

REF



SYSTEM

07027273190

07027273501

300

cobas e 801

English

For use in the USA only

System information

Short name	ACN (application code number)
FERR	10034

Intended use

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

The electrochemiluminescence immunoassay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Ferritin is a macromolecule 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 approximately 2500 Fe³⁺ ions (in liver and spleen ferritin).¹

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.² 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.^{1,3}

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.^{4,5,6}

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.⁷

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, microcytic anemia, then manifest iron deficiency is present.¹

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.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 6 µL of sample, a biotinylated monoclonal ferritin-specific antibody, and a monoclonal ferritin-specific antibody labeled with a ruthenium complex⁹ form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas link**.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Reagents - working solutions

The **cobas e** pack is labeled as FERR.

- M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-ferritin-Ab~biotin, 1 bottle, 21.0 mL:
Biotinylated monoclonal anti-ferritin antibody (mouse) 3.0 mg/L;
phosphate buffer 100 mmol/L, pH 7.2; preservative.
- R2 Anti-ferritin-Ab~Ru(bpy)₃²⁺, 1 bottle, 21.0 mL:
Monoclonal anti-ferritin antibody (mouse) labeled with ruthenium complex 6.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2;
preservative.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas link**.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the cobas e 801 analyzer	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K₂-EDTA and K₃-EDTA plasma.

Criterion: Slope 0.9-1.1 + intercept within ± 2x Limit of Blank + coefficient of correlation ≥ 0.95.

Stable for 48 hours at 20-25 °C, 7 days at 2-8 °C, 12 months at -20 °C (± 5 °C). The samples may be frozen twice.

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 samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

The claims, including those pertaining to sample stability made in the labeling of the cleared/approved reagents of Roche Diagnostics are part of the clearance of the overall IVD test system (assay). Sample stability was tested only for the temperatures/time frame as claimed by the manufacturer under the conditions claimed in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- [REF] 03737586190, Ferritin CalSet, 4 x 1.0 mL
- [REF] 11776452160, PreciControl Tumor Marker, for 4 x 3.0 mL or [REF] 05618860160, PreciControl Varia, for 4 x 3.0 mL
- [REF] 07299001190, Diluent Universal, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e 801** analyzer

Accessories for the **cobas e 801** analyzer:

- [REF] 06908799190, ProCell II M, 2 x 2 L system solution
- [REF] 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- [REF] 07485409001, Reservoir Cups, 8 cups to supply ProCell II M and CleanCell M
- [REF] 06908853190, PreClean II M, 2 x 2 L wash solution
- [REF] 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- [REF] 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- [REF] 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- [REF] 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the Elecsys Ferritin assay ([REF] 11820982122). The Elecsys Ferritin assay ([REF] 11820982122) has been standardized against the Enzymun-Test Ferritin method. This in turn has been standardized against the 1st International Standard (IS) NIBSC (National Institute for Biological Standards and Control) "Reagent for Ferritin (human liver)" 80/602.

Recovery studies, including a published study⁸, to assess traceability of the Elecsys Ferritin assay to more recent international standards (2nd IS 80/578 and 3rd IS 94/572) have been conducted, with results showing very good agreement.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 8 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer

- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Tumor Marker or PreciControl Varia.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in µg/L or ng/mL).

Limitations - interference

Do not use hemolyzed samples.

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1112 µmol/L or ≤ 65 mg/dL
Hemoglobin	≤ 0.062 mmol/L or ≤ 100 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 205 nmol/L or ≤ 50 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: Recovery within ± 10 % of initial value for samples ≥ 25 ng/mL or ± 2.5 ng/mL for samples < 25 ng/mL.

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.

There is no high-dose hook effect at ferritin concentrations up to 100000 µg/L (ng/mL).

Iron²⁺- and iron³⁺-ions at therapeutic concentrations do not interfere with the Elecsys Ferritin assay.

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

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

0.50-2000 µg/L (ng/mL) (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.50 µg/L (ng/mL). Values above the measuring range are reported as > 2000 µg/L (ng/mL) (or up to 100000 µg/L (ng/mL) for 50-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.25 µg/L (ng/mL)

Limit of Detection = 0.50 µg/L (ng/mL)

Limit of Quantitation = 2.0 µg/L (ng/mL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

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The Limit of Blank is the 95th percentile value from $n \geq 60$ measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation (functional sensitivity) is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples with ferritin concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:50 (either automatically by the analyzer or manually). The concentration of the diluted sample must be ≥ 40 $\mu\text{g/L}$ (ng/mL).

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzer, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

Results of a study with the Enzymun-Test Ferritin method on samples from 224 healthy test subjects (104 women - mainly premenopausal - and 120 men) are given below. The values correspond to the 5th and 95th percentiles.⁹

Men, 20-60 years: 30-400 $\mu\text{g/L}$ (ng/mL)

Women, 17-60 years: 13-150 $\mu\text{g/L}$ (ng/mL)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days ($n = 84$). The following results were obtained:

cobas e 801 analyzer					
Sample	Mean $\mu\text{g/L}$ (ng/mL)	Repeatability		Intermediate precision	
		SD $\mu\text{g/L}$ (ng/mL)	CV %	SD $\mu\text{g/L}$ (ng/mL)	CV %
Human serum 1	1.80	0.115	6.4	0.127	7.1
Human serum 2	3.02	0.0540	1.8	0.0625	2.1
Human serum 3	23.2	0.458	2.0	0.620	2.7
Human serum 4	414	6.06	1.5	10.9	2.6
Human serum 5	878	22.6	2.6	34.9	4.0
Human serum 6	1406	39.4	2.8	62.5	4.4
PreciControl Varia 1	147	1.84	1.3	3.48	2.4
PreciControl Varia 2	858	16.5	1.9	32.0	3.7
PreciControl TM ^{b)} 1	28.2	0.411	1.5	0.686	2.4
PreciControl TM 2	187	2.12	1.1	4.77	2.6

b) TM = Tumor Marker

Method comparison

A comparison of the Elecsys Ferritin assay on the **cobas e 801** analyzer (y) with the Elecsys Ferritin assay on the **cobas e 601** analyzer (x) gave the following correlations ($\mu\text{g/L}$):

Number of samples measured: 167

Passing/Bablok¹⁰

Linear regression

$$y = 0.999x + 0.425$$

$$r = 0.981$$

$$y = 0.931x + 18.6$$

$$r = 0.996$$

The sample concentrations were between 1.05 and 1972 $\mu\text{g/L}$ (ng/mL).

Analytical specificity

Human liver ferritin: 100 % recovery

Human spleen ferritin: 85 % recovery

Human heart ferritin: 1 % recovery

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see <https://usdiagnostics.roche.com> for definition of symbols used):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing
	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

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