

## ORGENTEC Diagnostika GmbH

Carl-Zeiss-Straße 49  
55129 Mainz - Germany

Phone: +496131/92580  
Fax: +496131/925858  
Internet : www.orgentec.com



Instruction for use  
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## ORG 5FE Ferritin

Immunometric Enzyme Immunoassay for the quantitative determination of Ferritin in serum or plasma

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### NAME AND INTENDED USE

Ferritin is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of Ferritin in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis and therapy control of iron deficiency.

### 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 various tissues, i.e. liver, spleen, bone marrow or 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 hydroxyphosphate residues. The uncombined 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 of the patient's age and sex. Regular loss 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 the iron-deficiency. A 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
- latent iron deficiency	- liver diseases
- risk groups	- tumors
- iron therapy	

### PRINCIPLE OF THE TEST

Anti-human-ferritin antibodies are bound to microwells. Ferritin, if present in diluted serum or plasma, bind to the respective antibody. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human ferritin immunologically detects the bound patient ferritin forming a conjugate/ferritin/antibody complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of ferritin present in the original sample.

## WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro diagnostic use only.
2. Do not interchange kit components from different lots.
3. 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.
4. Avoid contact with the TMB (3,3',5,5'-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is hydrochloric acid (1 M). If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide ( $\text{NaN}_3$ ) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.)
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

## CONTENTS OF THE KIT

Package size	96 determ.
Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified specific anti-human-ferritin antibodies (rabbit, polyclonal). Ready to use.
6 vials, 0.75 ml each	Ferritin-Calibrators (A-F) in a PBS/BSA matrix ( $\text{NaN}_3 <0,1\%$ (w/w)) containing ferritin: 0; 15; 50; 150; 500 and 1500 ng/ml. Ready to use.
2 vials, 0.75 ml each	Ferritin Controls in a PBS/BSA matrix ( $\text{NaN}_3 <0,1\%$ (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed package insert. Ready to use.

1 vial, 15 ml	Sample buffer (Tris, $\text{NaN}_3 <0,1\%$ (w/w)), yellow. Ready to use.
1 vial, 15 ml	Enzyme conjugate solution (PBS, PROCLIN 300 $<0,5\%$ (v/v)), (light red) containing polyclonal rabbit anti-human ferritin; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution. Ready to use.
1 vial, 15 ml	Stop solution (1 M hydrochloric acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, $\text{NaN}_3 <0,1\%$ (w/w)), concentrate (50x).

## STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

## MATERIALS REQUIRED

### Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100  $\mu\text{l}$
- Vortex mixer
- Pipets for 10  $\mu\text{l}$ , 100  $\mu\text{l}$  and 1000  $\mu\text{l}$
- Laboratory timing device
- Data reduction software

### Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

## SPECIMEN COLLECTION, STORAGE AND HANDLING

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

autoantibody activity.

6. Testing of heat-inactivated sera is not recommended.

#### PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

#### PREPARATION OF REAGENTS

##### Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

#### TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and patient samples.
2. Pipet **25 µl** of calibrators, controls and patient samples in duplicate into the wells.

	1	2	3	4	5	6
A	SA	SE	P1	P5		
B	SA	SE	P1	P5		
C	SB	SF	P2	P..		
D	SB	SF	P2	P..		
E	SC	C1	P3			
F	SC	C1	P3			
G	SD	C2	P4			
H	SD	C2	P4			

SA-SF: standards A to F  
 P1, P2...C: patient sample 1, 2 ...  
 C1: positive control  
 C2: negative control

3. Add **100 µl** sample buffer to each well.
4. Incubate for 30 minutes at room temperature (20-28 °C).
5. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
6. Dispense **100 µl** of enzyme conjugate into each well.
7. Incubate for 15 minutes at room temperature.
8. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
9. Dispense **100 µl** of TMB substrate solution into each well.
10. Incubate for 15 minutes at room temperature.
11. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature.

12. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

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

#### Automation

The ORGENTEC Ferritin ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

#### INTERPRETATION OF RESULTS

##### Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

##### Calculation of results

For Ferritin ELISA a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

##### Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

## Calculation example

The figures below show typical results for Ferritin ELISA. These data are intended for illustration only and should not be used to calculate results from another run.

Calibrators									
No	Position	OD 1	OD 2	Mean	Conc. 1	Conc. 2	Mean	decl.Conc.	CV %
ST1	A 1/A 2	0.015	0.013	0.014	0.0	0.0	0.0	0.0	10
ST2	B 1/B 2	0.050	0.048	0.049	16.6	15.8	16.2	15.0	3
ST3	C 1/C 2	0.151	0.161	0.156	49.4	52.4	50.9	50.0	5
ST4	D 1/D 2	0.482	0.518	0.500	143	154	149	150	5
ST5	E 1/E 2	1.339	1.293	1.316	521	488	505	500	3
ST6	F 1/F 2	1.912	1.901	1.907	1509	1463	1486	1500	0

## Interpretation of results

In a normal range study with urine and serum/plasma samples from healthy donors the following ranges have been established with the Ferritin test:

Female: 20 – 50 years	22 – 112 ng/ml	Female: 65 – 90 years	13 – 651 ng/ml
Male: 20 – 50 years	34 – 310 ng/ml	Male: 65 – 87 years	4 – 665 ng/ml
Umbilical cord blood:	30 – 276 ng/ml	Infants: 0,5 month	90 – 628 ng/ml
Infants: 1 month	144 – 399 ng/ml	Infants: 2 month	87 – 430 ng/ml
Infants: 4 month	37 – 223 ng/ml	Infants: 6 month	19 – 142 ng/ml
Infants: 9 month	14 – 103 ng/ml	Infants: 12 month	1 – 99 ng/ml
Children: 6 mon. – 15 Years	7 – 142 ng/ml		

taken from: Thomas: Labor und Diagnose. Joachim P. Kaltwasser

It is recommended that each laboratory establishes its own normal and pathological ranges. The reference ranges should be regarded as guidelines only.

## PERFORMANCE CHARACTERISTICS

### Parallelism

In dilution experiments sera with high ferritin concentrations were assayed in the Ferritin kit. The assay shows linearity over the full measuring range.

### Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations of each sample:

Intra-Assay		
Sample No	Mean [ng/ml]	CV [%]
1	31.9	5.0
2	94.1	4.7
3	300.1	3.4

Inter-Assay		
Sample No	Mean [ng/ml]	CV [%]
1	35.6	5.9
2	99.8	2.6
3	300.5	1.7

## Sensitivity

The lower detection limit for Ferritin has been determined at 5 ng/ml.

## Specificity

The antisera (polyclonal, rabbit) used for coating of the microplate and in the enzyme conjugate are highly specific for the human ferritin molecule.

## LIMITATIONS OF PROCEDURE

The Ferritin ELISA 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.

## INTERFERING SUBSTANCES

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

## REFERENCES

1. Beard, J.L. Iron Biology in immune function, muscle metabolism and neuronal functioning. J.Nutr., 2001, 131:568S-580S.
2. Dawson, D.W. et al. The accuracy and clinical interpretation of serum ferritin assays. Clin.Lab.Haematol., 1992, 14(1):47-52.
3. Powell, L.W. et al. Diagnosis of Hemochromatosis. Ann.Intern.Med., 1998, 129:925-931.

## INCUBATION SCHEME

- 1 Pipet **25 µl** calibrator, control or patient sample, add **100 µl** sample buffer  
→ Incubate for **30 minutes** at room temperature  
→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- 2 Pipet **100 µl** enzyme conjugate  
→ Incubate for **15 minutes** at room temperature  
→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- 3 Pipet **100 µl** substrate solution  
→ Incubate for **15 minutes** at room temperature
- 4 Add **100 µl** stop solution  
→ Leave untouched for **5 minutes**  
→ Read at **450 nm**