ab108698 – Ferritin Human ELISA Kit

Instructions for Use

An immunoenzymatic assay for the quantitative measurement of Ferritin in serum and plasma.

This product is for research use only and is not intended for diagnostic use.

Version 4d Last Updated 2 June 2023

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1. BACKGROUND

Abcam's Ferritin Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Ferritin in serum and plasma.

A 96-well plate has been precoated with anti-Ferritin IgG antibodies. Samples, standards and the Ferritin-HRP conjugate are added to the wells, where Ferritin in the sample and standards binds to the precoated antibody and added Ferritin-HRP conjugate binds to this antibody-AFP complex. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is directly proportional to the amount of Ferritin in the sample and the intensity is measured at 450 nm.

Ferritin is a globular protein found mainly in the liver, which can store about 2'250 iron (Fe3+) 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 tissues, with major concentrations found in the liver, spleen, bone marrow, and intestinal mucosa

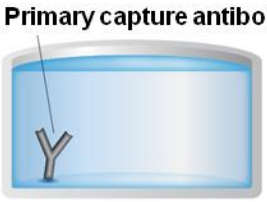
The ferritin levels measured have a direct correlation with the total amount of iron stored in the body. If ferritin is high there is iron in excess, which is excreted in stools. If ferritin is low there is a risk of iron deficiency, which can lead to anaemia.

Serum ferritin is the most sensitive lab test for iron deficiency anaemia, however, 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's lymphoma). High serum ferritin levels have also been associated with an elevated risk for myocardial infarction. Ferritin is also used as a marker for iron overload disorders, such as haemochromatosis in which ferritin levels may be abnormally raised.

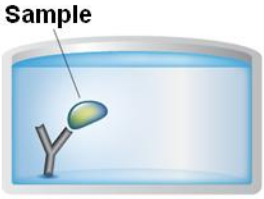
Ferritin is an acute-phase reactant; it is often elevated in the course of disease.

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.

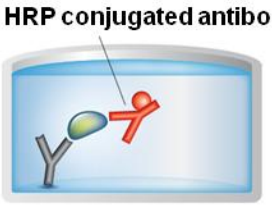
2. ASSAY SUMMARY



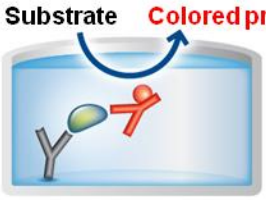
Prepare all reagents, samples and standards as instructed.



Add samples, standards and controls to wells used.



Add prepared labeled HRP-Conjugate to each well. Incubate at room temperature.



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-Ferritin IgG Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Stop Solution	15 mL	2-8°C
Anti-Ferritin-HRP Conjugate	12 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
10X Washing Solution	50 mL	2-8°C
Ferritin Control	1 mL	2-8°C
Ferritin Standard 0 – 0 ng/mL	3 mL	2-8°C
Ferritin Standard 1 – 5 ng/mL	1 mL	2-8°C
Ferritin Standard 2 – 20 ng/mL	1 mL	2-8°C
Ferritin Standard 3 – 100 ng/mL	1 mL	2-8°C
Ferritin Standard 4 – 400 ng/mL	1 mL	2-8°C
Ferritin Standard 5 – 800 ng/mL	1 mL	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- 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
- 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
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **1X Washing Solution**

Prepare 1X Washing Solution by diluting 10X Washing Solution with deionized water. To make 200 mL 1X Washing Solution combine 20 mL 10X Washing Solution with 180 mL deionized water. Mix thoroughly and gently.

- All other solutions are supplied ready to use

10. SAMPLE COLLECTION AND STORAGE

- The determination of Ferritin can be performed in plasma as well as in serum. If the assay is performed on the same day as sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20°C). If samples are stored frozen, mix thawed samples gently for 5 min. before testing. Avoid repeated freezing and thawing

  Samples with concentration of Ferritin over 1000 ng/ml have to be diluted with standard 0.

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3. Add 20 µL standard, control or sample into their respective wells. Add 100 µL anti-Ferritin-HRP Conjugate to each well. Leave a blank well for substrate blank.

13.4. Cover wells with the foil supplied in the kit and incubate for 1 hour at room temperature.

13.5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

13.6. Note: Complete removal of liquid at each step is essential for good assay performance.

13.7. Add 100 µL TMB Substrate Solution into all wells.

13.8. Incubate for exactly 10 minutes at room temperature in the dark.

13.9. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.

13.10. Measure the absorbance of the sample at 450 nm against a reference wavelength of 620-630 nm or against the blank within 5 minutes.

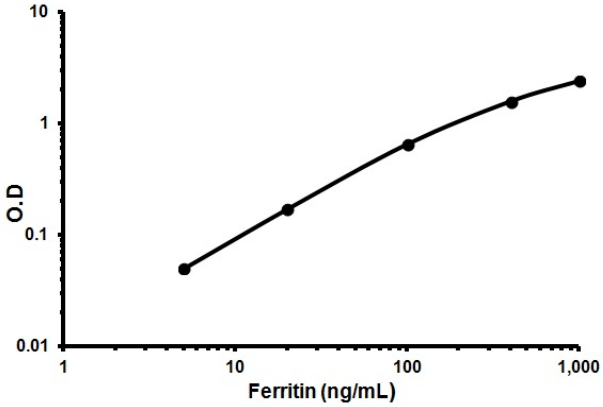
13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



Conc. (ng/mL)	O.D
0	0.01
5	0.05
20	0.17
100	0.65
400	1.57
1,000	2.39

15. TYPICAL SAMPLE VALUES

REFERENCE VALUES-

Human serum or plasma Ferritin reference values:

	Range (ng/mL)	Mean (ng/mL)
Premenopausal females	6 – 180	53
Post-menopausal females	8 – 350	105
Males	20 – 400	175

SENSITIVITY –

The lowest detectable concentration of Ferritin that can be distinguished from the zero standard is 0.53 ng/mL at the 95 % confidence limit.

PRECISION –

Control Serum	**Intra-Assay**	**Inter-Assay**
n=	48	48
%CV	≤ 5.4	≤ 6.1

RECOVERY –

The recovery of 12.5, 25, 50, 100 and 200 ng/mL of Ferritin added to samples gave an average value (±SD) of 98.66 % ± 2.9 % with reference to the original concentrations.

16. ASSAY SPECIFICITY

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

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

Abcam's Ferritin Human ELISA Kit shows no Hook Effect up to 50,000 ng/mL.

This method allows the determination of Ferritin from 5 – 1,000 ng/mL.

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. NOTES

For all technical and commercial enquires please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)