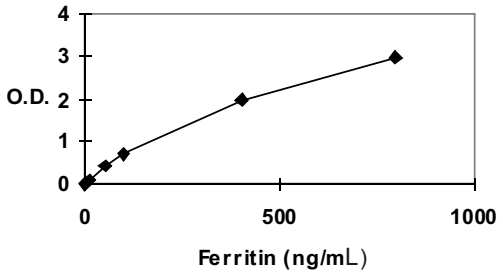
Calculation of results

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 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.

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.

Ferritin (ng/mL)	Absorbance (450nm)
0.0	0.003
10	0.093
50	0.401
100	0.714
400	1.995
800	2.963



This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Expected values and sensitivity

Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on a limited number of healthy adult blood specimens. The minimal sensitivity of the test is 5.0 ng/mL.

	Male	Female
Number	80	90
Mean (ng/mL)	170.0	71.0
Mean (ng/mL)	32.0-501.0	10-223.5

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) = 195.1
Mean (Bio-Rad) = 243.4

2.Precision.
1]. Intra-Assay:

Concentrations	Replicates	Mean	S.D.	% CV
Level	20	326.28	15.20	4.66
Level I	20	189.08	10.41	5.51
LevelII	20	26.52	1.72	6.49

2]. Inter-Assay:

Concentrations	Replicates	Mean	S.D.	% CV
Level	20	332.83	20.46	6.15
Level I	20	192.18	14.33	7.46
LevelII	20	24.24	3.58	14.73

3.Linearity
Two patient sera were serially diluted with 0 ng/mL standard in a linearity study. The average recovery was 101.0 %.

Sample A			
Dilution	Expected	Observed	% Recov.
Undiluted	186.94	186.94	
2x	93.47	94.16	100.7
4x	46.74	48.76	104.3
8x	23.37	24.53	105.0
16x	11.69	12.34	105.6
32x	5.85	5.87	100.3
Average Recovery: 103.2 %			

Sample B			
Dilution	Expected	Observed	% Recov.
Undiluted	146.25	146.25	
2x	73.13	76.51	104.6
4x	36.57	37.50	102.5
8x	18.29	18.01	98.5
16x	9.15	8.73	95.5
32x	4.58	4.27	93.2
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	99.6
9.30	8.79	94.5
21.27	20.11	94.5
43.13	48.03	89.7
85.34	90.00	105.5
166.60	174.06	104.4
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

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