



# AssayMax Human Ferritin ELISA Kit

Catalog # EF2003-1

## Introduction

Ferritin is an iron storage protein. It consists of 24 subunits with combined molecular weight of 474,000. Serum ferritin level is related to body iron stores and is influenced by several diseases. High serum ferritin levels associate with iron overload [1], diabetes mellitus [2], Adult-onset Still disease (AOSD) [3], excessive macrophage activation [4], alcohol intake [5]. On the other hand, low level of ferritin is an indication of Iron Deficiency Anemia [6].

## Principal of the Assay

The AssayMax Ferritin ELISA kit is designed for detection of human ferritin in plasma, serum and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique which measures ferritin in 3.5 hours. A polyclonal antibody specific for ferritin has been pre-coated onto a microplate. Ferritin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for ferritin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

## Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

## Reagents

- **Ferritin Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Ferritin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes which can be cut to fit the format of the individual assay.
- **Ferritin Standard:** Human Ferritin in a buffered protein base (100 ng, lyophilized).
- **Biotinylated Ferritin Antibody (100x):** A 100-fold biotinylated polyclonal antibody against Ferritin (80 µl).

- **Streptavidin-Peroxidase Conjugate (S. P. Conjugate):** A 100-fold concentrate (90  $\mu$ l).
- **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (20 ml).
- **Wash Buffer Concentrate (10x):** A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

## Storage Condition

- Store unopened kit at 2 - 8<sup>0</sup>C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2 - 8<sup>0</sup>C. Store reconstituted standard and S. P. Conjugate concentrate at -20<sup>0</sup>C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

## Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20  $\mu$ l, 20-200  $\mu$ l, and multiple channel).
- Deionized or distilled reagent grade water.

## Sample Collection and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:10 into EIA Diluent. The undiluted samples can be stored at -20<sup>0</sup>C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:10 into EIA Diluent. The undiluted samples can be stored at -20<sup>0</sup>C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Dilute samples 1:5 into EIA Diluent. Store samples at -20<sup>0</sup>C or below. Avoid repeated freeze-thaw cycles.

## Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **Ferritin Standard:** Reconstitute the 100 ng of human Ferritin Standard with 2 ml of EIA Diluent to generate a stock solution of 50 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard solution (50 ng/ml) twofold with equal volume of EIA Diluent to produce 25, 12.5, 6.25, 3.125, 1.563 and 0.781 ng/ml. Sample Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[Ferritin] (ng/ml)
P1	1 part Standard (50 ng/ml)	50.00
P2	1 part P1 + 1 part EIA Diluent	25.00
P3	1 part P2 + 1 part EIA Diluent	12.50
P4	1 part P3 + 1 part EIA Diluent	6.250
P5	1 part P4 + 1 part EIA Diluent	3.125
P6	1 part P5 + 1 part EIA Diluent	1.563
P7	1 part P6 + 1 part EIA Diluent	0.781
P8	EIA Diluent	0.000

- **Biotinylated Ferritin Antibody (100x):** Dilute the antibody 1:100 with EIA Diluent.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent Concentrate 1:10 with reagent grade water.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 with reagent grade water.
- **S. P. Conjugate (100x):** Dilute the conjugate 1:100 with EIA Diluent.

## Assay Procedure

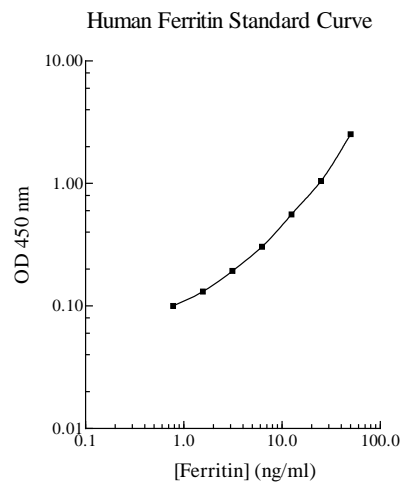
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well, and cover wells and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and blot it on absorbent paper towel to completely remove liquid at each step.
- Add 50 µl of Biotinylated Ferritin Antibody to each well and incubate for 60 minutes.
- Wash five times with 200 µl of Wash Buffer.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

## Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a Standard Curve, plot 4-parameter graph or log-log graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis and draw a best fit curve through the points on the graph. The best-fit line can be determined by regression analysis using 4-parameter or semi-log curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor of 10.

## Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



## Performance Characteristics

- The minimum detectable dose of Ferritin is typically 500 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0 % and 7.7 % respectively.
- This assay recognizes both natural and recombinant human Ferritin.

## References

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3. Omagari K. *et al* (2003) *Am J Med Sci.* 326(3): 148-51
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6. Eskeland B. *et al* (1999) *Acta Paediatr.* 88(8):815-21

Revision 2.3