SPECTRUM

The Creative Approach to Bioscience

Total Iron chromazurol B (Single Reagent)

REF: 269 001 (50 test) 2 x 25 ml
REF: 269 002 (100 test) 4 x 25 ml

SYMBOLS IN PRODUCT LABELLING

EC REP Authorised Representative	Use by/Expiration Date
IVD For in-vitro diagnostic use	CAUTION. Consult instructions for use
LOT Batch Code/Lot number	
REF Catalogue Number	Manufactured by
Consult instructions for use	(Xi) - Irritant
Temperature Limitation	

Intended Use

Spectrum Iron reagent is intended for the in-vitro quantitative, diagnostic determination of total iron in human serum or heparinized plasma on manual and automated systems.

Background

The majority of iron in the body (~3 – 3.5 g) is found in the haemoglobin 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 particulary 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 it 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

Colorimetric CAB Method.

Assay 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.

Reagents

Standard Iron (ST)	200 µg/dL 35.8 µmol/L
Acetate buffer PH 4.7	50 mM
CAB	0.13 mM
CTMA	0.82 mM
Preservatives and Stabilizers	

Precautions and Warnings

Do not ingest or inhalate. 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.
Iron test is very sensitive against contamination: Use only bidistilled water.
Contaminated glasswares are a source of error.
Disposable plastic ware is recommended for the test.

Reagent Preparation, Storage and Stability

All reagents are supplied ready to use and stable until expiration date stated on label when stored at 15 - 25 ^{o}C.Once opened, the reagent and the standard vials are stable for 3 months at specified temperature.

Deterioration

Failure to recover control values within assigned range may indicate reagent deteriortion

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 preferrable 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 ^{o}C ; 3 weeks at 2 – 8 ^{o}C;
1 year at -20 ^{o}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	25 ^{o}C ,30 ^{o}C or 37 ^{o}C
Incubation time	5 minutes
Zero adjustment	Reagent Blank
Reagent Blank Limits	Less than 1 AU
Sensitivity	12 µg/dL
Linearity	500 µg/dL

Procedure

	Reagent Blank	Standard	Sample
Reagent (R)	1.0 ml	1.0 ml	1.0 ml
Standard	------	40 µl	------
Sample	------	------	40 µl

Mix, and incubate for 5 minutes at 25,30 or 37 ^{o}C. Read the absorbance of the standard and sample against reagent blank.

Calculation

$$\text{Iron conc. (µg/dL)} = \frac{A_{sample}}{A_{standard}} \times 200$$

SI units

(µg/dL) x 0.1791 = µmol/L

Quality Control

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Important notes

1. Make sure that the distilled (double distilled) water is absolutely iron free.
2. Do not use turbid or hemolytic sera or plasma.

3. This iron test is very sensitive.
   To avoid contamination the glassware used must be iron free. We strongly recommend to use disposable laboratory materials when performing this test.
4. Bilirubin up to 15 mg/dL and copper up to 500 µg/dL do not interfere.

Performance Characteristics

Precision

Within run (Repeatability)

	Total Iron	
	Level 1	**Level 2**
n	20	20
Mean (µg/dL)	159	344
SD	2.1	1.9
CV%	1.32	0.55

Run to run (Reproducibility)

	Total Iron	
	Level 1	**Level 2**
n	20	20
Mean (µg/dL)	162	351
SD	2.9	2.6
CV%	1.79	0.74

Methods Comparison

A comparison between Spectrum Iron reagent and a commercial reagent of the same methodology was performed on 200 human sera. A correlation of 0.983 was obtained.

Sensitivity

When run as recommended, the sensitivity of this assay is 12 µg/dL for serum iron.

Linearity

The reaction is linear up to iron concentration of 500 µg/dL, Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result × 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

1- Neonates : 36 – 184 µg/dL (6. 4 - 33 µmol/L)
2- < 7 months : 37 – 145 µg/dL (7.7 - 33 µmol/L)
3- Adults
   a) Women : 37 – 145 µg/dL (6.6 - 26 µmol/L)
   b) Men : 59 – 158 µg/dL (10.6 - 28 µmol/L)

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature reference.

Analytical Range

Iron : 12 – 500 µg/dl (0.9 – 89.5 µmol/ L).

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct 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. refer to special instructions/safety data sheets.

References

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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 Fe2+ and Cu2+ in serum denatured with guanidine hydrochloride. Clin Chem.1977;23:237-240.

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
269 001	2 x 25 ml
269 002	4 x 25 ml

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