IRON

(Ferrozine Method)

ENZOPAK

Last update 09-2020

Ref. CC1-IRN.055, 20 Test

INTENDED USE

Reagent kit for quantitative estimation of iron in serum.

DIAGNOSTIC SIGNIFICANCE

Iron is usually bound to protein. Approximately 73% of the total iron is circulating in the erythrocyte bound to haemoglobin. The normal body contains approximately 51 to 73 mmol (3.2 to 4.3 gms) of iron and as free iron is toxic for body, approximately 27% is stored in the liver, spleen or bone marrow associated with the iron storage compound ferritin. Only 51-73 µmol (3.2 to 4.3 mg) of the total body iron is circulating in the serum bound to the transport protein transferrin. The remaining iron is incorporated into myoglobin, iron containing enzymes and cytochromes.

Increased iron concentrations occur in iron loading disorders such as haemochromatosis, acute iron poisoning in children and acute hepatitis among others. Decreased iron concentrations are seen in many but all patients with iron deficiency, anaemia and chronic inflammatory disorders.

PRINCIPLE

Transferrin bound iron breaks into free ferric ions in an acidic medium. These ferric ions react with Hydroxylamine Hydrochloride reduces into ferrous ions which react with Ferrozine to form a purple coloured complex measured at 560 nm. The difference before and after the addition of ferrozine is proportional to iron concentration reaction in the specimen.

PRESENTATION

Store all reagents at 2-8^{0}C	No. of Bottles 20 Tests
• 1 Iron (Buffer Reagent)	2
• 2 Iron (Color Reagent)	1
• 3 Iron (Enhancer)	2
• Iron Standard (80 µmol/L)	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Sodium Acetate	50 mmol/L
• Hydroxylamine Hydrochloride	300 mmol/L
• Detergent	5 mmol/L
• Ferrozine	4 mmol/L
• Enhancer	10 mmol/L
• pH 4.5 ± 0.1 at 25^0 C	

Iron Standard (80 µmol/L)

Also contains non-reactive fillers and Stabilizers.

PRECAUTION

It is essential that all the glasswares used for assay should be Iron-free. Glasswares should be soaked in 0.1N HNO_3 or 1N HCl & rinsed thoroughly with iron-free deionized water.

PREPARATION OF WORKING REAGENT

Carefully transfer the content of one vial of 3 Iron to one bottle of 1 Iron. Mix to dissolve. Wait for 15 minutes before use.

REAGENT STORAGE AND STABILITY

All the reagents included in the kit are stable at 2-8°C until the expiry date indicated on the label, when protected from light.

The working reagent is stable for 60 days at 2-8^{0}C.

SPECIMEN COLLECTION

Fresh clear serum with no hemolysis should be used. Plasma should not be used. Specimens are stable for one day at room temperature or one week at 2-8^{0}C.

REACTION PARAMETERS

Monochromatic

Temperature	: 37^0 C
Wavelength	: 560 nm
Type of reaction	: End point (Increase)
Incubation	: 10 min at 37^{0}C.
Std. Concentration	: 80 µmol/L (446 µg/dl)
Std./Sample volume	: 200 µl (0.200 ml)
Reagent 1 Volume	: 1.0 ml
Reagent 2 Volume	: 0.050 ml (50 µl)
Light path	: 1.0 cm
Zero setting with	: Reagent blank

Bichromatic

Other parameters as above	
Wavelength	: 560 nm and 630 nm
Sample Blank	: No
Zero setting with	: Distilled water

Set the instrument using above system parameters

TEST PROCEDURE

A) Monochromatic Method

Pipette Into Test Tubes	BLANK	STD.	TEST	
			Sample Blank(A1)	Sample Test(A2)
Working Reagent (ml)	1.0	1.0	1.0	1.0
Sample (ml)	-	-	0.2	0.2
Standard (ml)	-	0.2	-	-
Dis. Water (ml)	0.2	-	-	-
Rgt.-2 (ml)	0.05	0.05	-	0.05

Mix and allow to stand for 10 minutes at 37^{0}C. Read absorbance of test (A1& A2) and standard against reagent blank at 560 nm.

B) Bichromatic Method

Pipette Into Test Tubes	STANDARD	TEST
Working Reagent (ml)	1.0	1.0
Sample (ml)	-	0.2
Standard (ml)	0.2	-
Reagent-2 (ml)	0.05	0.05

Mix and allow to stand for 10 minutes at 37^{0}C. Read absorbance of test and standard against distilled water at 560 nm & 630 nm.

TEST RESULTS

$$\text{Serum Iron (µmol/L)} = \frac{\text{Abs or } \Delta \text{ Absorbance of test}}{\text{Absorbance of standard}} \times 80 \text{ (µmol/L)}$$

Where

Δ Abs. = (A2-A1)

80 µmol/L = Concentration of Standard

To convert (µg/dl) = µmol/L x 5.585

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FORMULA

$$\% \text{ Saturation of Transferrin} = \frac{\text{Serum Iron}}{\text{TIBC}} \times 100$$

LIMITATIONS FOR INTERFERENCE

- Haemolysis causes falsely elevated results.
- Iron medications (oral, intravenous or intravascular) affect serum levels.

NORMAL VALUES

Serum Iron

Male : 12.5 - 32.2 µmol/L (70-180 µg/dl)
Female : 10.7 - 32.2 µmol/L (60-180 µg/dl)

% Saturation of Transferrin

Male : 20 - 50 %
Female : 15 – 50 %

LINEARITY

This procedure is linear upto 89 µmol/L (500 µg/dl). For sample values higher than 89 µmol/L (500 µg/dl), dilute the sample suitably with 0.9% saline and repeat the assay. Apply dilution factor to obtain test results.

REFERENCES

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