REFERENCES

1. Polson RJ; Kenna JG; Shears IP; Bomford A; Williams R. Measurement of ferritin in serum by an indirect competitive enzyme-linked immunosorbant assay. Clin Chem 1988; 34(4):661-4.
2. Iacobello C; Ruggeri G; Signorini C; Di Cello GC; Albertini A. A semi-automated ELISA for serum ferritin determination. Clin Chem 1987; 32(5):899.
3. Aulbert E; Steffens O. Serum ferritin--a tumor marker in malignant lymphomas? Onkologie 1990; 13(2):102-8.
4. Essen A; Ozen H; Ayhan A; Ergen A; Tasar C; Remzi F. Serum ferritin: a tumor marker for renal cell carcinoma. J Urol 1990;145(6):1134-7.
5. Borque L; Rus A; del Cura J; Maside C; Escanero J. Automated quantitative nephelometric latex immunoassay for determining ferritin in human serum. J Clin Lab Anal 1992; 6(4):239-44.
6. Dawson DW; Fish DI; Shackleton P The accuracy and clinical interpretation of serum ferritin assays. Clin Lab Haematol 1992; 14(1):47-52.

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Cat#: FR248T (96 Tests)
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CALBIOTECH
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Ferritin ELISA

Catalog No. FR248T (96 Tests)

INTENDED USE

The Calbiotech, Inc. (CBI) Ferritin SA ELISA Kit is intended for the quantitative measurement of Ferritin in human serum or plasma.

Summary and explanation

Human Ferritin is a large molecule with 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, Ferrritin 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

This Ferritin ELISA kit is a solid phase sandwich assay method, based on a streptavidin-biotin principle. The standards, samples and the biotinylated Anti-Ferritin antibody reagent are added into designated wells, coated with Streptavidin. Endogenous Ferritin in the patient's serum binds to the antigenic site of the biotinylated Anti-Ferritin antibody. Simultaneously, the biotinylated antibody is immobilized onto the wells through the high affinity Streptavidin-Biotin interaction. Unbound protein and excess biotin conjugated antibody are washed off by wash buffer. Upon the addition of the Peroxidase (HRP) conjugated Anti-Ferritin antibody reagent, a sandwich complex is formed, the analyte of interest being in between the two highly specific antibodies, labeled with Biotin and HRP. Unbound protein excess enzyme conjugated antibody reagent is washed off by wash buffer. Upon the addition of the substrate, the intensity of color developed is directly proportional to the concentration of Ferritin in the samples. A standard curve is prepared relating color intensity to the concentration of the Ferritin.

	MATERIALS PROVIDED	96 Tests
1.	Microwells coated with Streptavidin	12x8x1
2.	Ferritin Standards: 6 vials (ready to use)	0.5ml
3.	Anti-Ferritin Biotin Reagent (ready to use)	12 ml
4.	Anti-Ferritin Enzyme Reagent (ready to use)	12ml
5.	TMB Substrate: 1 bottle (ready to use)	12ml
6.	Stop Solution: 1 bottle (ready to use)	12ml
7.	20X Wash concentrate: 1 bottle	25ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagent to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials:
   The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Typically, specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature (20-25°C).
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipette 25 µl of Ferritin standards, controls, and samples in to appropriate wells.
3. Add 100 µl of Biotin Reagent into each well. Shake the plate for (10-30) sec.
4. Cover the plate and incubate for 30 minutes at room temperature (20-25°C).
5. Remove liquid from all wells. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
6. Add 100 µl of Enzyme Reagent into each well.
7. Cover the plate and incubate for 30 minutes at room temperature (20-25°C).
8. Remove liquid from all wells. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
9. Add 100 µl of TMB substrate to all wells.
10. Incubate for 15 minutes at room temperature.
11. Add 50 µl of stop solution to all wells. Shake the plate 10-20 seconds to mix the solution.
12. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

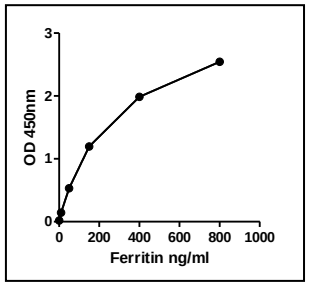
CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check Ferritin standard value on each standard vial. This value may vary from lot to lot. Make sure you check the value on every kit. See example of the standard below**.**
2. To construct the standard curve, plot the absorbance for the Ferritin standards (vertical axis) versus the Ferritin standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Data

	OD 450 nm	Conc. ng/mL
Std 1	0.019	0
Std 2	0.144	10
Std 3	0.531	50
Std 4	1.197	150
Std 5	1.987	400
Std 6	2.543	800



LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.