



## Ferritin MICRO-ELISA Test Kit

**Prod. No.:** T178  
**Pkg. Size:** 96 Tests

### Description

The MICRO-ELISA FERRITIN test is a solid phase sandwich-type enzyme immunoassay (ELISA) Diagnostic Kit for the *in vitro* quantitative determination of ferritin concentration in human serum.

### Summary and Explanation of the Test

Measurement of serum ferritin is made primarily to assess the level of body iron stores.<sup>1-6</sup> Serum ferritin levels correlate well with other direct measurements of iron stores like liver biopsy and microscopic examination of bone marrow aspirates for stainable iron deposits.<sup>1,2</sup> Indirect measurements of iron stores, such as serum iron, transferrin saturation (TIBC, UIBC and % saturation), and hemoglobin concentration do not reflect decreasing iron stores until the stores are exhausted.<sup>7</sup>

Serum ferritin measurements are used in the diagnosis of iron deficiency and excess.<sup>3-6</sup> It can be used to discriminate iron deficiency anemia from anemia due to other causes.<sup>8</sup> Virtually all patients with low serum iron and low ferritin have iron deficiency. In the interpretation of serum ferritin results it is important to note that ferritin is an acute phase reactant. A normal serum ferritin value cannot be used to exclude iron deficiency if a hepatic, malignant, or inflammatory condition is present.<sup>9-11</sup>

Elevated serum ferritin levels occur in hereditary hemochromatosis and other iron-overload conditions.<sup>3-6</sup> Moderate elevation of serum ferritin may reflect hepatic, malignant, or inflammatory conditions.<sup>9-11</sup>

The highest concentrations of ferritin are in the cells of the liver and the RE system, where ferritin serves as a storehouse for surplus iron scavenged from erythrocytes. Ferritin is a high-molecular weight protein (460,000 daltons) that contains approximately 20% iron. The protein shell consists of 24 subunits with variable combinations of two types of subunits, the H (heart) and L (liver) subunits. The charge heterogeneity of the various tissue isoforms is related to their subunit composition. Storage of iron within the protein shell of the ferritin molecule protects the body from the toxic effects of excess iron.<sup>12-14</sup>

### Principle of the Procedure

The MICRO-ELISA FERRITIN test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes two unique antibodies (a mouse monoclonal and a goat polyclonal) directed against distinct antigenic determinants on the ferritin molecule.

Specifically, plastic wells are coated with anti-ferritin (mouse monoclonal). With the addition of a test sample, calibrators or appropriate controls containing ferritin, immune complexes are formed between ferritin in the sample and the solid phase anti-ferritin. Anti-ferritin (goat polyclonal) enzyme-labeled with horseradish peroxidase is added to each well. During an incubation period (45 minutes at room temperature), the ferritin molecule is sandwiched between the solid phase and enzyme-labeled antibodies. The sample is then decanted and the wells are washed to remove unbound-labeled antibody. An enzyme substrate-chromogen (hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>, and tetramethylbenzidine, TMB) is added to the well and incubated for 15 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H<sub>2</sub>SO<sub>4</sub> stops the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of ferritin in the sample.

### Reagents

#### Components in Each 96-Test

#### Micro-Elisa Ferritin Diagnostic Kit

- 96 wells, FERRITIN ANTIBODY COATED WELLS: Coated with anti-ferritin (mouse monoclonal); contained in a pack with silica gel desiccant.
- 1 bottle, 27 ml, FERRITIN ENZYME Ab CONJUGATE: anti-ferritin (goat polyclonal) labeled with horseradish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C red # 40 as coloring agent.
- 1 bottle, 12 ml, SUBSTRATE-CHROMOGEN Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.
- 1 bottle, 12 ml, STOP SOLUTION 1 N H<sub>2</sub>SO<sub>4</sub>.
- 1 vial, 3 ml, 0 ng/ml FERRITIN CALIBRATOR/ SAMPLE DILUENT: Bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
- 3 vials, 0.5 ml, FERRITIN CALIBRATORS: Ferritin in bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. 30, 225 and 500 ng/ml.
- 1 bottle, 60 ml, WASH BUFFER CONCENTRATE (20X): Buffered detergent solution, contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. Dilute bottle to 1200 ml with deionized water.

### Additional Materials Required

Disposable tip precision pipets - 0.01, 0.10 and 0.25 ml.  
microtiter plate reader.



Distilled or deionized water.

## Storage and Stability

Store all components at 2°-8°C when not in use. The following components may be stored at ambient temperature: WELLS, SUBSTRATE-CHROMOGEN, WASH BUFFER and STOP SOLUTION. Expiration date printed on the kit indicates limits of stability.

The FERRITIN ANTIBODY COATED WELLS are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2° - 30°C.

## Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent contamination or deterioration.

## Instruments

Performance of the MICRO-ELISA FERRITIN test requires use of a precision microtiter plate reader with a wavelength of 450 ± 20 nm:

## Specimen Collection and Preparation

Serum samples are used in the MICRO-ELISA FERRITIN Diagnostic Kit procedure. No special preparation of the patient is necessary; fasting is not required. Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

**STORAGE:** Specimens may be stored in a tightly sealed tube at 2°-8°C for three days. If the serum is not assayed within 3 days, store frozen (-20°C) in a tightly sealed tube for up to 3 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

## Micro-ELISA Ferritin Procedure

### Reagent Preparation

Dilute bottle of WASH BUFFER CONCENTRATE (20X) solution to 1200 ml with deionized water. Diluted wash solution will be stable until the expiration date stamped on the kit.

### Preliminary Comments and Precautions

Patient sample may contain pathogens: treat all samples as potentially infectious.

Reagents contain thimerosal; avoid contact with skin.

Avoid contact with SUBSTRATE-CHROMOGEN (tetramethyl-benzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).

**CAUTION:** Source material used to prepare Calibrators and Controls was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. **HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS.** Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

### Procedural Notes

1. All test kit components used in an assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18°-30°C) before testing begins.
2. All calibrators, controls, and samples should be tested in duplicate simultaneously. The test samples and controls must be well mixed before use.
3. A separate disposable tip should be used for each sample to avoid cross-contamination. All pipetting steps should be performed with the utmost care and accuracy. Avoid contaminating the reagent pipette tip with the serum sample.
4. The duration of the incubation times must be the same for all wells within a run.
5. Run size should be limited to the number of samples that can be added to antibody coated wells within 5 minutes.
6. Samples should be pipetted to the bottom of the antibody coated wells.
7. If microtiter reader is not capable of reading absorbances greater than 2.0, the color should be read after a shorter incubation time with the SUBSTRATE/CHROMOGEN, i.e., 10 minutes.

## Test Procedure

1. Place sufficient COATED WELLS in a holder to run 0.0, 30, 225 and 500 ng/ml ferritin CALIBRATORS, Quality Control Sera and patient samples in duplicate. Limit run size to the number of samples that can be pipetted in 5 minutes.
2. Pipet 10 µl of the CALIBRATORS, Controls or Patient Sample to the corresponding COATED WELL.
3. Pipet or dispense 250 µl of the ENZYME ANTIBODY CONJUGATE solution to all the wells and mix gently.
4. Incubate at room temperature (18°-30°C) for 45 minutes ± 5 minutes.
5. Decant or aspirate and discard liquid contents of all wells. SLAP the inverted wells on a clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
6. Fill each well with diluted WASH BUFFER. Fill the wells to overflowing, you cannot cause any carry-over between the wells. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.



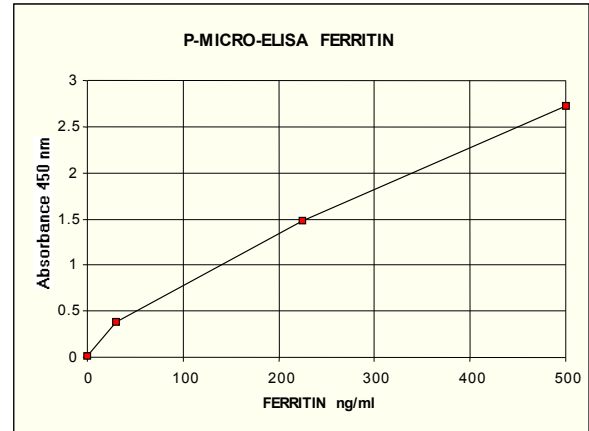
WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.

- 7. Repeat step 6 three more times (for a total of 4 washes).
8. Fill each well with deionized water. Fill the wells to overflowing. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
9. Pipet or dispense 100 µl (0.1 ml) of SUBSTRATE-CHROMOGEN solution into each well.
10. Mix thoroughly and incubate 15 minutes at room temperature (18°-30°C).
11. Pipet or dispense 100 µl (0.1 ml) of 1 N H2SO4 STOP SOLUTION into each well and mix thoroughly.
12. Read the absorbance of each well at 450nm ± 20 nm against water.

Calculation of Results

- 1. Calculate the mean value for each duplicate sample absorbance. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
2. Construct the standard curve by plotting the mean absorbance obtained for each FERRITIN CALIBRATOR on the vertical (Y) axis versus the corresponding FERRITIN concentration on the horizontal (X) axis, using rectilinear graph paper.
3. Connect the points with straight-line segments.
4. Using the mean absorbance for each sample, read the corresponding FERRITIN concentration in ng/ml from the curve. Multiply the value by the dilution factor if required.

EXAMPLE DATA
DO NOT USE IN PLACE OF CUREVE
DETERMINED AT THE TIME OF ASSAY.
Table with columns: Specimen I.D., A450, Mean A450, FERRITIN (ng/ml)
Rows include CALIBRATOR (0, 30, 225, 500 ng/ml) and SAMPLES (#1, #2, #3 UNKNOWN).



The range of this assay is 0 - 500 ng/ml. For specimen with ferritin concentrations beyond the standard curve (500 ng/ml), repeat the test by diluting the specimen with the 0 ng/ml CALIBRATOR. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor.

Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls containing azide can not be used. Two controls with normal and elevated values should be used. Pooled human serum or commercially available control sera without azide are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 ng/ml CALIBRATOR exceeds 0.060 it is an indication of careless washing and the assay must be repeated.

Standardization

The MICRO-ELISA FERRITIN CALIBRATORS have been standardized against the World Health Organization International Reference Preparation (1st IS 80/602).

Limitations of the Procedure

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.

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). These samples should not be assayed with



the MICRO-ELISA FERRITIN test as erroneous results may be obtained.<sup>21-23</sup> These conditions should be ruled out prior to clinical evaluation of test results.<sup>16-20</sup>

The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.

**FINAL REACTION STABILITY:** The spectrophotometric measurement should be made within 30 minutes after the addition of the H<sub>2</sub>SO<sub>4</sub> solution.

Samples with elevated levels of ferritin (up to 50,000 ng/ml) will always assay as >500 ng/ml when tested, and will not result in a "high dose hook effect". When it is necessary to measure levels of ferritin greater than the 500 ng/ml CALIBRATOR, the sample should be diluted with the 0 ng/ml CALIBRATOR and re-assayed.

**Expected Values**

Ferritin values were measured in serum samples from apparently normal individuals and the following results were obtained:

	N	Ferritin (ng/ml)	
		Geometric	Observed
		Mean	Value
ADULT FEMALE	152	54	4 – 760
Under 46 Years Old	40	24	5 – 257
Over 46 Years Old	112	72	4 – 760
ADULT MALE	124	138	11 – 785

These values are consistent with those reported in the literature. Values of 1-12 ng/ml are reported to be consistent with iron deficiency<sup>15</sup>. Values greater than 1350 ng/ml are reported to be consistent with iron overload<sup>1</sup>.

It is recommended that each laboratory determine its own normal range.

**Performance Characteristics of the Test**

**Assay Specificity**

The antibodies used in the MICRO-ELISA FERRITIN kit were selected to react with both liver and spleen ferritin. Cross-reactivity to human heart ferritin is less than 1%.

**Assay Sensitivity**

The sensitivity of this assay is defined as the smallest single value that can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty-one replicates at the zero concentration. The calculated sensitivity is <1.0 ng/ml.

**Assay Reproducibility**

Intra-assay reproducibility was determined by measurement of 20 replicates of three serum pools in a single run.

	Mean FERRITIN (ng/ml)	SD	%CV
Serum A	67.92	5.46	8.0
Serum B	199.62	9.69	4.9
Serum C	475.95	57.51	12.1

The interassay reproducibility was determined by duplicate measurement of three serum pools in nine separate runs.

	Mean FERRITIN (ng/ml)	SD	%CV
Serum I	23.84	1.36	5.7
Serum II	153.68	11.99	7.8
Serum III	525.74	63.61	12.1

**Assay Linearity**

A study was performed diluting a serum sample containing an elevated level of ferritin with the 0 ng/ml CALIBRATOR to determine the linearity of the MICRO-ELISA FERRITIN.

Dilution Factor	FERRITIN ng/ml		
	Expected Value	Observed Value	% of Expected Value
Undiluted	-	527	-
1:2	264	268	102 %
1:4	132	139	105 %
1:8	66	68	103 %
1:16	33	31	96 %
1:32	16	15	94 %

**Assay Recovery**

Three aliquots of human sera with a FERRITIN concentration of 19.9 ng/ml were spiked with 10, 25 and 100 µl of a human serum with a ferritin concentration 2550 ng/ml. The samples were assayed in duplicate.

Added Serum	FERRITIN ng/ml		
	Expected Value	Observed Value	% Recovery
2550 ng/ml			
0 µl	19.9		
10 µl	69.5	70.8	102 %
25 µl	140.3	150.4	107 %
100 µl	441.5	413.2	94 %

**Comparison to Other Ferritin Tests**

Correlation studies on a random group of 280 serum samples with a range of values from 3 - 833 ng/ml, were performed using the quantitative results from the MICRO-ELISA FERRITIN Test and another ferritin (IRMA) test. The correlation coefficient of the test results was 0.99.

	Slope	Y-Intercept	Correlation Coefficient
n= 280	0.989	-2.60	0.99



## References

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## Warranty

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