05169291500V13.0

IRON2

cobas®

Iron Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05169291190*	05169291500	Iron Gen.2 (750 tests)	System-ID 05 6596 1	**cobas c** 701/702
05169291214*	05169291500	Iron Gen.2 (750 tests)	System-ID 05 6596 1	**cobas c** 701/702

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
12149435122	Precinorm U plus (10 x 3 mL)	Code 300	
12149443122	Precipath U plus (10 x 3 mL)	Code 301	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	

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

English

System information

IRON2: ACN 8661

Intended use

In vitro test for the quantitative determination of iron in human serum and plasma on **cobas c** systems.

Summary

Iron measurements performed with this assay in human serum and plasma are used as an aid in diagnosis and monitoring of iron deficiency and iron overload disorders.

Iron is essential for many metabolic and biochemical processes. Similar to other micronutrients in the human body, iron is supplied with food. Ingested iron is mainly absorbed in the form of Fe^{2+} in the duodenum and proximal jejunum. The trivalent form and the heme-bound Fe^{3+}-component of iron in food has to be reduced by duodenal cytochrome B. About 1-2 mg of iron is absorbed and lost daily. Upon reaching the mucosal cells, Fe^{2+} ions become bound to transport proteins. In the cellular phase iron is either stored in cellular ferritin or transported to the circulation. Iron export into the circulation requires Fe^{2+} oxidation to Fe^{3+} by hephaestin (on cellular membrane) or ceruloplasmin (in the circulation), for loading onto transferrin. Circulating Fe ions are transported by transferrin-iron complexes. A maximum of 2 Fe^{3+} ions per protein molecule can be transported.[1]

Serum iron fluctuates with dietary intake and normal diurnal variation. Clinically, dysregulation of serum/plasma iron levels can be divided into iron deficiency and iron overload.[1,2] Iron deficiency disorders can be due to increased demands (e.g. growth, pregnancy), limited external supply (e.g. malnutrition, inappropriate diet, malabsorption), increased loss (e.g. hemorrhage, hemodialysis, blood donation), or other conditions, such as chronic kidney disease resulting in renal anemia, inflammatory bowel disease, heart failure, obesity, bone marrow disease.[2,3] Iron deficiency occurs in several stages, defined by the extent of depletion, first of iron stores and then of iron available for hemoglobin synthesis. In the first stage, iron stores can be completely depleted without causing anemia. Further iron loss causes anemia (iron deficiency anemia, IDA), which is initially normocytic, with a normal absolute reticulocyte count. Deeper deficiency results in classic anemia findings with hypochromic (low mean corpuscular hemoglobin) and microcytic (low mean corpuscular volume) red blood cells.[3,4,5] Another type of anemia is macrocytic anemia (elevated mean corpuscular volume), which is not directly due to iron deficiency but is rather related to other causes, such as vitamin B12 and folate deficiency, bone marrow disorders (myelodysplasia), use of certain medications, alcohol abuse, liver disease, marked reticulocytosis, and hypothyroidism. Iron measurements can help define different causes of anemia.[6,7]

Iron overload disorders normally result in increased serum/plasma iron concentration, and can be due to a number of underlying conditions, most commonly hereditary haemochromatosis (excess iron derived from increased gastrointestinal absorption due to inactivating mutations in components of the hepcidin pathway) and thalassemia (increased concentrations of iron mainly caused from regular red blood cell transfusions and to a lesser extent by increased iron absorption).[2,8]

Numerous photometric methods have been described for the determination of iron. All have the following in common:

- Liberation of Fe^{3+} ions from the transferrin complex using acids or detergents.
- Reduction of Fe^{3+} ions to Fe^{2+} ions.
- Reaction of the Fe^{2+} ions to give a colored complex.[1]

The method described here is based on the FerroZine method without deproteinization.

Test principle

Colorimetric assay.

$$\text{Transferrin-Fe-complex} \xrightarrow{\text{pH} < 2.0} \text{apotransferrin} + Fe^{3+}$$

$$Fe^{3+} \xrightarrow{\text{ascorbate}} Fe^{2+}$$

$$Fe^{2+} + \text{FerroZine} \longrightarrow \text{colored complex}$$

Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released Fe^{3+} ions to Fe^{2+} ions which then react with FerroZine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically.

Reagents - working solutions

R1 Citric acid: 200 mmol/L; thiourea: 115 mmol/L; detergent

R3 Sodium ascorbate: 150 mmol/L; FerroZine: 6 mmol/L; preservative

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.

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

IRON2

Iron Gen.2

Danger

H318	Causes serious eye damage.

Prevention:

P280	Wear eye protection/ face protection.

Response:

P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.
EUH 208	Contains DIAZOLIDINYL UREA. May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

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

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	2 weeks
On-board on the Reagent Manager:	24 hours

When removing the **cobas c** pack from the instrument during use, please immediately store at 2-8 °C.

Do not shake the **cobas c** pack to avoid foaming.

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 plasma. Do not use EDTA or oxalate plasma.

Separate serum or plasma from the clot or cells within 1 hour.

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.

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

Stability:[9,10]	7 days at 15-25 °C
	3 weeks at 2-8 °C
	several years at (-15)-(-25) °C

Freeze only once.

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 701/702 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 18-30		
Wavelength (sub/main)	700/570 nm		
Reaction direction	Increase		
Units	µmol/L (µg/dL, mg/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R3	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	8.5 µL	–	–
Decreased	4.0 µL	–	–
Increased	17.0 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration
	- after **cobas c** pack change
	- and 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 a primary reference material (SRM 937).

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

cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factors:	µmol/L x 5.59 = µg/dL
	µmol/L x 0.0559 = mg/L

Limitations - interference

Criterion: Recovery within ± 2.7 µmol/L of initial values of samples ≤ 26.9 µmol/L and within ± 10 % for samples > 26.9 µmol/L.

Icterus:[11] 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:[11] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 125 µmol/L or 200 mg/dL). Higher hemoglobin concentrations lead to artificially increased values due to contamination of the sample with hemoglobin-bound iron.

Lipemia (Intralipid):[11] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12, 13]

In patients treated with iron supplements or metal-binding drugs, the drug-bound iron may not properly react in the test, resulting in artificially low values.

In the presence of high ferritin concentrations > 1200 µg/L the assumption that serum iron is almost completely bound to transferrin is not valid anymore. Therefore, such iron results should not be used to calculate Total Iron Binding Capacity (TIBC) or percent transferrin saturation (% SAT).[14]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[15]

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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator's manual.

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

Limits and ranges

Measuring range

0.90-179 µmol/L (5.00-1000 µg/dL, 0.05-10.0 mg/L)

Determine samples having higher concentrations via the rerun function. For samples with higher concentrations, the rerun function decreases the sample volume by a factor of 2.1. The results are automatically multiplied by this factor.

Lower limits of measurement

Lower detection limit of the test

0.90 µmol/L (5.00 µg/dL, 0.05 mg/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Values below the lower detection limit (< 0.90 µmol/L) will not be flagged by the instrument.

Expected values[16]

Adults:	5.83-34.5 µmol/L (33-193 µg/dL)

The concentration of iron in serum/plasma is dependent on ingestion of iron and is subject to circadian variations.[17]

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 an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained on the **cobas c** 701 analyzer:

Repeatability	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
Precinorm U	20.1 (112)	0.1 (1)	0.6
Precipath U	29.9 (167)	0.3 (2)	1.1
Human serum A	11.2 (62.6)	0.1 (0.6)	0.8
Human serum B	81.1 (453)	0.6 (3)	0.7
Human serum C	155 (866)	1 (6)	0.3

Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
Precinorm U	20.1 (112)	0.3 (2)	1.5
Precipath U	33.5 (187)	0.5 (3)	1.5
Human serum 1	11.8 (66.0)	0.2 (1.1)	1.8
Human serum 2	55.1 (308)	0.7 (4)	1.3

Results for intermediate precision were obtained on the **cobas c** 501 analyzer. The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 701 analyzer(s).

Method comparison

Iron values for human serum and plasma samples obtained on a **cobas c** 701 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 85

Passing/Bablok[18]	Linear regression
y = 0.987x + 0.245 µmol/L	y = 0.988x + 0.117 µmol/L
τ = 0.942	r = 0.999

The sample concentrations were between 2.73 and 167 µmol/L (15.1 and 934 µg/dL).

References

1 Swinkels DW. Iron Metabolism. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 40, p. 418-418.e40.

2 Muñoz M, García-Erce JA, Remacha ÁF. Disorders of iron metabolism. Part II: iron deficiency and iron overload. J Clin Pathol 2011 Apr;64(4):287-296.

3 Camaschella C. Iron deficiency: new insights into diagnosis and treatment. Hematology Am Soc Hematol Educ Program. 2015;2015:8-13. doi: 10.1182/asheducation-2015.1.8.

4 Cook JD, Skikne BS. Iron deficiency: definition and diagnosis. J Intern Med 1989 Nov;226(5):349-355.

5 Camaschella C. Iron-deficiency anemia. N Engl J Med 2015 May 7;372(19):1832-1843.

6 Kujovich JL. Evaluation of Anemia. Obstet Gynecol Clin North Am. 2016 Jun;43(2):247-264.

7 Short MW, Domagalski JE. Iron deficiency anemia: evaluation and management. Am Fam Physician 2013 Jan 15;87(2):98-104.

8 Kolnagou A, Kontoghiorghe CN, Kontoghiorghes GJ. New targeted therapies and diagnostic methods for iron overload diseases. Front Biosci (Schol Ed). 2018 Jan 1;10(1):1-20.

9 Guder WG, Narayanan S, Wisser H, et al. List of Analytes; Preanalytical Variables. Brochure in: Samples: From the Patient to the Laboratory. Darmstadt: GIT-Verlag 1996.

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

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

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

13 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.

14 Tietz NW, Rinker AD, Morrison SR. When Is a Serum Iron Really a Serum Iron? A Follow-up Study on the Status of Iron Measurements in Serum. Clin Chem 1996;42(1):109-111.

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

16 Löhr B, El-Samalouti V, Junge W, et al. Reference Range Study for Various Parameters on Roche Clinical Chemistry Analyzers. Clin Lab 2009;55:465-471.

17 Burtis CA, Ashwood ER, Bruns DE (eds.). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. St Louis, Missouri; Elsevier Saunders 2006;1190.

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:

CONTENT	Contents of kit
→	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

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606