Ferritin ELISA Assay Kit

Catalog Number:
FRR31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 2.1 (26 FEB 24)

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INTENDED USE

The Eagle Biosciences Ferritin ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of Ferritin in human serum. The Eagle Biosciences Ferritin ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Ferritin is a protein which serves as a storage center for iron. It is found in many tissues but the highest concentrations are in the liver, spleen and bone marrow. The total body iron stores in normal people correlate well with the concentration of ferritin in serum. If there is a deficiency in iron, that is the concentration of iron is low in the blood, the ferritin result will be decreased. Likewise, an overload of iron indicates an increase in the level of ferritin. However, in some conditions like liver disease ferritin will be elevated.

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for ferritin is immobilized onto the microplate and another monoclonal antibody specific for a different region of ferritin is conjugated to horse radish peroxidase (HRP). Ferritin from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of ferritin in the sample. A set of standards is used to plot a standard curve from which the amount of ferritin in patient samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    c. Wear protective clothing and disposable gloves.
    d. Wash hands thoroughly after performing the test.
    e. Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.

8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit should be reported as > 40 pg/ml and must not be diluted. Dilution will alter the existing equilibrium and may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

LIMITATIONS

1. This kit is intended for research use only and should not be used as a diagnostic tool.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.2 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 20, 50, 150, 200 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS PROVIDED

1. **Mouse Anti-Ferritin Antibody-Coated Break-Apart Well Microplate** — Ready To Use

Contents:	One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
Storage:	Refrigerate at 2–8°C
Stability:	12 months or as indicated on label.

2. **Mouse Anti-Ferritin Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate** — Requires Preparation x51

Contents:	Anti-Ferritin monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume:	600 µL/vial
Storage:	Refrigerate at 2–8°C
Stability:	12 months or as indicated on label.
Preparation:	Dilute 1:51 in assay buffer before use (eg. 40 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 480 µL of HRP in 24 mL of assay buffer. Discard any that is left over.

3. **Ferritin Calibrators** — Ready To Use

Contents: Six vials containing ferritin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of ferritin.

* Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
A	0 ng/mL	2.0 mL
B	10 ng/mL	0.5 mL
C	50 ng/mL	0.5 mL
D	150 ng/mL	0.5 mL
E	400 ng/mL	0.5 mL
F	800 ng/mL	0.5 mL

Storage:	Refrigerate at 2–8°C.
Stability:	12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Controls — Ready to Use

Contents:	Two vials containing ferritin in a protein-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of ferritin. Refer to vial labels for the acceptable range.
Volume:	0.5 mL/vial
Storage:	Refrigerate at 2–8°C
Stability:	12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. **Wash Buffer Concentrate** — Requires Preparation x10

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume:	50 mL/bottle
Storage:	Refrigerate at 2–8°C
Stability:	12 months or as indicated on label.

Preparation: Dilute the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. **Assay Buffer** – Ready to Use

   Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
   Volume: 25 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

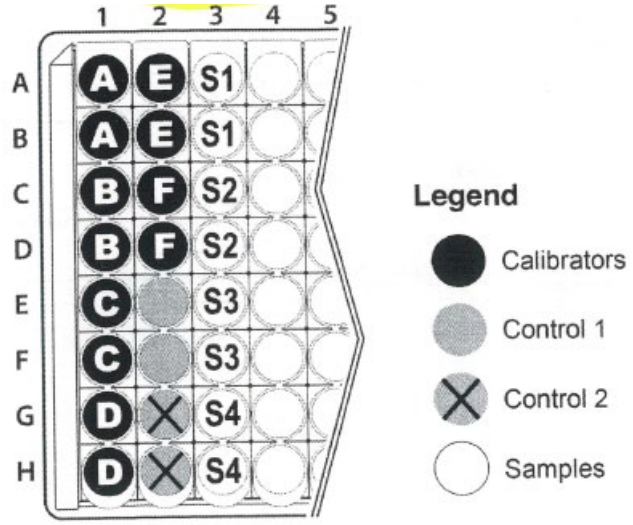
7. **TMB Substrate** — Ready To Use

   Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   Volume: 16 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

8. **Stopping Solution** — Ready To Use

   Contents: One bottle containing 1M sulfuric acid.
   Volume: 6 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

Specimen Pretreatment: None

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the anti-ferritin-HRP conjugate and wash buffer.
2. Remove the required number of well strips from the microplate. Reseal the bag and return any unused strips to the refrigerator.
3. **Pipette 20 µL** of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. **Pipette 200 µL** of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. **Incubate** on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
6. **Wash** the wells 5 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. **Pipette 150 µL** of the TMB substrate into each well at timed intervals.
8. **Incubate** on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD).
9. **Pipette 50 µL** of stopping solution into each well at the same timed intervals as in step 7.
10. **Read** the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm fi lter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the "0" calibrator from the mean absorbance values of the calibrators, controls and serum samples.
4. Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4 - parameter or 5 -parameter curve is recommended.
5. Read the values of the unknowns directly off the calibrator curve.
6. If a sample reads more than 800 ng/mL, then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated.

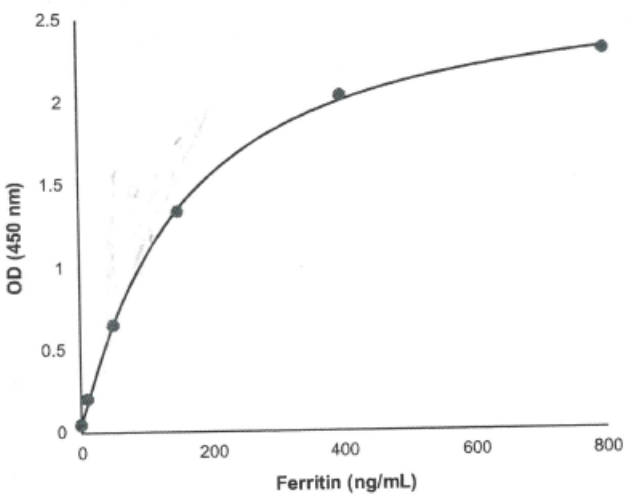
1. The calibrator A mean optical density meets the acceptable range as stated in the QC certificate.
2. The values obtained for the kit controls are within the acceptable ranges as stated in the kits QC certificate.
3. The results of any external controls that were used meet the acceptable ranges.

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD	% Binding	Value (ng/mL)
A	0.049	2	0
B	0.204	9	10
C	0.649	28	50
D	1.331	58	150
E	2.024	88	400
F	2.292	100	800
Unknown	0.985	-	91

TYPICAL CALIBRATOR CURVE



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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.