

Reagent for quantitative estimation of IRON in human 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.

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 578 nm.

SPECIMEN COLLECTION:

Fresh clear serum with no hemolysis should be used. **Plasma should not be used.**

KIT PRESENTATION:

Pack Size	25 Test	50 Test	2 X 50 ml
R1 – IRON	1 X 25 ml	1 X 50 ml	2 X 50 ml
R2 - IRON	1 X 1.4 ml	1 X 2.7 ml	1 X 5.2 ml
IRON Std.(100 μg/dl)	1 X 2 ml	1 X 2 ml	1 X 2 ml

REAGENT STORAGE & STABILITY:

IRON reagents and standard are stable at 2-8°C until the expiry date indicated on the label.

PRECAUTION:

It is essential that all the glassware used for assay should be iron-free. Glassware should be soaked in 0.1N HNO₃ or HCl & rinsed thoroughly with iron-free deionized water.

ASSAY PARAMETERS:

Reaction : End point	Sample Vol. : 200 μl
Wavelength 1 : 578 nm	R1 + R2 Vol. : 1.0 ml + 50 μl
Wavelength 2 : 630 nm	Std Conc. : 100 μg /dl
Zero Setting : Distilled Water	Linearity : 500 μg /dl
Incub. Time : 5+10 mins at 37 °C	Unit : μg /dl

PROCEDURE:

Pipette into TT	Standard	Test
R1 - IRON	1.0 ml	1.0 ml
IRON Std (100 μg/dl)	0.200ml (200 μl)	--
Sample (Test)	--	0.200ml (200 μl)
Mix & Incubate for 5 minutes at 37 °C		
R2 – IRON	0.05 ml (50 μl)	0.05 ml (50 μl)
Mix & Incubation for 10 minutes at 37 °C. Read absorbance of Standard (S) and Test (T) against Distilled Water at 578 nm & 630 nm.		

CALCULATION:

$$\text{IRON Conc. (}\mu\text{g/dl)} = \frac{\text{Absorbance of Test} \times 100}{\text{Absorbance of Standard}}$$

Unit Conversion

$$\mu\text{g/dl} \div 5.585 = \mu\text{Mol/L}$$

NORMAL VALUES:

Male : 70 – 180 μg/dl (12.5 – 32.2 μMol/L)

Female : 60 – 180 μg/dl (10.7 – 32.2 μMol/L)

Each laboratory should establish its own reference range.

LINEARITY:

The method is linear up to 500 μg/dl (89 μMol/L). For sample values higher than 500 μg/dl (89 μMol/L), dilute the sample suitably with 0.9% saline and repeat the assay. Apply dilution factor to result.

REFERENCES:

1. CaO G. and Prior R.L. Clinical Chemistry Anthocyanins and iron metabolism in human serum 1999b; 574-76.
2. Tietz NW "Text book of clinical chemistry 2nd Edition" Tietz NW (Ed) WB Saunders company Philadelphia 1994; 2059.

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