Elecsys Ferritin

cobas®

REF		Σ	SYSTEM
07027273190* 07027273214*	07027273500	300	**cobas e** 402 **cobas e** 801

* Some kits shown may not be available in all countries.

English

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 **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Ferritin measurements, performed with this assay in human serum and plasma, are used as an aid in diagnosis of iron deficiencies (e.g. iron deficiency anemia) and iron overload (e.g. hemochromatosis).

Ferritin is synthesized by many body cells and it is mainly found in the liver, spleen, muscle and bone marrow, with only a small fraction found in blood. The protein plays an important role in the cellular uptake, storage and release of iron.[1] The storage function is not only important for adequate amounts of bioavailable iron to be provided, but also to protect the cells from toxicity. Iron can generate reactive species which can directly damage DNA and proteins.[2,3]

The iron-free protein, apoferritin, consists of 24 subunits and has a molecular weight of approximately 450 kDa. The iron core of ferritin can contain up to approximately 4500 iron atoms in the form of Fe^{3+} ions.[4,5] Several isoforms of ferritin exist which are composed of different subunits that are partially tissue specific.[1,4]

Under steady-state conditions, the serum ferritin concentration is proportional to the total body iron stores: 1 ng of serum ferritin per mL corresponds to 10 mg of total iron stores.[6,7,8] Therefore, in the literature, the measurement of serum ferritin levels is proposed as the best and most convenient laboratory test to estimate iron stores and diagnose iron deficiency or iron related disorders.[5,6,8,9] It has substituted the invasive and semiquantitative histochemical examination of bone marrow aspirate or biopsy as the gold standard for diagnosis of iron deficiency anemia.[2,9] Serum ferritin is a good indicator of storage iron in the body; however it does not provide information about the amount of iron actually available for erythropoiesis.[10]

Decreased serum ferritin concentrations of < 15 µg/L always indicate iron deficiency and can be the result of prior blood loss, altered iron uptake, transferrin deficiency, reduced erythropoiesis (e.g. chronic kidney disease) or increased iron demand.[8,9,10,11,12,13,14] Different aetiologies can cause increased serum ferritin levels, like iron overload, inflammation, liver or renal disease, malignancy or metabolic syndrome.[15,16] An elevated serum ferritin in the absence of infection or inflammation may suggest the presence of an iron overload state due to clinical conditions such as hereditary hemochromatosis, transfusional iron overload or ineffective erythropoiesis (e.g. thalassemia).[10,15,16]

Serum ferritin may also be elevated as an acute phase reactant or due to massive cell and tissue death. Patients with infections, acute or chronic inflammation, and malignancies have increased serum ferritin levels. Clinical conditions unrelated to iron stores, such as alcoholic or viral hepatitis and chronic renal failure, exhibit increased serum ferritin levels. Diagnosis should be made looking at the entire clinical situation of the individual patient.[2,10,15]

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 complex[a)] 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 ($Ru(bpy)_3^{2+}$)

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~$Ru(bpy)_3^{2+}$, 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 for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

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Contact phone: all countries: +49-621-7590

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 analyzers	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, K_2-EDTA and K_3-EDTA plasma.

Li-heparin plasma tubes containing separating gel can be used.

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.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03737586190, Ferritin CalSet, 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL or REF 05618860190, PreciControl Varia, for 4 x 3.0 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- 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 Cup, 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 11298500316, 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 study[17], 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

Use PreciControl Tumor Marker, PreciControl Varia or other suitable controls for routine quality control procedures.

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

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

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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).

$Iron^{2+}$- and $iron^{3+}$-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.

The Limit of Blank is the 95th percentile value from n ≥ 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 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 analyzers or manually). The concentration of the diluted sample must be > 40 µg/L (ng/mL).

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

After dilution by the analyzers, 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.[18]

Men, 20-60 years: 30-400 µg/L (ng/mL)

Women, 17-60 years: 13-150 µg/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 analyzers are 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 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD µg/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) A comparison of the Elecsys Ferritin assay, REF 04491785190 (y) with the Elecsys Ferritin assay, REF 11820982122 (x) using clinical samples gave the following correlations (µg/L):

Number of samples measured: 134

Passing/Bablok[19]	Linear regression
y = 1.00x + 0.72	y = 0.99x + 4.11
τ = 0.984	r = 0.999

The sample concentrations were between 2.68 and 1891 µg/L (ng/mL).

b) A comparison of the Elecsys Ferritin assay, REF 07027273190 (**cobas e** 801 analyzer; y) with the Elecsys Ferritin assay, REF 03737551190 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured: 167

Passing/Bablok[19]	Linear regression
y = 0.999x + 0.425	y = 0.931x + 18.6
τ = 0.981	r = 0.996

The sample concentrations were between 1.05 and 1972 µg/L (ng/mL).

c) A comparison of the Elecsys Ferritin assay, REF 07027273190 (**cobas e** 402 analyzer; y) with the Elecsys Ferritin assay, REF 07027273190 (**cobas e** 801 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured: 122

Passing/Bablok[19]	Linear regression
y = 1.05x + 0.131	y = 1.00x + 4.44
τ = 0.987	r = 0.999

The sample concentrations were between 2.16 and 1882 ng/mL.

Analytical specificity

Human liver ferritin: 100 % recovery

Human spleen ferritin: 85 % recovery

Human heart ferritin: 1 % recovery

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References

1 Wick M, Pinggera W, Lehmann P. Clinical aspects and laboratory. Iron metabolism, anemias. Springer Verlag, Wien, New York, 7th edition, 2011:pp.8-11.

2 Thomas L. Clinical Laboratory Diagnostics: Use and Assessment of Clinical Laboratory Results; 1st Edition, Frankfurt/Main: TH-Books-Verl.-Ges.,1998:278-281.

3 Knovich MA, Storey JA, Coffman LG, et al. Ferritin for the clinician. Blood Rev 2009;23(3):95-104.

4 Sargent PJ, Farnaud S, Evans RW. Structure/function overview of proteins involved in iron storage and transport. Curr Med Chem 2005;12(23):2683-2693.

5 Ferraro S, Mozzi R, Panteghini M. Revaluating serum ferritin as a marker of body iron stores in the traceability era. Clin Chem Lab Med 2012;50(11):1911-1916.

6 http://accessmedicine.mhmedical.com/content.aspx?bookid=331&sectionid=40726841

7 Małyszko J, Levin-Iaina N, Myśliwiec M, et al. Iron metabolism in solid-organ transplantation: how far are we from solving the mystery? Pol Arch Med Wewn 2012;122(10):504-511.

8 Polin V, Coriat R, Perkins G, et al. Iron deficiency: from diagnosis to treatment. Dig Liver Dis 2013;45(10):803-809.

9 Castel R, Tax MG, Droogendijk J, et al. The transferrin/log(ferritin) ratio: a new tool for the diagnosis of iron deficiency anemia. Clin Chem Lab Med 2012;50(8):1343-1349.

10 Wang W, Knovich MA, Coffman LG, et al. Serum ferritin: Past, present and future. Biochim Biophys Acta 2010;1800(8):760-769.

11 Liu K, Kaffes AJ. Iron deficiency anaemia: a review of diagnosis, investigation and management. Eur J Gastroenterol Hepatol 2012;24:109-116.

12 Fletcher A, Forbes A, Svenson N, et al. A British Society for Haematology Good Practice Paper. Guideline for the laboratory diagnosis of iron deficiency in adults (excluding pregnancy) and children. Br J Haematol 2022 Feb;196(3):523-529.

13 Wojtaszek E, Glogowski T, Malyszko J. Iron and Chronic Kidney Disease: Still a Challenge. Front Med (Lausanne) 2020 Dec 18;7:565135.

14 Chronic kidney disease: assessment and management. London: National Institute for Health and Care Excellence (NICE); 2021 Nov 24.

15 Cullis JO, Fitzsimons EJ, Griffiths WJ, et al. British Society for Haematology. Investigation and management of a raised serum ferritin. Br J Haematol 2018 May;181(3):331-340.

16 Fitzsimons EJ, Cullis JO, Thomas DW, et al. British Society for Haematology. Diagnosis and therapy of genetic haemochromatosis (review and 2017 update). Br J Haematol 2018 May;181(3):293-303.

17 Blackmore S, Hamilton M, Lee A, et al. Automated immunoassay methods for ferritin: recovery studies to assess traceability to an international standard. Clin Chem Lab Med 2008;46(10):1450-1457.

18 Lotz J, Hafner G, Prellwitz W. Reference Study for Ferritin Assays. Kurzmitteilung Clin Lab 1997;43(11):993-994.

19 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

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

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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