Iron

DiaSystem Scandinavia AB

Diagnostic reagent for quantitative in vitro determination of iron in serum and plasma on photometric systems

Order Information

Cat. No.	Kit size						
01 00028 70 04 0100	R1	4 x	20 mL	+	R2	1 x	20 mL
01 00028 70 04 0500	R1	5 x	80 mL	+	R2	1 x	100 mL
01 00028 70 10 0100	R1	2 x	40 mL	+	R2	2 x	10 mL
CDT-Iron	R1	4 x	20 mL	+	R2	1 x	20 mL

06 00109 70 04 0018 Iron Standard 6x3 mL

Summary [1,2]

Iron exists in the body as a component of hemoglobin and myoglobin as well as bound to transferrin for the transport in plasma and stored in ferritin. Increased iron concentrations occur in hemochromatosis and liver damage. Malabsorption due to gastrointestinal diseases can cause decreased iron levels, and may thus lead to anemia. Blood loss after gastrointestinal lesions or heavy menstrual bleeding can generate anemia, too.

Method

Photometric test using Ferene

Principle

Iron bound to transferrin is released in an acidic medium as ferric iron and is then reduced to ferrous iron in the presence of ascorbic acid. Ferrous iron forms a blue complex with Ferene. The absorbance at 595 nm is directly proportional to the iron concentration.

$Transferrin(Fe^{3+})_2 \xrightarrow{\text{Ascorbic acid, Buffer}} 2\ Fe^{2+} + Transferrin$

$Fe^{2+} + 3\ Ferene \longrightarrow$ Ferrous Ferene (blue complex)

Reagents

Components and Concentrations

R1:	Acetate buffer	pH 4.5	1 mol/L
	Thiourea		120 mmol/L
R2:	Ascorbic acid		240 mmol/L
	Ferene		3 mmol/L
	Thiourea		120 mmol/L
Standard			100 µg/dL (17.9 µmol/L)

Storage Instructions and Reagent Stability

Reagents and standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. Reagent 1: Danger. H315 Causes skin irritation. H318 Causes serious eye damage. P264 Wash hands and face thoroughly after handling. P280 Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a poison center or doctor/physician.
2. Standard: Warning. H290 May be corrosive to metals. P234 Keep only in original container. P280 Wear protective gloves/protective clothing/eye protection/face protection. P390 Absorb spillage to prevent material damage.
3. Use only disposable material to avoid iron contamination. Rinse glass material with diluted HCl and copious dist. water.
4. In very rare cases, samples of patients with gammopathy might give falsified results [8].
5. PlPlease refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patients' medical history, clinical examinations and other findings.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents and the standard are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma
Separate serum/plasma at the latest 2 h after blood collection to minimize hemolysis.

Stability [3]:			
	7 days	at	20 - 25 °C
	3 weeks	at	4 - 8 °C
	1 year	at	-20 °C

Discard contaminated specimens! Only freeze once!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	595 nm, 600 nm, Hg 623 nm
Optical path	1 cm
Temperature	20 – 25 °C, 37 °C
Measurement	Against reagent blank

	Blank	Sample or standard
Sample or standard	-	100 µL
Dist. Water	100 µL	-
Reagent 1	1000 µL	1000 µL
Mix, read absorbance A1 after 1 - 5 min, then add:		
Reagent 2	250 µL	250 µL
Mix, read absorbance A2 after 10 min.		

$\Delta A = (A2 - 0.82\ A1)$ Sample/Standard

The factor 0.82 compensates the decrease of the absorbance by addition of reagent 2. The factor is calculated as follows: (Sample + R1)/Total volume. This compensation is necessary as a high sample volume is used.

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Calculation

With standard or calibrator

$$Iron\ [\mu g/dL] = \frac{\Delta A\ Sample}{\Delta A\ Std./Cal.} \times Conc.\ Std./Cal.\ [\mu g/dL]$$

Conversion factor

Iron [µg/dL] x 0.1791 = [µmol/L]

Calibrators and Controls

For the calibration of automated photometric systems, DiaSystem UniCal CC calibrator is recommended. The assigned values of the calibrator have been made traceable to the NIST-SRM®682 reference material. DiaSystem UniLab N and DiaSystem UniLab P controls should be assayed for internal quality. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat.-No.	Kit size
UniCal CC	06 00122 70 04 0018	6 x 3 mL
UniLab N	07 00123 70 05 0030	6 x 5 mL
UniLab P	07 00124 70 05 0030	6 x 5 mL

Performance Characteristics

Measuring range

The test has been developed to determine iron concentrations within a measuring range from 5 – 1000 µg/dL (0.9 – 179 µmol/L). When values exceed this value samples should be diluted 1 + 2 with NaCl solution (9 g/L) and the result multiplied by 3.

Specificity/Interferences

No interference was observed by conjugated and free bilirubin up to 60 mg/dL, hemoglobin up to 100 mg/dL, lipemia up to 2000 mg/dL triglycerides, copper up to 200 µg/dL and zinc up to 400 µg/dL. For further information on interfering substances refer to Young DS [7].

Sensitivity/Limit of Detection

The lower limit of detection is 5 µg/dL (0.9 µmol/L).

Precision

Intra-assay precision n = 20	Mean [µg/dL]	SD [µg/dL]	CV [%]
Sample 1	98.0	1.00	1.02
Sample 2	164	2.01	1.22
Sample 3	216	2.11	0.98

Inter-assay precision n = 20	Mean [µg/dL]	SD [µg/L]	CV [%]
Sample 1	85.8	2.13	2.48
Sample 2	144	3.16	2.19
Sample 3	195	3.86	1.98

Method Comparison

A comparison of DiaSystem Iron (y) with a commercially available test (x) using 70 samples gave following results:
y = 0.99 x - 0.33 µg/dL; r = 0.999.

Reference Range [4]

	µg/dL	µmol/L
Children		
2 weeks	63-201	11-36
6 months	28-135	5-24
12 months	35-155	6-28
2 –12 years	22-135	4-24
Women		
25 years	37-165	6.6-29.5
40 years	23-134	4.1-24.0
60 years	39-149	7.0-26.7
Pregnant women		
12th gestational week	42-177	7.6-31.6
At term	25-137	4.5-24.5
6 weeks postpartum	16-150	2.9-26.9
Men		
25 years	40-155	7.2-27.7
40 years	35-168	6.3-30.1
60 years	40-120	7.2-21.5

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Wick M. Iron metabolism and its disorders. In: Thomas L, editor. Clinical laboratory diagnostics. 1st ed. Frankfurt: THBooks Verlagsgesellschaft; 1998. p. 268-73.
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