Human Ferritin ELISA Kit

Enzyme Immunoassay for the quantification of Ferritin in human serum and plasma

Catalog number: ARG80501

Package: 96 wells

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INTRODUCTION

Ferritin is a globular protein found mainly in the liver, which can store about 2'250 iron (Fe^{3+}) ions. The ferritin molecule consists of a protein shell (apoferritin) composed of heavy and light subunits, which surrounds a crystalline core containing iron oxide and phosphate.

Ferritin is synthesized in the liver, spleen and numerous other body tissues, with major concentrations found in the liver, spleen, bone marrow, and intestinal mucosa

The ferritin levels measured have a direct correlation with the total amount of iron stored in the body. If ferritin is high there is iron in excess, which would be excreted in the stool. If ferritin is low there is a risk for lack in iron, which sooner or later could lead to anemia.

In the setting of anemia, serum ferritin is the most sensitive lab test for iron deficiency anemia. In contrast, serum ferritin levels are normal or increased in anemia associated with chronic disease. Elevated serum ferritin levels have been observed in acute and chronic liver disease and lymphoid malignancy (leukemia and Hodgkin lymphoma). High serum ferritin levels have also been associated with an elevated risk for myocardial infarction in men. Ferritin is also used as a marker for iron overload disorders, such as haemochromatosis in which the ferritin level may be abnormally raised.

Ferritin is an acute-phase reactant, it is often elevated in the course of disease.

Free iron is toxic to cells, and the body has an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored complexed to protein as ferritin or hemosiderin. Apoferritin binds to free ferrous iron and stores it in the ferric state. Under steady state conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin level is the most convenient laboratory test to estimate iron stores.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Ferritin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ferritin present is bound by the immobilized antibody. After washing away any unbound substances, an HRP conjugated antibody specific for Ferritin is added to each well and incubate. A substrate solution (TMB) is then added to the wells and color develops in proportion to the amount of Ferritin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Ferritin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C
Standard 1-5 (5, 20, 100, 400, 1000 ng/ml)	5 X 1ml (Ready-to-use)	4°C
Zero Standard	3 ml (Ready-to-use)	4°C
Ferritin Control (101.25 ng/ml; acc. range: 70.9-131.6 ng/ml)	1 ml (Ready-to-use)	4°C
HRP-Conjugated antibody	12 ml (Ready-to-use)	4°C
10X Wash buffer	50 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

Store the unopened kit at 2-8 °C in dark. Use the kit before expiration date.

Open the bag of antibody-coated microplate only when it is at room temperature and close it immediately after use, once opened, it is stable until the expiry date of the kit. Do not remove the adhesive sheets on the strips unutilized.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620-630 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- All reagents should be stored refrigerated at 2 °C- 8 °C in their original container (in dark). Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated. Once opened, the standards are stable for 6 months when stored at 2 °C - 8 °C.
- Allow all kit components and specimens to reach room temperature (22 °C- 28 °C) and mix well prior to use.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C for 15min or until the crystals are completely dissolved.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.

- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 10X Wash buffer + 450 ml of distilled water.) Diluted wash buffer is stable for 30 days at 2-8°C.
- Sample: If the initial assay found samples contain Ferritin higher than the highest standard, the samples can be diluted with Zero Standard and then reassay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 22-28°C) for at least 30 min. All reagents should be store at 2 °C- 8 °C immediately after used and avoid long exposure to room temperature. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it and stored at 2°C - 8°C.
2. Add **20 µl** of **standards, controls and samples** in duplicate into wells. Keep one well empty as blank.
3. Add **100 µl** of **HRP-conjugated antibody** into each well, except blank well. Gently tap the plate to mix well. **Incubate for 1 hours at RT.**
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Gently shake the plate for 5 seconds. Then complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

   Note: if you use automated equipment, wash the wells at least 5 times.
5. Add **100 µl** of **TMB Substrate Reagent** to each well (including blank well). Incubate for **10 minutes at room temperature in dark.**
6. Add **100 µl** of **Stop Solution** to each well. Shake the microplate gently. The color of the solution should change from blue to yellow.
7. **Read** the OD with a microplate reader **at 450 nm** against a reference

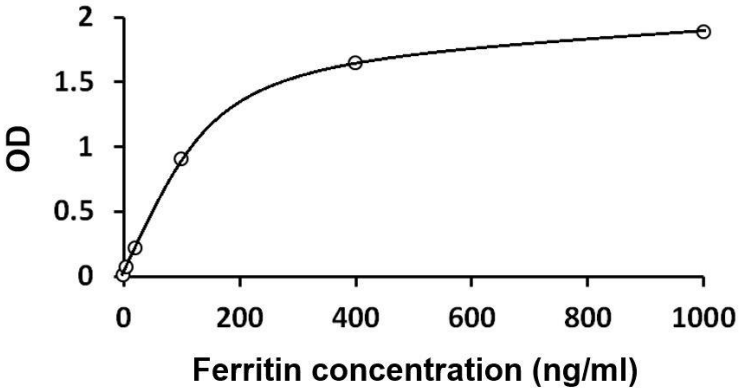
wavelength of 620-630 nm or against Blank. It is recommended read the absorbance within 5 minutes after adding stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value 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 from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



Acc. Range for standards:

S1 (5 ng/ml): OD should ≥ 0.030

S2 (20 ng/ml): OD should ≥ 0.105

S3 (100 ng/ml): OD should ≥ 0.350

S4 (400 ng/ml): OD should ≥ 0.605

S5 (1000 ng/ml): OD should ≥ 0.725

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Ferritin ranged from 5-1000 ng/ml. The mean MDD was 0.4 ng /ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was <7.5 % and inter-assay precision was < 6.1.

Specificity

The cross reaction of the antibody calculated on a weight/weight basis are shown in the table:

Liver Human Iso-Ferritin	100 %
Spleen Human Iso-Ferritin	80 %
Heart Human Iso-Ferritin	12 %

Recovery

95.76-107.43%