

**Tina-quant Ferritin Gen.4****Order information**

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
04885317 190	Tina-quant Ferritin Gen.4 250 tests	System-ID 07 6966 5 Roche/Hitachi <b>cobas c</b> 311, <b>cobas c</b> 501/502
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**<sup>1,2,3,4,5,6,7,8,9</sup>

Ferritin is the iron storage protein. It has a molecular weight of  $\geq 440000$  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  $\text{Fe}^{3+}$  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 isoforms are responsible for the long-term iron storage function and are mainly detectable in the liver, spleen and bone marrow. Acid isoforms 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**<sup>9</sup>

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.

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.

**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,  $\text{K}_2$ - or  $\text{K}_3$ -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  $\text{K}_3$ -EDTA tubes pay particular attention that the tubes are adequately filled.

Centrifuge samples containing precipitates before performing the assay.

Do not thaw frozen specimens in a 37 °C bath. Violent mixing may denature ferritin.<sup>10</sup>

Stability:<sup>11</sup>

7 days at 15-25 °C

7 days at 2-8 °C

1 year at (-15)-(-25) °C

**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 <sub>2</sub> O)
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R1	80 µL	–
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R3	80 µL	–
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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 <sub>2</sub> O)
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R1	80 µL	–
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R3	80 µL	–
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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 <sub>2</sub> O)
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R1	80 µL	–
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R3	80 µL	–
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Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	10 µL	–	–
Decreased	10 µL	20 µL	140 µL
Increased	–	–	–

**Calibration**

Calibrators	S1: H <sub>2</sub> O S2-6: C.f.a.s. Proteins
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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
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S3: 0.1120	S6: 1.3000
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S4: 0.2300	
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Calibration mode	Spline
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Calibration frequency	Full calibration • after reagent lot change • as required following quality control procedures
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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: <sup>12</sup>	µg/L = ng/mL µg/L × 2.247 = pmol/L µmol/L × 445000 = ng/mL
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**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:<sup>13</sup> 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:<sup>13</sup> No significant interference up to an H index of 500 (approximate hemoglobin concentration: 310 µmol/L or 500 mg/dL).

Lipemia (Intralipid):<sup>13</sup> 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 up to 1200 IU/mL do not interfere.

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>14,15</sup>

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.<sup>16</sup>

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/Multiclean/SCCS or 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 not required.

**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 (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)*

LoB = 3 µg/L (6.7 pmol/L, 3 ng/mL)

LoD = 5 µg/L (11.2 pmol/L, 5 ng/mL)

LoQ = 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 95<sup>th</sup> 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 %).

Values below the Limit of Detection ( $< 5 \mu\text{g/L}$  (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  $\leq 20\%$ . It has been determined using low concentration ferritin samples.

##### Expected values<sup>17</sup>

**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 5<sup>th</sup> and 95<sup>th</sup> 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

##### 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 <sup>18</sup>	Linear regression
$y = 0.904x + 7.73 \mu\text{g/L}$	$y = 0.901x + 8.68 \mu\text{g/L}$
$\tau = 0.983$	$r = 0.998$

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 <sup>18</sup>	Linear regression
$y = 0.949x + 5.96 \mu\text{g/L}$	$y = 0.950x + 5.10 \mu\text{g/L}$
$\tau = 0.989$	$r = 1.000$

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

##### References

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# FERR4

## Tina-quant Ferritin Gen.4

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- 12 Young DS, Huth EJ. SI Units For Clinical Measurement. American College of Physicians 1998.
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- 17 Lotz J, Hafner G, Prellwitz W. Reference Study for Ferritin Assays. Kurzzmitteilung Clin Lab 1997;43:993-994.
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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.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

	Contents of kit
	Volume after reconstitution or mixing

COBAS, COBAS C, PRECICONTROL, PRECINORM, PRECIPATH and TINA-QUANT are trademarks of Roche.

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Significant additions or changes are indicated by a change bar in the margin.

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