













# 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

	Authorised Representative		Temperature Limitation
	For in vitro diagnostic use		Use by/Expiration Date
	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
	Catalogue number		Manufactured by
	Consult instructions for use		Xi - Irritant
	Reagent A (Buffer)		
	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.<sup>1</sup>

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.<sup>2</sup>

#### 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 coloured purple complex with an absorption maximum near 560 nm. The difference in colour 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 coloured complex. Neocuproine is therefore also present in the colour reagent to prevent copper interference.

#### REAGENT COMPOSITION

##### Active Ingredients

##### 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

**WARNING:** Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. For further information consult the Iron Reagent Material Safety Data Sheet.

##### Reagent A and Reagent B:

R43	May cause sensitisation by skin contact.
S24	Avoid contact with skin.
S37	Wear suitable gloves.

#### 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<sup>3</sup>

#### ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) measuring absorbance at 560nm and accommodating a 2 reagent assay system
- Analyser specific consumables, eg: sample cups.
- Normal and Abnormal assayed control material.
- Calibrator or a suitable aqueous Iron standard.
- Distilled or deionised 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
eg: 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).

#### 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 colour 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<sup>4</sup> and Constantino.<sup>5</sup>

## EXPECTED VALUES<sup>6</sup>

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 analyser. Users should establish product performance on their specific analyser used.

## IMPRECISION

Imprecision was evaluated using three levels of commercial control and following the NCCLS EP5-T procedure.<sup>7</sup>

	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.



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REF

## Reorder Information

REAG A REAG B

TR46101 2 x 125 mL 1 x 14 mL