

**Human Ferritin
ELISA Kit Protocol**

(Cat. No.:EK-310-25)

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INTENDED USE

For the quantitative determination of Human Ferritin concentration in human serum. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

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INTRODUCTION AND PROTOCOL OVERVIEW

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.

PRINCIPLE OF THE TEST

The Ferritin Quantitative Test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horse-radish 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 45-minute incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, 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.

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

STORAGE

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 described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement. **DO NOT FREEZE**

LIST OF COMPONENTS

Materials Provided with the Kit:

- Antibody coated microtiter plate with 96 wells.
- Enzyme Conjugate Reagent, 13 ml.
- Ferritin reference standards, containing 0, 15, 80, 250, 500, and 1,000 ng/ml, (NIBSC-WHO 80/602, human liver standard) liquid, 0.5 ml, ready to use.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes: 20 µl, 100 µl and 1.0 ml
- Distilled water
- Disposable pipette tips
- Vortex mixer or equivalent
- Absorbent paper or paper towel
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater
- Graph paper

SPECIMEN COLLECTION AND PREPARATION

1. 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.
2. **Samples with expected values greater than 1,000 ng/mL (e.g. dialysis patients) should be diluted with Zero Standard prior to assaying. A 1:10 initial dilution is recommended.**

REAGENT PREPARATION

All reagents should be allowed to reach room temperature (18-25°C) before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 20 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Gently mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into sink.
7. Rinse and flick the microtiter wells 5 times with **distilled or deionized water.** (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 100 µl 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 the optical density at 450 nm with a microtiter plate reader **within 15 minutes.**

CALCULATION OF RESULTS

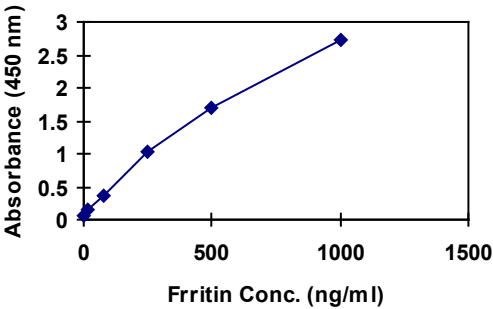
1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of AFP in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y-axis against Ferritin concentrations shown in the X-axis. 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 standard curve and patient data in each experiment.

Ferritin Conc. (ng/ml)	Absorbance (450 nm)
0	0.074
15	0.150
80	0.362
250	1.017
500	1.699
1000	2.728

Each laboratory must establish its own normal ranges based on patient population.



EXPECTED VALUES AND SENSITIVITY

The information provided below is cited from Reference #6:

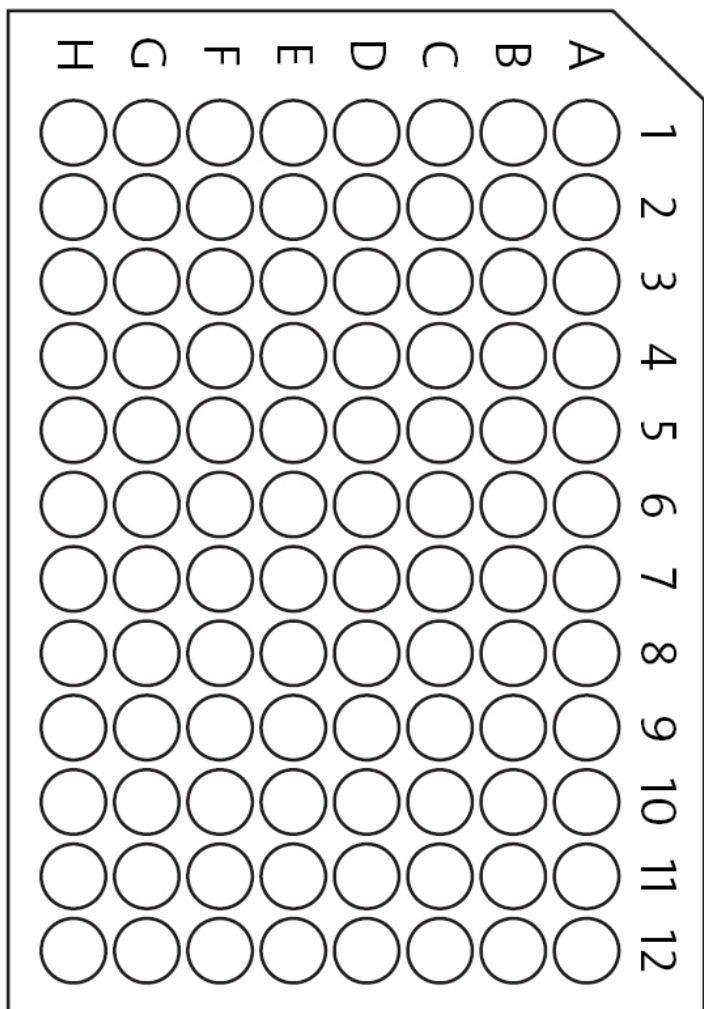
Male	20-250 ng/ml
Female	10-120 ng/ml
Children 6 mon. to 15 yr.	7-140 ng/ml
Infants, 2-5 month	50-200 ng/ml
Infants, 1 month	200-600 ng/ml
Newborn	25-200 ng/ml

The minimal detectable concentration of human ferritin by this assay is estimated to be 5 ng/ml.

REFERENCES

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ASSAY DIAGRAM



NOTES