

FERRITIN ENZYME IMMUNOASSAY TEST KIT**Catalog Number: BC-1025**

BioCheck, Inc.
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Enzyme Immunoassay for the Quantitative Determination of Ferritin in Human Serum

FOR IN VITRO DIAGNOSTIC USE

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

BioCheck Ferritin Enzyme Immunoassay

INTENDED USE

The BioCheck Ferritin ELISA is intended for the quantitative determination of ferritin in human serum. This assay is to be used in the diagnosis of diseases affecting iron metabolism.

INTRODUCTION

One of the most prevalent disorders of man is the dietary deficiency of iron and the resulting anemia.¹ Therefore, the assays of iron, total iron binding capacity, and other assessments of iron compounds in the body are clinically significant.²

Iron-storage compounds in the body include hemoglobin, hemosiderin, myoglobin, and the cytochromes. In most tissues, ferritin is a major iron-storage protein.³ 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.⁴ Under normal conditions, this may represent 25% of the total iron found in the body. In addition, ferritin can be found in several isomers.⁵

High concentrations of ferritin are found in the cytoplasm of the reticuloendothelial system, the liver, spleen, and the bone marrow.⁶ Methods previously used to measure iron in such tissues are invasive, cause patient trauma, and lack adequate sensitivity.⁷

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.^{8,9}

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.¹⁰ Ferritin levels in serum have also been used to evaluate clinical conditions not related to iron storage, including inflammation, chronic liver disease, and malignancy.¹¹

The Biocheck Ferritin Enzyme Immunoassay provides a rapid, sensitive, and reliable assay. The antibodies developed for the test will determine a minimal concentration of human ferritin of 5.0 ng/mL. There is minimal cross-reactivity with human serum

albumin, alpha-fetoprotein, human hemoglobin, human transferrin, and ferric chloride.

PRINCIPLE OF THE ASSAY

The BioCheck Ferritin Quantitative Test is based on the principle of a solid phase enzyme-linked immunosorbent assay.^{12,13,14} The assay system utilizes rabbit anti-ferritin for the solid phase (microtiter wells) immobilization and mouse monoclonal anti-ferritin 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 45 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of 3,3',5,5'-Tetramethylbenzidine (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 1N HCl, and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of ferritin is directly proportional to the color intensity of the test sample.

REAGENTS AND MATERIALS PROVIDED

1. Antibody-Coated Wells (1 plate, 96 wells)
Microtiter wells coated with rabbit anti-ferritin.
2. Enzyme Conjugate Reagent (13 mL)
Contains mouse monoclonal anti-ferritin conjugated to horseradish peroxidase.
3. Reference Standard Set (1 mL/vial)
Contains 0, 15, 80, 250, 500, and 1000 ng/mL human liver or spleen ferritin in bovine serum with preservatives. 1 mL each, liquid, ready to use.
4. TMB Reagent (One-Step) (1 bottle, 11 mL)
Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
5. Stop Solution (1N HCl) (1 bottle, 11 mL)
Contains diluted hydrochloric acid.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes: 0.02, 0.05, 0.1, 0.2 and 1 mL
3. Disposable pipette tips
4. Glass tube or flask for preparing TMB solution
5. Microtiter well reader capable of reading absorbance at 450nm.
6. Vortex mixer, or equivalent
7. Absorbent paper
8. Graph paper
9. Quality control material (e.g., BioRad Lyphochek Control sera)

WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.¹⁵
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
6. Do not pipette reagents by mouth.
7. The reference standards and sample diluent contain sodium azide (NaN₃) which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of these materials always flush with large volumes of water to prevent azide build-up.
8. For in vitro diagnostic use.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

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. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25 °C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Samples with expected values greater than 1000 ng/mL should be diluted with Zero Standard prior to assaying. A 1:10 initial dilution is recommended.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 20µL of standards, samples, and controls into appropriate wells.
3. Dispense 100µL of Enzyme Conjugate Reagent into each well.
4. Gently mix for 30 seconds. It is very important to have complete mixing.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking well contents into a suitable waste container.
7. Rinse the wells 5 times with distilled or dionized water. (Please do not use tap water.)
8. Strike the wells sharply on absorbent paper to remove residual water droplets.
9. Dispense 100 µL TMB Reagent 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 (1N HCl) into each well.
12. Gently mix for 5 seconds.
13. Read OD at 450nm with a microtiter well reader **within 15 minutes.**

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD₄₅₀) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on 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 ferritin in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further converted by the appropriate dilution factor.

PROCEDURAL NOTES

1. Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

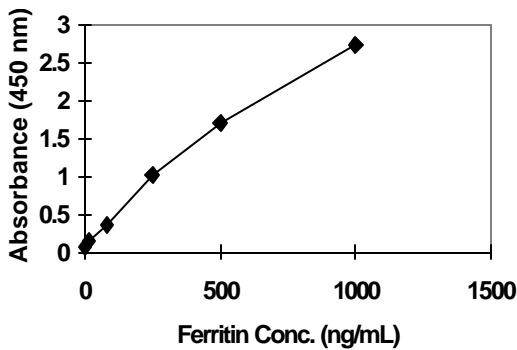
Abbott Mean = 165.4 ng/mL

EXAMPLE OF STANDARD CURVE

1. Results of a typical standard run of the assay are shown below.

| Ferritin (ng/mL) | Absorbance (450nm) |
|------------------|--------------------|
| 0 | 0.074 |
| 15 | 0.150 |
| 80 | 0.362 |
| 250 | 1.017 |
| 500 | 1.699 |
| 1000 | 2.728 |

2. This standard curve is for illustration only, and should not be used to calculate unknowns.



EXPECTED VALUES

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.

| | Male | Female |
|---------------|--------------|-------------|
| Number | 80 | 90 |
| Mean (ng/mL) | 170.0 | 71.0 |
| Range (ng/mL) | 32.0 – 501.0 | 3.5 – 223.5 |

PERFORMANCE CHARACTERISTICS

1. Accuracy

A statistical study using 98 healthy patient samples, ranging in ferritin concentration from 1 ng/mL to 831 ng/mL, demonstrated good correlation with a commercially available kit as shown below.

a. Comparison between the BioCheck Ferritin ELISA Test Kits and the Abbott AxSYM® Ferritin MEIA kit provided the following data:

N = 98
 Correlation coefficient = 0.999
 Slope = 0.993
 Intercept = 1.013
 BioCheck Mean = 165.5 ng/mL

2. Sensitivity

The minimum detectable concentration of the BioCheck Ferritin ELISA assay as measured by 2SD from the mean of a zero standard is estimated to be at least 5.0 ng/mL.

3. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of three different serum samples in one assay. Within-assay variability is shown below:

| Serum Sample | 1 | 2 | 3 |
|------------------------------|-------|-------|------|
| Number of Replicates | 24 | 24 | 24 |
| Mean Ferritin (ng/mL) | 341.6 | 231.3 | 40.0 |
| Standard Deviation | 12.2 | 12.1 | 1.4 |
| Coefficient of Variation (%) | 3.6% | 5.7% | 3.5% |

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of three different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

| Serum Sample | 1 | 2 | 3 |
|------------------------------|-------|-------|------|
| Number of Replicates | 24 | 24 | 24 |
| Mean Ferritin (ng/mL) | 340.1 | 220.6 | 37.3 |
| Standard Deviation | 14.3 | 11.3 | 2.5 |
| Coefficient of Variation (%) | 4.2% | 5.1% | 6.6% |

4. Recovery and Linearity Studies

a. Recovery

Various patient serum samples of known ferritin levels were combined and assayed in duplicate. The mean recovery was 98.0%.

| | Expected Concentration (ng/mL) | Observed Concentration (ng/mL) | % Recovery |
|--|--------------------------------|--------------------------------|--------------------|
| | 744.4 | 745.1 | 100.1% |
| | 350.1 | 341.6 | 97.6% |
| | 165.8 | 156.0 | 94.1% |
| | 84.9 | 82.5 | 97.2% |
| | 39.3 | 38.9 | 99.1% |
| | 20.4 | 19.4 | 95.2% |
| | 10.6 | 10.2 | 96.1% |
| | | | Mean: 97.1% |
| | 753.7 | 765.6 | 101.6% |
| | 374.3 | 371.1 | 99.1% |
| | 182.9 | 177.3 | 96.9% |
| | 91.0 | 94.5 | 103.9% |
| | 46.7 | 46.5 | 99.4% |
| | 24.1 | 23.3 | 96.3% |
| | 12.3 | 11.7 | 95.2% |
| | | | Mean: 98.9% |

b. *Linearity*

Three patient samples were serially diluted to determine linearity. The mean recovery was 103.7%.

| # | Dilution | Expected Conc. (ng/mL) | Observed Conc. (ng/mL) | % Expected |
|----|-----------|------------------------|------------------------|---------------|
| 1. | Undiluted | ----- | 672.9 | ----- |
| | 1:2 | 336.5 | 310.1 | 92.2% |
| | 1:4 | 168.2 | 159.3 | 94.7% |
| | 1:8 | 84.1 | 84.6 | 100.5% |
| | 1:16 | 42.1 | 43.0 | 102.3% |
| | 1:32 | 21.0 | 22.1 | 105.1% |
| | 1:64 | 10.5 | 11.6 | 110.3% |
| | | | | Mean = 100.9% |

PERFORMANCE CHARACTERISTICS

b. *Linearity*

| # | Dilution | Expected Conc. (ng/mL) | Observed Conc. (ng/mL) | % Expected |
|----|-----------|------------------------|------------------------|---------------|
| 2. | Undiluted | ---- | 828.1 | ---- |
| | 1:2 | 414.1 | 450.2 | 108.7% |
| | 1:4 | 207.0 | 223.5 | 108.0% |
| | 1:8 | 103.5 | 115.2 | 111.3% |
| | 1:16 | 51.8 | 53.9 | 104.2% |
| | 1:32 | 25.9 | 27.7 | 107.1% |
| | 1:64 | 12.9 | 15.0 | 116.4% |
| | | | | Mean = 109.2% |
| 3. | Undiluted | ---- | 423.8 | ---- |
| | 1:2 | 211.9 | 209.3 | 98.8% |
| | 1:4 | 106.0 | 102.5 | 96.7% |
| | 1:8 | 53.0 | 52.2 | 98.4% |
| | 1:16 | 26.5 | 26.8 | 101.0% |
| | 1:32 | 13.2 | 14.3 | 100.6% |
| | | | | Mean = 100.6% |

5. **Hook Effect**

No high dose hook effect is observed in this assay at ferritin levels up to 12,000 ng/mL.

6. **Specificity**

The following hormones were tested for cross-reactivity:

| HORMONE TESTED | CONCENTRATION | READ AS TOTAL FERRITIN (LIVER) IN SERUM (ng/mL) |
|----------------------------|---------------|---|
| <i>Human Serum Albumin</i> | 2.50 g/dL | 0 |
| | 5.00 g/dL | 0 |
| | 10.00 g/dL | 0 |
| <i>Alpha-Fetoprotein</i> | 1,000 ng/mL | 0.0 |
| | 4,000 ng/mL | 0.0 |
| | 8,000 ng/mL | 0.0 |

| | | |
|--------------------------|-----------|-----|
| <i>Human Hemoglobin</i> | 125 mg/dL | 0.0 |
| | 250 mg/dL | 0.0 |
| | 500 mg/dL | 0.0 |
| <i>Human Transferrin</i> | 1.0 mg/dL | 0.0 |
| | 10 mg/dL | 0.0 |
| | 100 mg/dL | 0.0 |
| <i>Ferric Chloride</i> | 1.0 mg/dL | 0.0 |
| | 10 mg/dL | 0.0 |
| | 100 mg/dL | 0.0 |

STANDARDIZATION

The Reference Standards are calibrated against the International Committee for Standardization in Hematology (ICSH) Expert Panel on Iron, human liver standard (NIBSC-WHO 80/602).

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges.

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 results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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