Innovative RESEARCH Results Driven Research

Human Ferritin ELISA Kit

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], and alcohol intake [5]. On the other hand, a low level of ferritin is an indication of Iron Deficiency Anemia [6].

Principal of the Assay

The Ferritin ELISA kit is designed for detection of human ferritin in plasma, serum, milk, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique, which measures ferritin in less than 4 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

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.**
- 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 that 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).

- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (20 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store components of the kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20^{0}C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8^{0}C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8^{0}C.
- Store Standard at 2-8^{0}C before reconstituting with Diluent and at -20^{0}C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000µl 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^{0}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^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute milk 1:4 into EIA Diluent. The undiluted samples can be stored at -20^{0}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 1:10 into EIA Diluent. Store samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x)**: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.

- **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 duplicate or 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 and 1.563 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C and use within 30 days.

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	EIA Diluent	0.000

- **Biotinylated Ferritin Antibody (100x):** Dilute the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1: 20 with reagent grade water.
- **SP Conjugate (100x):** Dilute the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20^{0}C.

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^{0}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. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated Ferritin Antibody to each well and incubate for one hour.
- Wash the microplate as described above.
- 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 the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 15 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. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some

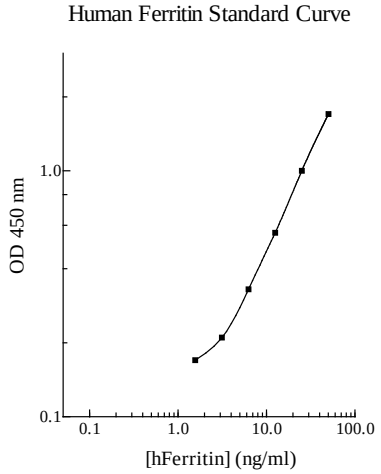
unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

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

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 1.5 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9 % and 7.1 % respectively.
- This assay recognizes both natural and recombinant human Ferritin.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:5	89%	88%
1:10	98%	100%
1:20	105%	108%

	Average Percentage of Expected Value
Sample Dilution	**Milk**
1:2	96%
1:4	100%
1:8	101%

Recovery

Standard Added Value	2 – 20 ng/ml
Recovery %	82-111 %
Average Recovery %	97 %

Cross-Reactivity

Species	% Cross Reactivity
Canine	10%
Monkey	100%
Mouse	5%
Rat	5%
Swine	10%
Rabbit	None
Bovine	5%

References

(1) Solis-Herruzo JA. (2003) *Rev Esp Enferm Dig*. 95(5): 351-7, 343-509
(2) Canturk Z. *et al* (2003) *Endocr Res*. 29(3): 299-306
(3) Omagari K *et al* (2003) *Am J Med Sci*. 326(3): 148-51
(4) Lambotte O *et al* (2003) *J Rheumatol*. 30(5): 1027-8
(5) Whitfield JB *et al* (2001) *Alcohol Clin Exp Res*. 25(7): 1037-45
(6) Eskeland B *et al* (1999) *Acta Paediatr*. 88(8): 815-21

Version 4.5