IRON FERROZINE LS 2nd GEN 4+1

IVD

Quantitative determination of Iron in Serum or Plasma. Colorimetric method. Ferrozine®.

REF CC1230 R1: 3x40 mL + R2: 1x30 mL + R3:1x3 mL (standard)

METHOD AND PRINCIPLE

In an acidic environment, iron is released from Transferrin, its carrier protein, in the form of a trivalent Iron ion (Fe^{3+}) and is reduced to a bi-valent Iron ion (Fe^{2+}) by ascorbate. The bi-valent ion reacts with Ferrozine® to form a colored complex. The intensity of color that develops, measured photometric at the wavelength of 578 nm, is directly proportional to the total amount of Iron present in the sample.

$$\text{Transferrin-Fe-complex} \xrightarrow{pH < 2,0} \text{Apotransferrin} + Fe^{3+}$$

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

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

CLINICAL SIGNIFICANCE

Following intestinal absorption of iron or erythrocyte destruction, iron ions are released into the plasma where they bind to either Apo Transferrin or Apo Ferritin proteins to form Transferrin and Ferritin, respectively. The former helps transport iron to bone marrow for erythropoiesis; the latter stores iron in tissues, until is needed. An increase in the iron level in plasma due to rapid destruction of erythrocytes or excessive uptake of iron may also lead to iron overload. The latter causes iron deposition disorders in tissue known as hemosiderosis or hemochromatosis. Conversely, a decrease in the iron level in plasma due to malnutrition or malabsorption may lead to excessive depletion in iron storage, resulting in anaemia such as iron-deficiency anaemia.

REAGENT COMPOSITION

Reagent (R1)	
Citric acid, pH 1.8	200 mmol/L
Thiourea	115 mmol/L
Na-Ascorbate	150 mmol/L
Detergent	< 1 %
Reagent (R2)	
Citric acid, pH 1.8	200 mmol/L
FerroZine®	6 mmol/L
Detergent	< 1 %
Reagent (R3)	
Standard (iron)	value on label

REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents.
Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera.
After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

SPECIMEN

Serum, heparin plasma.
Separate serum / plasma at the latest 2 h after blood collection to minimize hemolysis. EDTA and oxalate plasma cause decreased values.
Stability: 7 days at 2 - 8° C, 4 days at 15 - 25° C.
Discard contaminated specimens.

PROCEDURE

Wavelength:	578 nm (546-600)
Temperature:	37° C
Measurement:	Against Distilled Water

Pipette as follow:

Reagent (R1)	800 µL
Sample, Std or Cal	100 µL

Mix and incubate 5 minutes. Read the Absorbance (Abs1), then add:

Reagent (R2)	200 µL

Mix and incubate 5 minutes. Read again the Absorbance (Abs2).
Calculate Δ Abs (Abs2 – Abs1) for calibrator and samples.

CALCULATION

$$\text{Iron} = \frac{\Delta \text{ Abs Sample}}{\Delta \text{ Abs Calibrator}} \times \text{Concentration Std/Cal}$$

Conversion Factor: Iron [µg/dL] x 0.1791 = [µmol/L]

CALIBRATION WITH CALIBRATOR

The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.

As an alternative to the standard included in the package, it is possible to use **MTD Diagnostics Calibrator**:

Chemistry Multicalibrator - REF CAL1010 (10 x 3 mL)

QUALITY CONTROL

Normal and abnormal control sera of known concentration should be analysed routinely with each group of unknown samples utilizing **MTD Diagnostics** Quality Control Material:

Chemistry Control N - REF CNN1010 10 x 5 mL (Level 1)
Chemistry Control P - REF CNP1020 10 x 5 mL (Level 2)

EXPECTED VALUES

	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		
12 th 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 establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:
Low Level: Samples= 20; Average = 120.97; S.D. = 2.36; CV = 1.95%
High Level: Samples = 20; Average = 191.58; S.D. = 3.02; CV = 1.58%

ACCURACY (CORRELATION): A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

y=1.001x – 0.493 ; r=0.997

SENSITIVITY: 5.0 µg/dL

LINEARITY: 5.0 – 500.0 µg/dL

SPECIFICITY / INTERFERENCES

No interference was observed by conjugated and free bilirubin up to 60 mg/dL, haemoglobin 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.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact your distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.
3. ® Trade mark of Hach Chemical Co. Ames, Iowa.

PRECAUTIONS

R1 e R2 contein:
CITRIC ACID 200 mmol/L – pH 1.8 - CAS. N. 77-92-9 - Xi
R35 (H314): Causes severe skin burns and eye damage
R36/37/38 (H319 – H335 – H315): Irritating to eyes, respiratory system and skin

The product contains dangerous substances or mixtures according to the EC regulation n ° 1272/2008 (CLP), therefore it needs the special labeling required by the aforementioned regulation. The product is labeled according to the directive for CE marking (98/79 / CE).
Sodium Azide less than 0.1%.
However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:
S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.

FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

SIMBOLOGY

CE	CE Mark (EC Directive 98/79)		
IVD	In Vitro Diagnostic		Temperature Limitation
	Consult instructions for use		Contains sufficient for <n> test
REF	Catalog Number		Use By
LOT	Batch Code		Manufacturer

BIBLIOGRAPHY

Zilva JR, Pannall PR. “Iron Metabolism.” Clinical Chemistry in Diagnosis and Treatment (Ed) Lloyd-Luke 1979; Ch18:378-92.
Tietz NW “Textbook of Clinical Chemistry 2nd Edition.” Tietz NW (Ed) WBSaunders Company Philadelphia 1994; 2059.
Henry RJ “Clinical Chemistry: Principles and Techniques”, Second Edition (Ed) Harper and Row 1974; 682-95.
Young DS, et al. “Effects of Drugs on Clinical Chemistry Laboratory Tests.” Clinical Chemistry (Ed) Pestaner L.C. and Gibberman V. 1975; 21:321D.
Constantino NV, Kabat HF “Drug Modifi cation of Laboratory Test Values” (Ed) American Journal of Pharm. 1973; 30.
Tietz NW “Textbook of Clinical Chemistry.” (Ed) WB Saunders CompanyPhiladelphia 1986; 1582.