DRG® Ferritin (EIA-1872)

CE

REVISED 30 DEC. 2009 RM (VERS. 6.1) IVD

Please use only the valid version of the package insert provided with the kit.

FOR IN VITRO DIAGNOSTIC USE

Store at 2 °C to 8 °C.

I. INTENDED USE

The 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.

II. 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, myoglobulin, 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 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.

III. PRINCIPLE OF THE ASSAY

The 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.

IV. 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 (0.5 mL/vial)
   Contains 0, 15, 80, 250, 500, and 1,000 ng/mL human liver or spleen ferritin in bovine serum with preservatives. 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.

V. 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. Microtiter well reader capable of reading absorbance at 450nm.
5. Vortex mixer, or equivalent
6. Absorbent paper
7. Graph paper
8. Quality control material (e.g., BioRad Lyphochek Control sera)

VI. 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 and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.21
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.

5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. 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.
10. For in vitro diagnostic use.

VII.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. Opened kits retain activity for two months if stored at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

VIII.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°. Specimens held for a longer time should be frozen only once at -20° prior to assay. Thawed samples should be inverted several times prior to testing.
3. **Samples with expected values greater than 1,000 ng/mL (e.g. dialyisis patients) should be diluted with Zero Standard prior to assaying. A 1:10 initial dilution is recommended.**

IX. 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.

X. 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.

XI. ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 20 µl of standards, samples, and controls into appropriate well.
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.
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 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 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 (1N HCl) 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**.

XII.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.

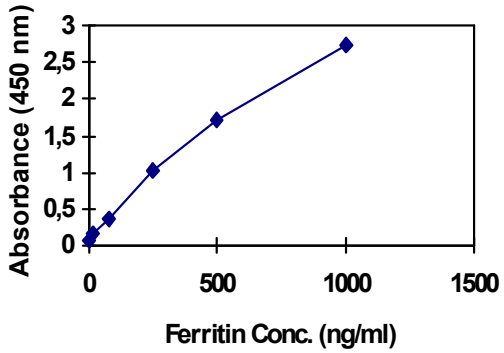
XIII.PROCEDURAL NOTES

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.

XIV.EXAMPLE OF STANDARD CURVE

Results of a typical standard run of the assay are shown below.
This standard curve is for illustration only, and should not be used to calculate unknowns.

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



XV.Expected Values and Sensitivity

Each laboratory must establish its own normal ranges based on patient population. The information provided below is cited from Reference #16:

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

XVI.Performance Characteristics

A. 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.

Comparison between the Ferritin ELISA (EIA-1872) 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
(EIA-1872) Mean = 165.5 ng/mL

Abbott Mean = 165.4 ng/mL

B. Sensitivity

The minimum detectable concentration of the Ferritn ELISA (EIA-1872) assay as measured by 2SD from the mean of a zero standard is estimated to be at least 5.0 ng/mL.

C. 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%

D. 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%
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%

E. Hook Effect

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

F. Specificity

The following hormones were tested for cross-reactivity:

HORMONE TESTED	CONCENTRATION	READ AS TOTAL FERRITIN (LIVER) IN SERUM (NGML)
Human Serum Albumin	2.50 g/dL 5.00 g/dL 10.00 g/dL	0 0 0
Alpha-Fetoprotein	1,000 ng/mL 4,000 ng/mL 8,000 ng/mL	0.0 0.0 0.0
Human Hemoglobin	125 mg/dL 250 mg/dL 500 mg/dL	0.0 0.0 0.0
Human Transferrin	1.0 mg/dL 10 mg/dL 100 mg/dL	0.0 0.0 0.0
Ferric Chloride	1.0 mg/dL 10 mg/dL 100 mg/dL	0.0 0.0 0.0

XVII.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).

XVIII.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.

XIX.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.

XX.References/Literature

1. Fairbanks, V. F. and Bentler, E., "Hematology," (Williams, Bentler, Erslev and Rundles, Ed.), McGraw-Hill, New York, 305-326 (1972).
2. Colltrell, D. B., Lab. Mgmt., (Feb. 1978).
3. Jacobs, A. and Worwood, M., *Prog. Hematol*., 9, 1 (1975).
4. Forman, D. T. and Vye, M. V., *Clin. Chem*., 26/1, 145-147 (1980).
5. Hazard, J. T., et. al., *J. Blood*, 49, 139 (1977).
6. Baur, J. D., Gradwohls, *Clin. Meth, and Diag*., 22, 1, 436 (1970).
7. Jacobs, A., *Fed. Proc*., 36, 7 (1977).
8. Schrier, S., "Hematology," in: Scientific American (Rubenstein, E. and Federman, D., eds). New York: Scientific American Inc., Chapter V (1980).
9. White, D., et. al., *Am. J. Clin. Path.* (Feb. 1986).
10. Valberg, L., CMA J., 122, 1240 (1980).
11. Forman, D. and Parker, S., *Ann. Clin. Lab. Sci*., 10, 345 (1980).
12. Engvall, E., Meth. In Enz., (VanVunakis, H. and Langone, J. J., eds.), Academic Press, New York, NY, 70, 419-492 (1980).
13. Uotila, M., Ruoslahti, E. and Engvall, E., J. Immunol. Methods, 42, 11 (1981).
14. Simes, M. A., Addiego, Jr. J. E. and Dallman, P. R., Blood, 43, 581 (1974).
15. USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", (1984).
16. Clinical Guide to Laboratory Tests. Ed. N.W. Tietz, 4th Ed., W.B. Saunders Company, 2006.