HUMAN FERRITIN ENZYME IMMUNOASSAY TEST KIT

Enzyme Immunoassay for the Quantitative Determination of Human Ferritin Concentration in Human Serum

IVD *For In Vitro Diagnostic Use Only*

2°C 8°C ***Store at 2 to 8°C.***

Σ **96 Tests**

INTENDED USE

Immunoassay for the in vitro quantitative determination of ferritin in human serum.

INTRODUCTION

Ferritin is a macro molecule with a molecular weight of at least 440 kD (depending on the iron content) and consists of a protein shell (apoferritin) of 24 subunits and an iron core containing an average of approx. 2500 Fe3+ ions (in liver and spleen ferritin). Ferritin tends to form oligomers, and when it is present in excess in the cells of the storage organs there is a tendency for condensation to semicrystalline hemosiderin to occur in the lysosomes. At least 20 isoferritins can be distinguished with the aid of isoelectric focusing. This microheterogeneity is due to differences in the contents of the acidic H and weakly basic L subunits. The basic isoferritins are responsible for the long-term iron storage function, and are found mainly in the liver, spleen, and bone marrow. Acidic isoferritins are found mainly in the myocardium, placenta, and tumor tissue. They have a lower iron content and presumably function as intermediaries for the transfer of iron in various syntheses.The determination of ferritin is a suitable method for ascertaining the iron metabolism situation. Determination of ferritin at the beginning of therapy provides a representative measure of the body's iron reserves. A storage deficiency in the reticulo-endothelial system (RES) can be detected at a very early stage Clinically, a threshold value of 20 µg/L (ng/mL) has proved useful in the detection of prelatent iron deficiency. This value provides a reliable indication of exhaustion of the iron reserves that can be mobilized for hemoglobin synthesis. Latent iron deficiency is defined as a fall below the 12 µg/L (ng/mL) ferritin threshold. These two values necessitate no further laboratory elucidation, even when the blood picture is still morphologically normal. If the depressed ferritin level is accompanied by hypochromic, microcytal anemia, then manifest iron deficiency is present. When the ferritin level is elevated and the possibility of a distribution disorder can be ruled out, this is a manifestation of iron overloading in the body. 400 µg/L (ng/mL) ferritin is used as the threshold value. Elevated ferritin values are also encountered with the following tumors: acute leukemia, Hodgkin's disease and carcinoma of the lung, colon, liver and prostate. The determination of ferritin has proved to be of value in liver metastasis. Studies indicate that 76 % of all patients with liver metastasis have ferritin values above 400 µg/L (ng/mL). Reasons for the elevated values could be cell necrosis, blocked erythropoiesis or increased synthesis in tumor tissue. Two monoclonal mouse antibodies - M-4.184 and M-3.170 - are used to form the sandwich complex in the assay.

PRINCIPLE OF THE TEST

Sandwich principle. Total duration of assay: **80 minutes.**

- Sample, Anti-Ferritin coated microwells and enzyme labeled Anti-Ferritin are combined.
- During the incubation, ferritin presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies.
- After washing, a complex is generated between the solid phase, the TSH within the sample and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The absorbance is proportional to the amount of ferritin in the sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Coated Microplate: 8 x 12 strips, 96 wells, pre-coated with mouse anti-ferritin.
- Calibrators: 6 white cap vials, 1 ml each, ready to use; Concentrations: 0(A), 20(B), 50(C), 100(D), 300(E) and 600(F) ng/mL.
- Enzyme Conjugate: 1 red cap vial, 11 mL of HRP (horseradish peroxidase) labeled mouse monoclonal anti-ferritin.
- Substrate: 1 brown cap vial, 11ml, ready to use, (tetramethylbenzidine) TMB.
- Stop Solution: 1 yellow cap vial, 6.0 ml of 1 mol/l sulfuric acid.
- Wash Solution Concentrate: 1 transparent cap bottle, 25 ml (40X concentrated), PBS-Tween wash solution.
- IFU 1 copy.
- Plate Lid: 1 piece.

Materials required but not provided:

- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water.

Precautions and warnings

- For in vitro diagnostic use only. For professional use only.
- All products that contain human serum or plasma have been found to be non-reactive for HBsAg, HCV and HIVI/II. But all products should be reared as potential biohazards in use and for disposal.
- Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
- Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
- Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- If more than one plate is used, it is recommended to repeat the calibration curve.
- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Ensure that the bottom of the plate is clean and dry
- Ensure that no bubbles are present on the surface of the liquid before reading the plate.
- The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

STORAGE OF TEST KIT AND INSTRUMENTATION

- Store at 2-8°C.
- Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8 °C, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date whichever is earlier.
- Seal and return unused calibrators to 2-8°C, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20 °C. Avoid multiple freeze-thaw cycles.
- Seal and return all the other unused reagents to 2-8 °C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation

- Collect serum samples in accordance with correct medical practices.
- Cap and store the samples at 18-25 °C for no more than 24 hours. Stable for 7 days at 2-8 °C, and 1 month at -20 °C. Recovery within 90-110 % of serum value or slope 0.9-1.1. Freeze only once.
- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
- Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
- Avoid grossly hemolytic, lipemic or turbid samples.
- Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
- If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
- Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.
- Adjust the incubator to 37 °C.
- Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature.
- Don's use Substrate if it looks blue.
- Don't use reagents that are contaminated or have bacteria growth

Quality control

Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. There controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels

ASSAY PROCEDURE

1. Use only the number of wells required and format the microplates wells for each calibrator and sample to be assayed.
2. Add **25 µl of calibrators or samples** to each well.
3. Add **100 µl of enzyme conjugate** to each well.
4. Shake the microplate gently for 30 seconds to mix.
5. Cover the plate with a plate lid and incubate at 37 °C for 60 minutes.
6. Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add **350 µl of wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of 5 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
8. Add **100 µl of substrate** to each well.
9. Incubate at ambient temperature (18-25°C) in the dark for reaction for 20 minutes. Do not shake the plate after substrate addition.
10. Add **50 µl of stop solution** to each well.
11. Shake for 15-20 seconds to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
12. Read the absorbance of each well at 450 nm (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within 30 minutes of adding the stop solution.

CALCULATION OF RESULTS

- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in ng/ml for each calibrator.
- Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

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

Sample	Value (ng/mL)	Absorbance
Calibrator A	0	0.015
Calibrator B	20	0.188
Calibrator C	50	0.635
Calibrator D	100	1.174
Calibrator E	300	2.210
Calibrator F	600	3.279
Control 1	38.76	0.510
Control 2	268.84	1.250
Sample	88.39	0.740

LIMITATIONS OF THE PROCEDURE

- The assay is unaffected by icterus (bilirubin < 1112 µmol/L or 65 mg/dL), hemolysis (Hb < 0.31 mmol/L or < 0.5 g/dL), lipemia (Intralipid < 3300 mg/dL) and biotin (< 205 nmol/L or < 50 ng/mL).
- Criterion: Recovery within ± 10 % of initial value.
- Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
- No interference was observed from rheumatoid factors up to a concentration of 2500 IU/mL.
- There is no high-dose hook effect at ferritin concentrations of up to 50000 µg/L (ng/mL).
- In vitro tests were performed on 19 commonly used pharmaceuticals.
- No interference with the assay was found.
- Iron2+- and iron3+-ions at therapeutic concentrations do not interfere with the Atlas Ferritin assay.
- In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or

ruthenium can occur. These effects are minimized by suitable test design.

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.500-1000 µg/L (ng/mL) (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.500 µg/L (ng/mL). Values above the measuring range are reported as > 1000 µg/L (ng/mL) (or up to 25000 µg/L (ng/mL) for 25-fold diluted samples).

Lower limits of measurement

Lower detection limit

Lower detection limit: 0.1 ng/mL.

The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 20).

Expected values

Each laboratory must establish its own normal ranges based on patient population. The values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 869 healthy test subjects examined as below:

Men, 18-60 years: 27 - 430 µg/L(ng/mL)

Women, 18-60 years: 15-168 µg/L (ng/mL)

Specific performance data

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Atlas reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability*		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human Serum 1	2.85	0.243	8.53	0.257	9.01
Human Serum 2	64.71	3.436	5.31	4.349	6.72
Human Serum 3	292.55	13.925	4.76	17.056	5.83
PC Universal 1	48.73	2.558	5.25	3.133	6.43
PC Universal 2	223.75	8.682	3.88	11.792	5.27

*Repeatability = within-run precision

Method comparison

A comparison of the Atlas Ferritin assay (y) with the Roche Cobas Ferritin (x) using clinical samples gave the following correlations:

Number of samples measured: 156

Linear regression

y = 1.0733x + 0.0116

r = 0.9746

The sample concentrations were between approx. 0 and 964 ng/ mL.

Analytical specificity

Human liver ferritin 98 % recovery

Human spleen ferritin 90 % recovery

Human heart ferritin 1 % recovery

REFERENCES

1. Wick M, Pinggera W, Lehmann P. Ferritin in Iron Metabolism Diagnosis of Anemieas (second edition). Springer-Verlag 1995;ISBN 3-211-82525-8 and ISBN 0-387-82525-8.
2. Arosio P, Levi S, Gabri E, et al. Heterogeneity of ferritin II: Immunological aspects. In: Albertini A, Arosio P, Chiancone E, Drysdale J (eds). Ferritins and isoferritins as biochemical markers. Elsevier, Amsterdam 1984;33-47.
3. Kaltwasser JP, Werner E. Serumferritin: Methodische und Klinische Aspekte. Springer Verlag (1980).
4. Morikawa K, Oseko F, Morikawa S. A role for ferritin in hematopoiesis and the immune system. Leuk-Lymphoma 1995;18(5-6):429-433.
5. Borch-Iohnson B. Determination of Iron status: brief review of physiological effects on iron measures. Analyst 1995;120(3): 891-903.
6. Cook-JD, Skikne-BS, Baynes RD. Iron deficiency: the global perspective. Adv-Exp-Med-Biol 1994;356:219-228.
7. Albertini A, Arosio P, Chiancone E, Drysdale J (eds). Functional aspects of isoferritins. In: Jacobs A, Hodgetts J, Hoy TG. Ferritins and isoferritins as biochemical markers. Elsevier, Amsterdam 1984;113-127.

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry