

Total Iron Chromazurol B – Single Reagent

Diagnostic reagent for the in-vitro quantitative determination of total iron in human serum and plasma on manual systems.

REF: BS.1/TI02.025.0050	50 test	REF: BS.1/ TI02.050.0100	100 test
REF: BS.1/ TI04.025.0100	100 test		

CLINICAL SIGNIFICANCE

The majority of iron in the body (~ 3 - 3.5 g) is found in the hemoglobin of the red blood cells or their precursors in the bone marrow. Plasma contains very small fraction of iron (~ 2.5 mg). Iron is transported from one organ to another as a complex formed of ferric ions and a protein called apotransferrin, this iron-protein complex is called transferrin. The major iron-storage compound in the body is ferritin; it occurs in almost all body cells but particularly in hepatocytes. Serum iron is measured by the quantity of iron bound to transferrin, while TIBC is a direct measurement to transferrin. Elevated serum iron levels have been found in cases of hemochromatosis, hepatitis, hepatic necrosis and hemolytic anemia. Decreased levels have been associated with iron deficiency anemia, chronic blood loss, chronic disorders and insufficient dietary iron. The TIBC varies in disorders of iron metabolism so, TIBC is elevated in iron deficiency anemia. The measurements of both serum iron and TIBC is fundamental in evaluation and differential diagnosis of various types of anemia, liver disease and chronic illness.

METHOD PRINCIPLE

Iron reacts with chromazurol B and cetyltrimethyl-ammonium bromide (CTMA) to form a coloured ternary complex with an absorbance measured at 623 nm. The intensity of the colour produced, is directly proportional to the concentration of iron in the sample.

REAGENT COMPOSITION

R1: Standard	200 µg/dl (35.8 µmol/L)
R2: Reagent	
Acetate buffer PH 4.7	50 mM
CAB	0.13 mM
CTMA	0.82 mM

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the total iron reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien total iron reagents are supplied ready-to-use. All reagents are stable until expiration date stated on label when stored at 2-8°C.

Deterioration

The **BioScien** total iron reagent is normally clear, do not use reagent if it is turbid.

SPECIMEN COLLECTION AND PRESERVATION

The recommended specimen is serum or heparinized plasma. Plasma specimens collected with EDTA, oxalate, or citrate as anticoagulants are unsatisfactory since they bind iron, preventing its reaction with the chromogen. Morning specimen is preferable to avoid low result due to diurnal variation. The biological half-life of iron in blood is few hours.

Stability: 7 days at 15 –25 °C ; 3 weeks at 2 - 8 °C; 1 year at -20 °C.

SYSTEM PARAMETERS

Wavelength	623 nm
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample Reagent Ratio	1:25
e.g.: Reagent volume	1 ml
Sample volume	40 µl
Temperature	15-25°C
Incubation time	5 min at 25-37°C
Zero adjustment	Reagent blank
Sensitivity	12 µg/dl
Linearity	500 µg/dl

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Blank	Standard	Specimen
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard		40 µl	
Specimen			40 µl

Mix, incubate for 5 min at 25-37°C. Measure absorbance of specimen and standard against reagent blank.

CALCULATION

Total iron concentration ($\mu\text{g/dl}$) = $\frac{\text{Abs. specimen} \times 200}{\text{Abs. standard}}$

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **BioScien** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean $\mu\text{g/dl}$	159	344	162	351
SD.	2.1	1.9	2.9	2.6
CV. %	2.3	0.57	2.9	0.68

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)

Result obtained from **BioScien** total iron reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.983.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 12 $\mu\text{g/dl}$.

Linearity

The reaction is linear up to concentration of 500 $\mu\text{g/dl}$. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result $\times$ 2).

INTERFERING SUBSTANCES

Haemolysis

No interference up to haemoglobin level of 5 g/L (0.3 mmol/L) in determining serum iron and up to 1 g/L for TIBC.

Icterus

No significant interference up to a bilirubin level of 30 mg/dL.

Lipemia

Lipemic specimens are not recommended since they may cause negative bias. Lipemic specimens can be diluted before assay and the dilution factor should be considered during calculation.

Anticoagulants

Citrate, EDTA, and oxalate should be avoided.

EXPECTED VALUES

Serum / Plasma	$\mu\text{g/dl}$
< 7 months	150 – 220
Adults women	37 – 145
Men	55 – 175

DYNAMIC RANGE

12 – 500 $\mu\text{g/dl}$.

REFERENCES

1. Bauer JD. Haemoglobin, porphyrin, and iron metabolism. In: Kaplan LA, Pesce AJ, ed. Clinical Chemistry, theory, analysis, and correlation. ST. Louis: Mosby Company: 1984: 611-655.
2. Fairbanks VF, Klee GG. Biochemical aspects of hematology. In: Tietz NW, ed. Fundamentals of clinical chemistry. 3rd ed. Philadelphia: WB Saunders: 1987: 789-824.
3. Stookey LL. Ferrozine-a new spectrophotometric reagent for iron. Anal Chem. 1970; 42: 779-781.
4. Viollier MA, Gschwind H, Schläpfer P. Neue serumeisenbestimmung auf dem GSA II. Lab Med. 1980; 4: 240-244.
5. Williams HL, Johnson DJ, Haut MJ. Simultaneous spectrophotometry of Fe^{2+} and Cu^{2+} in serum denatured with guanidine hydrochloride. Clin Chem. 1977; 23: 237-240.

SYMBOLS IN PRODUCT LABELLING	
IVD	For in-vitro diagnostic use
LOT	Batch Code/Lot number
REF	Catalogue Number
	Temperature Limitation
	Expiration Date
	Manufactured by
	Number of <n> test in the pack
	Caution
	Do not use if package is damaged
	Consult Instruction for use



Medical Device Safety Service

MDSS GmbH

Schiffgraben 41

30175 Hannover, Germany

