Ferritin is an ELISA test system for the quantitative measurement of ferritin in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

**NAME AND INTENDED USE**

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**SYMBOLS USED ON LABELS**

- **MICROPLATE**: Microplate
- **CALIBRATOR A/B/C/D/E/F**: Calibrator
- **CONTROL M/N**: Control positive/negative
- **SAMPLE BUFFER**: Sample Buffer
- **ENZYME CONJUGATE**: Enzyme Conjugate
- **TMB SUBSTRATE**: TMB Substrate
- **STOP SOLUTION**: Stop solution
- **WASH BUFFER**: Wash Buffer
- **RTU**: Ready to use

**SUMMARY AND EXPLANATION OF THE TEST**

20 % of the human iron (total: 4-5 g) is reversely bound to ferritin as an intracellular storage protein. The remaining iron is bound to hemoglobin (60 %) and myoglobin or enzymes (20 %). Ferritin has a molecular weight of 450 kDa and is located in the liver, spleen, bone marrow, and the mucous of the bowels. Highly purified ferritin can develop red-brown crystals. Its 24 subunits form a hollow sphere to bind 4000 iron atoms connected to hydroxyporphosphate residues. The iron-free protein is called apo-ferritin. The iron-loaded ferritin is the most important and most specific iron storage of the cells and of the whole organism. In case of iron-deficiency iron can be released quickly from ferritin and it is served in a bioavailable status. [1]

Ferritin is found intracellular and in the blood stream. It is a reliable parameter to determine the iron concentration in the body. Serum ferritin concentrations remain constant during the biorhythm – in contrast to the alternating iron values. Ferritin values depend on the patient's age and sex. Regular losses of blood or blood donation decrease the ferritin values.

The determination of serum ferritin is an important parameter for the diagnosis and therapy control of an iron-deficiency. Negative iron-balance decreases the ferritin value. Ferritin contents below 12 ng/ml indicate a manifested iron-deficiency. During therapy with iron, ferritin values indicate the actual iron storage. Ferritin measurements are recommended for risk groups, like blood donors, pregnant women, hemodialysis patients and infants.

In some cases of iron-overloading serum ferritin values can exceed 500 ng/ml. Patients with hemochromatosis or secondary siderosis reveal elevated ferritin values. The whole clinical situation can only be evaluated by considering the entire diagnostic parameters [2, 3].

**Indications:** Iron-deficiency, iron-overloading, iron-deficiency anemia, hemochromatosis, liver disease, risk groups, tumors, iron therapy.

**PRINCIPLE OF THE TEST**

Anti-human-ferritin antibodies are bound to microwells. The reaction is based on indirect enzyme immuno assay (ELISA) method with these steps:

- Ferritin present in a patient sample binds to the antibody coated forming an antigen-antibody-complex. Washing of the microwells removes unbound unspecific serum and plasma components. During incubation with anti-ferritin enzyme-conjugate immunologically a conjugate/antibody/antigen complex is formed. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

**ORG 5FE Ferritin**

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CONTENTS OF THE KIT

ORG SFE I 96 Sufficient for 96 determinations

MICROPATE 1 One divisible microplate consisting of 12 modules of 8 wells each. Ready to use.

Product code on module: FER

CALIBRATOR A 1x 0.75 ml Calibrator A 0 ng/ml, containing serum/buffer matrix (PBS, BSA, detergent, Na3 0.09%), yellow. Ready to use.

CALIBRATOR B 1x 0.75 ml Calibrator B 15 ng/ml, containing ferritin in a serum/buffer matrix (PBS, BSA, detergent, Na3 0.09%), yellow. Ready to use.

CALIBRATOR C 1x 0.75 ml Calibrator C 50 ng/ml, containing ferritin in a serum/buffer matrix (PBS, BSA, detergent, Na3 0.09%), yellow. Ready to use.

CALIBRATOR D 1x 0.75 ml Calibrator D 150 ng/ml, containing ferritin in a serum/buffer matrix (PBS, BSA, detergent, Na3 0.09%), yellow. Ready to use.

CALIBRATOR E 1x 0.75 ml Calibrator E 500 ng/ml, containing ferritin in a serum/buffer matrix (PBS, BSA, Na3 0.09%), yellow. Ready to use.

CALIBRATOR F 1x 0.75 ml Calibrator F 1500 ng/ml, containing ferritin in a serum/buffer matrix (PBS, BSA, detergent, Na3 0.09%), yellow. Ready to use.

CONTROL H 1x 0.75 ml Control positive, containing ferritin in a serum/buffer matrix (PBS, BSA, detergent, Na3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.

CONTROL M 1x 0.75 ml Control negative, containing ferritin in a serum/buffer matrix (PBS, BSA, detergent, Na3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.

SILENT 20 ml Sample Buffer STP, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow. Ready to use.

CONJUGATE 15 ml Enzyme Conjugate containing anti-human ferritin antibodies, HRP labeled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.

WASH 20 ml Wash Buffer, containing Trit, detergent, preservative sodium azide 0.09%, 50 x conc.

STOP 15 ml Stop solution; contains acid. Ready to use.

1 1 Instruction for Use: ELISA Mini-DVD

1 Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 25 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopened test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3.3',5.5'-Tetramethylbenzidine).
- Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures: Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed. Observe the guidelines for performing quality control in medical laboratories by assaying control sera.
**PREPARATION OF REAGENTS**

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

Sample buffer STP is ready to use.

**Preparation of samples**

Use undiluted patient sample. Note: Calibrators / Controls are ready to use and need no dilution.

**TEST PROCEDURE**

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette 25 µl of calibrators, controls and patient samples into the wells. Add 100 µl of sample buffer.

   Incubate for 30 minutes at room temperature (20-28 °C).

   Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

2. Dispense 100 µl of enzyme conjugate into each well.

   Incubate for 15 minutes at room temperature.

   Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

3. Dispense 100 µl of TMB substrate solution into each well.

   Incubate for 15 minutes at room temperature.

   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.

   The developed colour is stable for at least 30 minutes. Read during this time.

**Expected values and interpretation of results**

<table>
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<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed</th>
<th>Expected</th>
<th>CV %</th>
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<td>1088.0</td>
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<tr>
<td></td>
<td>1:2</td>
<td>550.0</td>
<td>544.0</td>
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<td>272.0</td>
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</table>

**Limit of detection**

Functional sensitivity was determined to be: 5 ng/ml

**Reproducibility**

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

**Interfering substances**

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

**LIMITATIONS OF THE PROCEDURE**

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually. The above pathological
and normal reference ranges in patient samples should be regarded as recommendations only. Each laboratory
should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES
   131:5685-5805.