ISE S.r.l.
CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

IRON ferene – Instructions for use

R1: 2 x 18 mL • REF R3330000020
R2: 2 x 4,5 mL

CE IVD

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

TEST PARAMETERS

Method:	Colorimetric, endpoint, increasing reaction, Ferene
Wavelength:	595 nm, 600 nm, Hg 623 nm
Temperature:	20 – 25 °C, 37 °C
Sample:	Serum, heparin plasma
Linearity:	up to 1000 µg/dL (179 µmol/L)
Sensitivity:	The lower limit of detection is 5 µg/dL (0.9 µmol/L)

SUMMARY [1,2]

Iron exists in the body as a component of haemoglobin 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.

TEST 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.

$$\text{Transferrin } (Fe^{3+})_2 \xrightarrow{\text{Asc. Acid, Buffer}} 2\ Fe^{2+} + \text{Transferrin}$$

Fe^{2+} + 3 Ferene → Ferrous Ferene (blue complex)

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1:	
Acetate Buffer, pH 4.5	1 mol/L
Thiourea	120 mmol/L
Reagent 2:	
Ascorbic Acid	240 mmol/L
Ferene	3 mmol/L
Thiourea	120 mmol/L

REAGENT PREPARATION

Reagents are ready to use.

REAGENT STABILITY AND STORAGE

Conditions:	Protect from light (R2) Close immediately after use Do not freeze the reagents! Avoid contamination.
Storage:	at 2 – 8 °C
Stability:	up to the expiration date

SAMPLE STABILITY AND STORAGE

Separate serum/plasma at the latest 2 h after blood collection to minimize haemolysis.

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

Discard contaminated specimens. Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

STANDARD

(not included in the kit – has to be ordered separately)
Concentration: 100 µg/dL (17.9 µmol/L)
Storage: 2 – 25 °C
Stability: up to the expiration date
Close immediately after use! Avoid contamination.
Protect from light!

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Std./ Cal.	Sample
Sample	-	-	100 µL
StandardCalibrator	-	100 µL	-
Distilled Water	100 µL	-	-
Reagent 1	1000 µL	1000 µL	1000 µL
Mix, read absorbance A1 after 1 - 5 min against reagent blank. Then add:			
Reagent 2	250 µL	250 µL	250 µL
Mix, read absorbance A2 after 10 min. against reagent blank. ΔA = [(A2 – 0.82 A1) Sample or Std./Cal.]			

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.

CALCULATION

$$\text{Iron [µg/dL]} = \frac{\Delta A \text{ sample}}{\Delta A \text{ std/cal}} \times \text{Conc. Std/Cal [µg/dL]}$$

UNIT CONVERSION

µg/dL x 0.1791 = µmol/L

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
Females	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
Males	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.

PERFORMANCE CHARACTERISTICS

LINEARITY, 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.

SENSITIVITY/LIMIT OF DETECTION

I.S.E S.r.l.
Via Delle Driadi, 45 – 00133 Roma - Italy
Tel.+39 0774579365; FAX +39 0774579305
E-mail: info@logotech-ise.com
www.logotech-ise.com

Revision A R3330000020 11-2018

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

PRECISION

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

SPECIFICITY/INTERFERENCES

no interference up to:

Bilirubin	60 mg/dL
Hemoglobin	100 mg/dL
Triglyceride	2000 mg/dL
Copper	200 µg/dL
Zinc	400 µg/dL

For further information on interfering substances refer to Young DS [7].

METHOD COMPARISON

A comparison between this Iron Ferene (y) and a commercially available test (x) using 70 samples gave following results:
y = 0.99 x – 0.33 µg/dL; r = 0.999.

CALIBRATION

The assay requires the use of an Iron Standard or Calibrator.

QUALITY CONTROL

Control sera with iron values determined by this method can be used.
Each laboratory should establish corrective action in case of deviations in control recovery.

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. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
6. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

1. Wick M. Iron metabolism and its disorders. In: Thomas L, editor. Clinical laboratory diagnostics. 1 st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 268-73.
2. Fairbanks VF, Klee GG. Biochemical aspects of hematology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3 rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1642-1710.
3. Guder WG, Zawta B et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p34-5
4. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 273-5
5. Higgins T. Novel chromogen for serum iron determinations. Clin Chem 1981;27:1619.
6. Artiss JD, Vinogradov S, Zak B. Spectrophotometric study of several sensitive reagents for serum iron. Clin Biochem 1981;14:311-15.
7. Young DS. Effects of Drugs on Clinical laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.

Symbols used on labels and packaging

IVD = In vitro diagnostic medical device
REF = Catalogue Number
LOT = Lot Number
= Manufacturer
= Expiration date
= Temperature limitation
= Instruction for use

I.S.E S.r.l.
Via Delle Driadi, 45 – 00133 Roma - Italy
Tel.+39 0774579365; FAX +39 0774579305
E-mail: info@logotech-ise.com
www.logotech-ise.com