Iron Reagent
Liquid FerroZine® Method

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	Up to 179 µmol/L (1000 µg/dL)
Specimen Type	:	Serum
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		Exclamation Mark
REAG A	Reagent A (Buffer)		Health Hazard
REAG B	Reagent B (Chromogen)		

INTENDED USE
This reagent is intended for the in vitro quantitative, diagnostic determination of iron in human serum.

CLINICAL SIGNIFICANCE
The normal body contains approximately 50 - 70 mmol (3 - 4 g) of iron and, as free iron is toxic, it is usually bound to protein. About 70% of the total iron is circulating in the erythrocyte bound to haemoglobin. A further 25% is stored in the liver, spleen or bone marrow complexed with the iron storage compound ferritin. Only about 50 - 70 µmol (3 - 4mg) of the total body iron is circulating in the serum, bound to the iron transport protein Transferrin. It is this fraction which is measured in serum iron estimation. The remaining iron is incorporated into myoglobin, iron-containing enzymes and cytochromes.[1]
Decreased iron concentrations are seen in many but not all patients with iron deficiency anaemia, and chronic inflammatory disorders. Increased iron concentrations occur in iron loading disorders such as haemochromatosis, acute iron poisoning in children and acute hepatitis among others.[2]

METHODOLOGY
In an acidic medium transferrin bound iron dissociates into free ferric ions. Hydroxylamine hydrochloride reduces the ferric ions to ferrous ions which react with Ferrozine to form a strongly colored purple complex with an absorption maximum near 560 nm. The difference in color intensity measured at 560 nm, before and after the addition of Ferrozine, is proportional to the iron concentration in the sample.
In addition to iron, copper is the only other trace metal found in serum which reacts with Ferrozine to form a colored complex. Neocuproine is therefore also present in the color reagent to prevent copper interference.

REAGENT COMPOSITION

Active Ingredients	Concentration
Reagent A: Iron buffer	
Hydroxylamine hydrochloride	0.22 mol/L
Surfactant	
Acetate buffer, pH 4.5 (25°C)	
Reagent B: Chromogen	
Ferrozine	7.8 mmol/L
Hydroxylamine hydrochloride	0.22 mol/L
Neocuproine	14.4 mmol/L

Hazard Symbol: Exclamation Mark , Health Hazard
Signal Word: Warning

Hazard Statements
H302 Harmful if swallowed
H317 May cause an allergic skin reaction
H351 Suspected of causing cancer
H373 May cause damage to organs through prolonged or repeated exposure
Precautionary Statements - Prevention
Obtain special instructions before use
Do not handle until all safety precautions have been read and understood
Use personal protective equipment as required
Wash face, hands and any exposed skin thoroughly after handling
Do not eat, drink or smoke when using this product
Contaminated work clothing should not be allowed out of the workplace
Wear protective gloves
Do not breathe dust/fume/gas/mist/vapors/spray
Precautionary Statements - Response
IF exposed or concerned: Get medical advice/attention
Specific treatment (see supplemental first aid instructions on this label)
Skin
IF ON SKIN: Wash with plenty of soap and water
If skin irritation or rash occurs: Get medical advice/attention
Wash contaminated clothing before reuse
Ingestion
IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
Rinse mouth
Precautionary Statements - Storage
Store locked up
Precautionary Statements - Disposal
Dispose of contents/container to an approved waste disposal plant
Refer to the product Safety Data Sheet for additional information

REAGENT PREPARATION
Reagent A and Reagent B are ready to use as supplied.

STABILITY AND STORAGE
Prior to use:
When stored refrigerated at 2-8°C and protected from light, the reagents are stable until the expiry date stated on the bottle and kit box label.

Once the Reagent is Opened:
When stored capped at 2-8°C, the reagents are stable until expiry.

Indications of Reagent Deterioration:
- Turbidity,
- Presence of precipitate; and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING
Collection: Blood should be collected using materials (e.g.,syringes, test tubes) that are iron-free.
Serum: Use non-haemolysed serum.
Storage: Specimens are stable for at least 4 days at room temperature (18-25°C) or one week at 2-8°C.[3]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- A clinical chemistry analyzer capable of maintaining constant temperature (37°C), measuring absorbance at 560nm and accommodating a 2 reagent assay system
- Analyzer specific consumables, e.g.: sample cups.
- Normal and Abnormal assayed control material.
- Calibrator or a suitable aqueous Iron standard.
- Distilled or deionized water and related equipment, eg pipettes.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Wavelength	560 nm
Assay Type	Endpoint
Direction	Increase
Sample : Reagent Ratio	1 : 5
e.g.: Sample Vol	0.2 mL
Reagent 1 Vol	1.0 mL
Incubation Time	2 minutes
Reagent 2 Vol	0.04 mL
Delay Time	10 minutes

MANUAL PROCEDURE
The following instructions are designed for manual instrumentation.
1. Label one test tube or cuvette for a reagent blank, each standard, control and unknown sample.
2. Add 1.0 mL of Iron Buffer (Reagent A) to each tube or cuvette.
3. Add 0.2 mL of sample (water, standard, control, unknown) to appropriate tubes or cuvettes, mix and incubate for at least 2 minutes.
4. Zero the spectrophotometer at 560nm with the reagent blank tube.
5. Read and record the absorbance of each standard, control and unknown sample at 560nm to obtain sample blank readings (A1).
6. Add 0.04 mL of Chromogen (Reagent B) to all tubes, mix and incubate for 10 minutes.
7. Re-zero spectrophotometer against reagent blank with Chromogen (Reagent B) added.
8. Read and record absorbance of each tube to obtain test readings (A2).
9. Subtract the sample blank reading from the test reading to obtain Δ Absorbance (A2 - A1).

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CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

$$\text{Serum Iron } (\mu\text{mol/L}) = \frac{\Delta\text{Abs of Unknown}}{\Delta\text{Abs of Standard}} \times \text{Conc of Std } (\mu\text{mol/L})$$

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. The color development is stable for 30 minutes.
3. Specimens with iron concentrations greater than 179 µmol/L (1000 µg/dL) should be diluted with saline and reassayed. Multiply the results by the dilution factor.
4. Even though the reaction of iron with Ferrozine in fresh serum is instantaneous, in some lyophilised control sera the reaction is delayed and a 10 minute incubation is recommended.
5. Unit conversion: µmol/L x 5.585 = µg/dL.

CALIBRATION

Calibration is required. An aqueous standard or serum based calibrator, with and assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For Calibration Frequency on automated instruments refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin, lipaemia and ascorbic acid were carried out and the following results were obtained:
   Haemoglobin: Avoid the use of haemolysed samples.
   Bilirubin: No interference from bilirubin up to 1026 µmol/L (60 mg/dL).
   Lipaemia: Avoid the use of lipaemic samples.
   Ascorbic Acid: No interference from ascorbic acid up to 10 mg/dL.
2. For a more comprehensive review of factors affecting iron assays refer to the publications of Young[4] and Constantino.[5]

EXPECTED VALUES[6]

Adult Males:	12.5 - 32.2 µmol/L (70 - 180 µg/dL)
Adult Females:	10.7 - 32.2 µmol/L (60 - 180 µg/dL)

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.

PERFORMANCE DATA

The following data was obtained using the Liquid Iron Reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on their specific analyzer used.

IMPRECISION

Imprecision was evaluated using three levels of commercial control and following the NCCLS EP5-T procedure.[7]

	LEVEL I	LEVEL II	LEVEL III
Number of data points	20	20	20
Mean (µmol/L)	9.9	40.8	89.0
Mean (µg/dL)	55.5	228.0	497.0
C.V. (%) Within run	2.8	1.1	0.4
C.V. (%) Between day	5.4	1.4	0.6

ACCURACY

Comparison studies were carried out using a similar commercially available Iron reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	51
Range of sample results	5.4 - 64.5 µmol/L (30.0 - 360.0 µg/dL)
Mean of reference method results	21.7 µmol/L (121 µg/dL)
Mean of Iron results	21.7 µmol/L (121 µg/dL)
Slope	1.003
Intercept	-0.2 µmol/L (-1.2 µg/dL)
Correlation coefficient	0.9858

LINEARITY

When run as recommended the assay is linear up to 179 µmol/L (1000 µg/dL). Linearity on automated instruments will be dependent upon the ratio of sample volume to reagent volume used and the timing of measurements. The specific instrument application should be consulted.

SENSITIVITY

When run as recommended the sensitivity of this assay is 3.7 ΔmAbs per µmol/L or 0.67 ΔmAbs per µg/dL (1cm light path, 550nm).

REFERENCES

1. Zilva JR, Pannall PR. "Iron Metabolism." Clinical Chemistry in Diagnosis and Treatment (Ed) Lloyd-Luke 1979; Ch18:378-92.
2. Tietz NW "Textbook of Clinical Chemistry 2nd Edition." Tietz NW (Ed) WB Saunders Company Philadelphia 1994; 2059.
3. Henry RJ "Clinical Chemistry: Principles and Techniques", Second Edition (Ed) Harper and Row 1974; 682-95.
4. Young DS, et al. "Effects of Drugs on Clinical Chemistry Laboratory Tests." Clinical Chemistry (Ed) Pestaner L.C. and Gibberman V. 1975; 21:321D.
5. Constantino NV, Kabat HF "Drug Modification of Laboratory Test Values" (Ed) American Journal of Pharm. 1973; 30.
6. Tietz NW "Textbook of Clinical Chemistry." (Ed) WB Saunders Company Philadelphia 1986; 1582.
7. National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices. NCCLS, 1984, NCCLS Publication EP5-T.

Fisher Diagnostics
a division of Fisher Scientific Company, LLC
a part of Thermo Fisher Scientific Inc.
Middletown, VA 22645-1905 USA
Phone: 800-528-0494
540-869-3200
Fax: 540-869-8132

EC REP WMDE
Bergerweg 18
6085 AT Horn
The Netherlands

JL840885-en (R0)

REF

Reorder Information

	REAG A	REAG B
TR46101	2 x 125 mL	1 x 14 mL