

CALDON BIOTECH INC. Ferritin ELISA

Catalog No. FR065T
(96 tests)

INTENDED USE

The CALDON BIOTECH INC. (CBI), Ferritin ELISA kit is used for the quantitative measurement of Ferritin in human serum or plasma.

SUMMARY AND EXPLANATION

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. In addition, Ferritin 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

The Ferritin is a solid phase direct sandwich ELISA method. The samples and diluted anti-Ferritin-HRP conjugate are added to the wells coated with Mab to Ferritin. Ferritin in the patient's serum binds to anti-Ferritin MAb on the well and the anti-Ferritin-HRP second antibody then binds to Ferritin. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is 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

- Microwell coated Ferritin MAb (12x8x1 wells). Total 96 wells.
- Ferritin Standard: 6 vials (0.7 mL each). Ready to use
- Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
- TMB Substrate: 1 bottle (12 mL). Ready to use.
- Stop Solution: 1 bottle (8 mL). Ready to use.
- Wash Concentrate: One Bottle (50 mL, 10X).

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.

WARNINGS AND PRECAUTIONS

1. 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
2. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. 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. 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

10X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 450 mL of distilled water. Store at room temperature.

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 20 µL of Ferritin standards, control and patient's sera.
3. Add 100 µL of enzyme conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18 - 26° C).
5. Remove liquid from all wells. Fill wells with wash buffer. Wash three times. Blot on absorbent paper towels.
6. Add 100 µL of TMB substrate to all wells.
7. Incubate for 10 minutes at room temperature.
8. Add 50 µL of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 20 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 might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.

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.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:

Men:	20-300 ng/mL
Women:	15-120 ng/mL

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS

1. Precision

Intra-Assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation%
Normal	16	277	16.2	5.8
Low	16	253	11.6	4.6
High	16	87	6.5	7.5

Inter-assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation%
Normal	10	294	19.4	6.6
Low	10	260	18.2	7.0
High	10	82	7.9	9.6

2. Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.70	0.75	2.2 ng/mL

REFERENCES:

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