



Ferritin (human) ELISA Kit

(Catalog # K7420-100, 100 assays; Store at 2-8°C)

rev 08/16

I. Introduction:

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. BioVision's Ferritin kit is a solid phase direct sandwich ELISA Kit. The standards, samples, and controls are added to the wells coated with monoclonal antibody to Ferritin. Ferritin in the sample, standards & controls binds to anti-Ferritin Mab on the well and the anti-Ferritin HRP then binds to Ferritin. Unbound Protein and HRP conjugate is 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.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Human Ferritin

IV. Sample Type:

Serum & plasma

V. Kit Contents:

Components	K7420-100	Part No.
Microplate coated with Ferritin MAb, 96 wells	12 stripsx8 wells	K7420-100-1
Ferritin Standard: (0.5 ml) (ready to use)	6 vials	K7420-100-2.x
Anti-Ferritin Biotin Reagent (ready to use)	12 ml	K7420-100-3
Anti-Ferritin Enzyme Reagent (ready to use)	12 ml	K7420-100-4
Wash Concentrate (20X)	25 ml	K7420-100-5
TMB Substrate (ready to use)	12 ml	K7420-100-6
Stop Solution (ready to use)	12 ml	K7420-100-7

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

- **Wash Concentrate:** 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 (18-26° C).

VIII. Warning & Precautions:

- Potential biohazardous materials: The Standards set contains 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, 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.
- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.
- 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.

IX. Sample Preparation and Storage:

Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 5 days. Store frozen at (-20°C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Don't use grossly lipemic specimens.

X. Assay Protocol:

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use. 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.

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipet 25 µl of Ferritin Standard, control, and samples into designated wells.

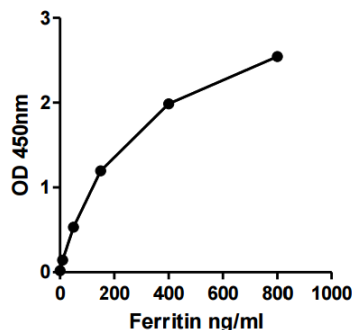


3. Add 100 µl of Biotin Reagent to all wells. Shake gently for 20-30 sec. to mix.
4. Cover the plate and incubate for 30 min. at room temperature (18-26°C).
5. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of Enzyme Reagent to all wells. Shake gently for 20-30 sec. to mix.
7. Cover the plate and incubate for 30 min. at room temperature (18-26°C).
8. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
9. Add 100 µl of TMB substrate to all wells & incubate for 15 min. at room temperature.
10. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
11. Read absorbance on ELISA Reader at 450 nm within 15 min. after adding the stop solution.

- XI. Calculation:** Construct the standard curve; plot the absorbance for the Ferritin standards (vertical axis) versus Ferritin standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of Standard Curve:

Standard	OD (450 nm)	Part No.
Standard 1 (0 ng/ml)	0.019	K7420-100-2.1
Standard 2 (10 ng/ml)	0.114	K7420-100-2.2
Standard 3 (50 ng/ml)	0.531	K7420-100-2.3
Standard 4 (150 ng/ml)	1.197	K7420-100-2.4
Standard 5 (400 ng/ml)	1.987	K7420-100-2.5
Standard 6 (800 ng/ml)	2.543	K7420-100-2.6



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 for Ferritin were established by the BioVision and may be used as initial guideline ranges only

Classification	Normal Range (ng/ml)
Males	25-350
Females	10-200

XII. RELATED PRODUCTS:

Iron Colorimetric Assay Kit (K390)
Deferoxamine Mesylate (1883)

Ferrostatin-1 (2230)

FOR RESEARCH USE ONLY! Not to be used on humans.