FERR4

cobas®

Tina-quant Ferritin Gen.4

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04885317 190	Tina-quant Ferritin Gen.4 250 tests	System-ID 07 6966 5	Roche/Hitachi **cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

11355279 216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 656	
10557897 122	Precinorm Protein (3 x 1 mL)	Code 302	
11333127 122	Precipath Protein (3 x 1 mL)	Code 303	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

FERR4: ACN 692

For **cobas c** 502 analyzer:

FERR4: ACN 8692

Intended use

In vitro test for the quantitative determination of ferritin in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6,7,8,9]

Ferritin is the iron storage protein. It has a molecular weight of ≥ 440000 daltons, depending upon the iron content, and consists of a protein shell (apoferritin) of 24 subunits and an iron core containing an average of approx. 2500 Fe^{3+} ions (in the basic isoforms). Common to all isoforms is their construction from two separate subunits, the acidic H (heavy)-type subunit and the weakly basic L (light)-type subunit. The basic isoferritins are responsible for the long-term iron storage function and are mainly detectable in the liver, spleen and bone marrow. Acid isoferritins are found mainly in the myocardium, placenta, tumor tissue and - to a lesser extent - in the depot organs.

The determination of ferritin is necessary above all in iron metabolism diagnosis, monitoring iron therapy, ascertaining the iron reserves in groups at risk and in the differential diagnosis of anemias. It encompasses prelatent and latent iron deficiency as well as iron overloading. It is also used to distinguish between hypoferric anemia and hypochromic anemia (chronic infection and tumor anemias, sideroblastic anemia or thalassemia).

Ferritin determinations are particularly suitable for monitoring renal anemia when iron utilization and distribution disorders are present during therapy with erythropoietin. The ferritin detectable in blood is in equilibrium with the body's depot iron and hence acts as an indicator for the level of the iron stores.

A variety of routine methods are available for determining ferritin, e.g. enzyme-linked immunosorbent assays (ELISA), fluorescence immunoassays (FIA), luminescence immunoassays (LIA), nephelometric and turbidimetric immunoassays.

The automated Roche ferritin assay is based on the immunological agglutination principle with enhancement of the reaction by latex.

Test principle[9]

Particle enhanced immunoturbidimetric assay

Human ferritin agglutinates with latex particles coated with anti-ferritin antibodies. The precipitate is determined turbidimetrically at 570/800 nm.

Reagents - working solutions

R1 TRIS buffer, pH 7.5; immunoglobulins (rabbit); preservative, stabilizers

R3 Aqueous matrix containing latex particles coated with anti-human ferritin antibodies (rabbit); preservative, stabilizers

R1 is in position B and R3 is in position C.

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.

Reagent handling

Ready for use

Mix **cobas c** pack well before placing on the analyzer.

Storage and stability

FERR4

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin, K_2- or K_3-EDTA plasma.

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; with K_3-EDTA tubes pay particular attention that the tubes are adequately filled.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Do not thaw frozen specimens in a 37 °C bath. Violent mixing may denature ferritin.[10]

Stability:[11]	7 days at 15-25 °C
	7 days at 2-8 °C
	1 year at (-15)-(-25) °C

FERR4

Tina-quant Ferritin Gen.4

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Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- See "Order information" section

General laboratory equipment

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.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 24-57	
Wavelength (sub/main)	800/570 nm	
Reaction direction	Increase	
Units	µg/L (pmol/L, ng/mL)	
Reagent pipetting		Diluent (H_2O)
R1	80 µL	–
R3	80 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	–	–
Decreased	10 µL	20 µL	140 µL
Increased	10 µL	–	–

cobas c 501 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 36-70	
Wavelength (sub/main)	800/570 nm	
Reaction direction	Increase	
Units	µg/L (pmol/L, ng/mL)	
Reagent pipetting		Diluent (H_2O)
R1	80 µL	–
R3	80 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	–	–
Decreased	10 µL	20 µL	140 µL
Increased	10 µL	–	–

cobas c 502 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 36-70	
Wavelength (sub/main)	800/570 nm	
Reaction direction	Increase	
Units	µg/L (pmol/L, ng/mL)	
Reagent pipetting		Diluent (H_2O)
R1	80 µL	–
R3	80 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	–	–
Decreased	10 µL	20 µL	140 µL
Increased	–	–	–

Calibration

Calibrators	S1: H_2O
	S2-6: C.f.a.s. Proteins
	Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the 6-point calibration curve:
	S2: 0.0270 S5: 0.5000
	S3: 0.1120 S6: 1.3000
	S4: 0.2300
Calibration mode	Spline
Calibration frequency	Full calibration • after reagent lot change • as required following quality control procedures

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

Traceability: This method has been standardized against the Elecsys Ferritin assay (immunological method) which is traceable to NIBSC (WHO).

Quality Control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

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.

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

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:[12]	µg/L = ng/mL
	µg/L × 2.247 = pmol/L
	µmol/L × 445000 = ng/mL

Limitations - interference

Criterion: Recovery within ± 4 µg/L (≤ 8.99 pmol/L, ≤ 4 ng/mL) of initial values for samples ≤ 40 µg/L (≤ 89.9 pmol/L, ≤ 40 ng/mL) and within ± 10 % for samples > 40 µg/L.

Icterus:[13] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[13] No significant interference up to an H index of 500 (approximate hemoglobin concentration: 310 µmol/L or 500 mg/dL).

Lipemia (Intralipid):[13] No significant interference up to an L index of 1000 (approximate intralipid concentration: 1000 mg/dL). There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 1200 IU/mL.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

High-dose hook effect: Using prozone check, no false result without a flag was observed up to a ferritin concentration of 80000 µg/L (80000 ng/mL).

The polyclonal antibodies used in this assay are specific for ferritin from human liver and also recognize ferritin from human spleen. The antibodies show no cross reactivity to the human ferritin H subunit, which is the major component of human heart ferritin.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

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

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

5-1000 µg/L (11.2-2247 pmol/L, 5-1000 ng/mL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:8 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 8.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 3 µg/L (6.7 pmol/L, 3 ng/mL)
Limit of Detection	= 5 µg/L (11.2 pmol/L, 5 ng/mL)
Limit of Quantitation	= 5 µg/L (11.2 pmol/L, 5 ng/mL)

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A 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 %).

Values below the Limit of Detection (< 5 µg/L (11.2 pmol/L, 5 ng/mL)) will not be flagged by the instrument.

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of ≤ 20 %. It has been determined using low concentration ferritin samples.

Expected values[17]

Adults: Expected values for ferritin concentrations in clinically healthy subjects are strongly dependent upon age and sex.

Results of a study with Tina-quant Ferritin on samples from 224 healthy test subjects (104 women, mainly premenopausal, and 120 men) are given below. These values correspond to the 5th and 95th percentiles.

Men (20-60 years)	30-400 µg/L (67-899 pmol/L, 30-400 ng/mL)
Women (17-60 years)	15-150 µg/L (34-337 pmol/L, 15-150 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 human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements with repeatability (n = 84) and intermediate precision (4 aliquots per run, 1 run per day, one lot of reagent, 21 days, on a Roche/Hitachi **cobas c** 501 analyzer). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	µg/L (pmol/L, ng/mL)	*µg/L (pmol/L, ng/mL)*	*%*
Precinorm Protein	125 (281, 125)	1 (2, 1)	0.8
Precipath Protein	306 (688, 306)	2 (4, 2)	0.6
Human serum 1	8.76 (19.7, 8.76)	0.83 (1.9, 0.83)	9.5
Human serum 2	26.1 (58.7, 26.1)	0.7 (1.6, 0.7)	2.8
Human serum 3	223 (501, 223)	1 (2, 1)	0.7
Human serum 4	568 (1276, 568)	5 (11, 5)	0.9
Human serum 5	781 (1755, 781)	7 (16, 7)	0.8

Intermediate precision	*Mean*	*SD*	*CV*
	µg/L (pmol/L, ng/mL)	*µg/L (pmol/L, ng/mL)*	*%*
Precinorm Protein	125 (281, 125)	1 (2, 1)	1.1
Precipath Protein	306 (688, 306)	4 (9, 4)	1.3
Human serum 1	8.76 (19.7, 8.76)	1.14 (2.6, 1.14)	13.0
Human serum 2	26.1 (58.7, 26.1)	0.7 (1.6, 0.7)	2.8
Human serum 3	223 (501, 223)	3 (7, 3)	1.2
Human serum 4	568 (1276, 568)	10 (22, 10)	1.7
Human serum 5	781 (1755, 781)	14 (31, 14)	1.8

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

Ferritin values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer using the Tina-quant Ferritin Gen.4 assay (y) were compared with those determined on a Roche/Hitachi 917 analyzer using the Tina-quant Ferritin assay (x).

Sample size (n) = 87

Passing/Bablok[18]	Linear regression
y = 0.904x + 7.73 µg/L	y = 0.901x + 8.68 µg/L
τ = 0.983	r = 0.998

The sample concentrations were between 19.5 and 775 µg/L (43.8 and 1741 pmol/L, 19.5 and 775 ng/mL).

In addition a comparison of the Tina-quant Ferritin Gen.4 assay on a Roche/Hitachi **cobas c** 501 analyzer (y) with the Tina-quant Ferritin Gen.3 assay on the same analyzer (x) using human serum and plasma samples gave the following correlations:

Sample size (n) = 88

Passing/Bablok[18]	Linear regression
y = 0.949x + 5.96 µg/L	y = 0.950x + 5.10 µg/L
τ = 0.989	r = 1.000

The sample concentrations were between 13.5 and 762 µg/L (30.3 and 1712 pmol/L, 13.5 and 762 ng/mL).

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

References

1 Wick M, Pinggera W, Lehmann P, eds. Iron Metabolism, Diagnosis and Therapy of Anemias. Clinical Aspects and Laboratory, 5th ed. Vienna/New York: Springer-Verlag 2003.

2 Kaltwasser IP, Werner E, eds. Serumferritin: Methodische und klinische Aspekte. Berlin/Heidelberg/New York: Springer-Verlag 1980.

3 Williams WJ, Beutler E, Ersler AJ, et al. eds. Hematology, 7th ed. New York: McGraw-Hill 2005.

4 Albertini A, Arosio P, Chiancone E, et al. eds. Ferritins and isoferritins as biochemical markers. Amsterdam/New York/Oxford: Elsevier 1984.

5 San Diego Declaration, Erythropoietin use and response in end-stage renal disease. The American Society of Nephrology, Annual meeting, San Diego. J Am Soc Nephrol 1995;3:35.

6 Finlayson NDC. Hereditary (primary) haemochromatosis. BMJ 1990;301:350-351.

7 Franco RS. Ferritin. In: Pesce AJ, Kaplan LA, eds. Methods in clinical chemistry. St. Louis/Washington/Toronto: CV Mosby Company 1987:1240-1242.

8 Dati F, Sauder U. Immunchemische Methoden im klinischen Labor. GIT Labor-Medizin 1990;7-8:357-372.

9 Dubois S, McGovern M, Ehrhardt V. Eisenstoffwechsel-Diagnostik mit Boehringer Mannheim/Hitachi-Analysensystemen: Ferritin, Transferrin und Eisen. GIT Labor-Medizin 1988;9:468-471.

10 Wu AHB, ed. Tietz Clinical Guide to Laboratory Tests. 4th ed. Philadelphia: WB Saunders; 2006:392.

11 Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB/99.1 Rev. 2: Jan 2002.

12 Young DS, Huth EJ. SI Units For Clinical Measurement. American College of Physicians 1998.

13 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

14 Breuer J. Report on the Symposium “Drug effects in Clinical Chemistry Methods”. Eur J Clin Chem Clin Biochem 1996;34:385-386.

15 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

16 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

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

18 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.

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 dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
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