IRON (Ferrozine Method)

Invitro Diagnostic reagent kit for quantitative determination of Iron in human serum/plasma sample on Photometric System.

Order Information

Cat. No.	Kit Configuration
OAR1118	Reagent 1: 2 x 20 mL Reagent 2: 1 x 12 mL Standard: 1 x 3 mL

Summary

The iron is the component of a great number of enzymes. The myoglobin, muscular protein, contains iron, as well as the liver. Iron is necessary for the hemoglobin production, molecule that transports oxygen inside red globules. Their deficit in the last causes the ferropenic anemia. High levels of iron are found in hemochromatosis, cirrhosis, hepatitis and in increased transferrin levels.

Method

Ferrozine Method.

Principle

The Fe^{+3} bound to serum ferritine once dissociated in a weak-acid medium by Teepol and guanidium chloride, is reduced by hydroxylamine to Fe^{+2}, forming the ferrous ion a colored complex with Ferrozine proportional to the concentration of iron present in the sample.

Transferrin -Fe^{+3} $\xrightarrow{\text{Teepol (pH<5)}}$ Apotransferrin+

Fe^{+3} Fe^{+3} + Hydroxylamine $\longrightarrow$ Fe^{+2}

Fe^{+2} + Ferrozine $\longrightarrow$ Fe (FerroZine) $^{+2}$ complex

Reagents

Storage Instructions and Reagent Stability
Reagent 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!
Reagent 1: Buffer Solution
Reagent 2: Chromogen
Solution
Standard: Iron Standard (100 µg/dL)

Composition

Guanidine chloride 1.0 mol/L, hydroxylamine 0.6 mol/L, acetate buffer 400 mmol/L pH 4.0, Teepol, Ferrozine 8 mmol/L, sodium acetate 400 mmol/L.

Warnings and Precautions

- Keep out of reach of children. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Take off immediately all contaminated clothing.
- The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For professional use only!

Waste Management

Please refer to latest Biological Medical Waste (BMW) guidelines and local legal requirements.

Reagent Preparation

Mix, 4 parts of reagent 1 and 1 part of reagent 2 = working reagent. The stability of the working reagent is 5 days at 15°-25°C. 4 weeks at 2°- 8°C.
Protect the reaction solution from light and contamination.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma separate at the latest 1h after blood collection from cellular contents.
15 days at 2° –8°C
30 days at –20°C
Only freeze once! Discard contaminated specimens.

Assay Procedure

Wavelength 560 nm (540 nm – 580 nm) Optical Path 10 mm
Temperature 37°C

Sample Start

	Reagent Blank	Sample Blank	Sample/Standard/ Calibrator
Sample/Standard/ Calibrator	--	200 µL	200 µL
Distilled water	200 µL	--	--
R1 Buffer Solution	--	1000 µL	--
Working Reagent	1000 µL	--	1000 µL
Mix, incubate for 5 min. at 37°C. Read absorbance of standard and sample against reagent blank.			

Calculation

$$\text{Iron (µg/dL)} = \frac{\text{A (Sample) - A (Sample blank)}}{\text{A (Standard)}} \times \text{C (Standard Conc.)}$$

Conversion Factor

Iron (µg/dL) x 0.179 = Iron (µmol/L)

Quality Controls

For internal quality control any normal and abnormal controls should be assayed with each batch of samples. Each laboratory should establish corrective action in case of deviations in control recovery.

Performance Characteristics Measuring Range

The test has been developed determine Iron concentration within a measuring range from 2.5 - 1000 µg/dL. If such value is exceeded the sample should be diluted 1 + 1 with NaCl solution (9 g/L) and results multiplied by 2.

Interferences

No interference was observed by, Ascorbic acid up to 30 mg/dL, Bilirubin up to 40 mg/dL and Triglycerides up to 2000 mg/dL. Contamination of glassware with iron will affect the test. Use acid- washed glassware or plastic tubes.

Sensitivity/Limit of Detection

The lower limit of detection is 2.5 µg/dL.

Linearity

The Linearity of detection is 1000 µg/dL.

Precision

Intra-assay n = 20	Mean (µg/dL)	SD (µg/dL)	CV (%)
Sample 1	107.68	3.14	2.92
Sample 2	185.26	2.32	1.25

Inter-assay n = 20	Mean (µg/dL)	SD (µg/dL)	CV (%)
Sample 1	86.45	3.55	4.11
Sample 2	155.75	1.56	1.00

Method Comparison

A comparison of Nucleus Diagnosys Iron (y) with a commercially available test (x) using 15 samples gave following results:

$y = 1.011x - 0.406$; $r^2 = 0.995$.

Reference Range

Women	50 – 170 µg/dL (9.0 - 30.4 µmol/L)
Men	60 – 175 µg/dL (10.7 - 31.3 µmol/L)

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

Literature

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5. Central Pollution Control Board (CPCB) Guidelines for Management of healthcare waste as per Biomedical Waste Management rules, 2016. https://www.cpcb.nic.in/uploads/hwmd/Guidelines_healthcar eJune_2018.pdf

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