IRON2

cobas®

Iron Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057931190*	08057931500	Iron Gen.2 (700 tests)	System-ID 2077 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057931214*	08057931500	Iron Gen.2 (700 tests)	System-ID 2077 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

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

English

System information

IRON2: ACN 20770

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 laboratory 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:

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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.

Hazardous components:

- Poly(oxy-1,2-ethanediyl), .alpha.-isotridecyl-.omega.-hydroxy-

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:	12 weeks

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 -20 °C (± 5 °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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/570 nm		
Reagent pipetting		Diluent (H_2O)	
R1	75 µL	–	
R3	15 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (NaCl)
Normal	6.4 µL	–	–
Decreased	9.0 µL	25 µL	50 µL
Increased	6.4 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change
	1-point recalibration using S1 - after **cobas c** pack green change - every 7 days on-board

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 12 weeks.

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 in the unit µmol/L (µg/dL, mg/L).

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. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.9 µmol/L (5.03 µg/dL)
Limit of Detection	= 0.9 µmol/L (5.03 µg/dL)
Limit of Quantitation	= 0.9 µmol/L (5.03 µg/dL)

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 a total error of 20 %. It has been determined using low concentration iron samples.

Expected values[16]

µmol/L

Adults:	5.83-34.5 µmol/L

µg/dL

Adults:	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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean* µmol/L	*SD* µmol/L	*CV* %
PCCC1[a)]	18.6	0.111	0.6
PCCC2[b)]	41.4	0.163	0.4
Human serum 1	2.37	0.0817	3.4
Human serum 2	6.01	0.0830	1.4
Human serum 3	35.1	0.135	0.4
Human serum 4	89.2	0.307	0.3
Human serum 5	158	0.655	0.4

Intermediate precision	*Mean* µmol/L	*SD* µmol/L	*CV* %
PCCC1[a)]	18.6	0.212	1.1
PCCC2[b)]	41.6	0.369	0.9
Human serum 1	2.32	0.120	5.2
Human serum 2	5.95	0.149	2.5
Human serum 3	35.1	0.187	0.5
Human serum 4	89.2	0.337	0.4
Human serum 5	158	0.673	0.4

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Iron values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 74

Passing/Bablok[18]	Linear regression
y = 1.004x + 0.0354 µmol/L	y = 1.003x + 0.00110 µmol/L
τ = 0.985	r = 1.000

The sample concentrations were between 1.20 and 169 µmol/L.

Iron values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 98

Passing/Bablok[18]	Linear regression
y = 1.011x - 0.0750 µmol/L	y = 1.011x - 0.0772 µmol/L
τ = 0.993	r = 1.000

The sample concentrations were between 1.72 and 172 µmol/L.

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Iron values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 75

Passing/Bablok[18]	Linear regression
y = 1.000x + 0.0000 µmol/L	y = 1.001x - 0.0494 µmol/L
τ = 0.998	r = 1.000

The sample concentrations were between 2.55 and 176 µmol/L.

References

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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.

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.

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