Ferritin

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for Quantitative Determination of Ferritin concentration in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Ferritin test is intended for the Quantitative Determination of Ferritin in Human Serum. For In Vitro Diagnostic Use Only.

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, 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.

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 ASSAY

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 standards and test specimen (serum) are added to the anti-ferritin antibody coated microtiter wells. Then anti-ferritin antibody labeled with horseradish peroxidase (conjugate) is added. If Ferritin is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed & bound enzyme is detected by adding substrate. The reaction is stopped after specified time with stop solution and absorbance is determined for each well using an ELISA reader. The concentration of Ferritin is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with anti-ferritin antibody.
- Enzyme Conjugate. Ready to use.
- TMB Substrate. Ready to use.
- Stop Solution. Ready to use.
- Ferritin Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).
- Control Set
- Pack Insert
- Plate Sealer
- Protocol Sheet
- Microwell Holder

Materials required but not provided

- Precision pipettes: 10µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **Ferritin** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to pink at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable for upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to Standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

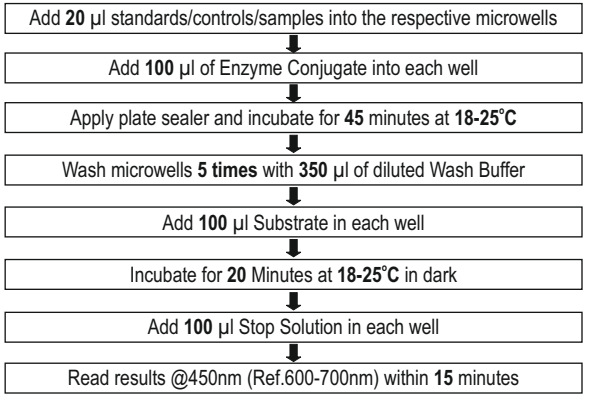
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens, standards and controls should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **20** µl of standards, controls and sera into the appropriate wells.
2. Dispense **100** µl of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for **45 minutes**.
3. After incubation, empty the microtitre wells and wash the plate **5 times** with **350 µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
4. Dispense **100** µl of TMB substrate into each well. Incubate at room temperature (18-25°C), in the dark, for **20 minutes**.
5. Stop the reaction by adding **100** µl of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
6. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.



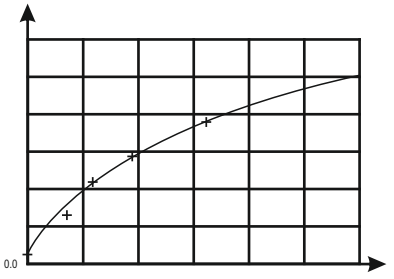
CALCULATION OF RESULTS

Construct a standard curve by plotting the absorbance obtained from each reference standard against its concentration in ng/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of Ferritin in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical standard run with optical density reading at 450nm (ref 600 – 700nm) shown in the Y axis against Ferritin concentrations shown in the X axis.

Ferritin (ng/ml)	Absorbance (450nm)
A	0.0083
B	0.0796
C	0.4204
D	0.6990
E	1.4296
F	1.8141



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

Expected Ranges of values

Each laboratory must establish its own normal ranges based on patient population. The results provided below are from the literatures, which are based on a limited number of healthy adult blood specimens.

Adult Males	16-220 ng/ml
Adult Females	10-124 ng/ml
Newborn	22-220 ng/ml
Children (6 months – 15 years)	7-140 ng/ml

The minimum detectable concentration of Ferritin by this assay is estimated to be 5 ng/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal Study **Ferritin** was evaluated against commercially available licensed kit with 90 random clinical samples and **Ferritin** has demonstrated 95% clinical correlation with the commercially available licensed kit.
2. Precision: **Ferritin** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with **Ferritin**	Coefficient of Variation (CV)
Level 1	10	75.44	3.36
Level 2	10	146.53	3.84
Level 3	10	324.40	5.21

B) External Evaluation:

Ferritin ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Ferritin** ELISA has demonstrated 95% correlation with the reference method.
*Data file: Orchid Biomedical Systems (P) Ltd.

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards, controls & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

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. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

1. White, D. ; Kramer, D.; Johnson, G.; Dick, F. And Hamilton, H. A.m. J. Clin. Path. 72: 346; 1986.
2. Valberg, L. CMAJ. 122:1240; 1980.
3. Forman, D. and Parker, S. Ann. Clin. Lab. Sci. 10: 345;1980.
4. Hazard, J.T.; Yokota, M.; Arosio, P. And Drysdale, J. Blood. 49:139; 1977.
5. Smimes, M.A.; Addiego, Jr.J.E. and Dallman, P.R. Blood. 43:581; 1974.
6. Data on file: Orchid Biomedical Systems (P) Ltd.

SYMBOL KEYS

	Temperature Limitation		Consult Instructions for use
	Manufacturer	IVD	*In vitro* Diagnostic Medical Device
	Use by	REF	Catalogue Number
	Date of Manufacture	LOT	Batch Number / Lot Number
	This side up	Σ	Contains sufficient for <n> tests
	Do not reuse		

Orchid Biomedical Systems (P) Ltd.*

M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.
Email id: sales@orchidbiomedical.com

***A Revvity Inc. Company**

0824/VER-01