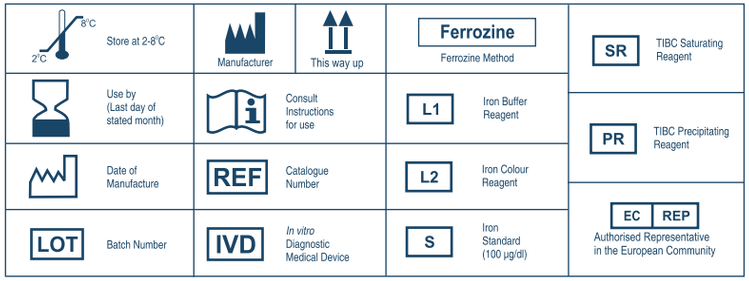
SBio IRON & TIBC KIT

(Ferrozine / Magnesium Carbonate Method)

(For invitro diagnostic use only)

REF	90550075	90560150
Pack Size	75 ml	150 ml

CE



INTENDED USE

Iron & TIBC Kit is used for the determination of Iron and Total Iron Binding Capacity in serum.

PRINCIPLE OF THE TEST

Iron, bound to Transferrin, is released in an acidic medium and the Ferric ions are reduced to Ferrous ions. The Fe (II) ions react with Ferrozine to form a violet coloured complex. Intensity of the complex formed is directly proportional to the amount of Iron present in the sample. For TIBC, the serum is treated with excess of Fe (II) to saturate the iron binding sites on transferrin. The excess Fe (II) is adsorbed and precipitated and the Iron content in the supernatant is measured to give the TIBC.

Fe (III) —(Acidic Medium)→ Fe (II)

Fe(II) + Ferrozine → Violet Coloured Complex

CLINICAL SIGNIFICANCE

Iron deficiency is the most common cause of anemia. It is usually the result of blood loss but may occasionally be secondary to iron malabsorption. Total iron binding capacity (TIBC) measures the total capacity of your blood to transport iron. TIBC correlates with the amount of the protein transferrin in your blood. TIBC is an indirect measurement of transferrin, a protein that binds and transports iron. It quantifies transferrin in terms of the amount of iron it can bind. Classically, TIBC is elevated in iron deficiency, pregnancy and by anovulatory agents. Because transferrin levels are depressed in patients who are malnourished or who have chronic disease states, it may be normal in many patients who are iron deficient.

PRESENTATION	75 ml	150 ml
Iron Reagents		
L1 : Iron Buffer Reagent	75 ml	150 ml
L2 : Iron Colour Reagent	4 ml	8 ml
S : Iron Standard (100 µg/dl)	2 ml	4 ml
TIBC Reagents	**75 ml**	**150 ml**
SR : TIBC Saturating Reagent	20 ml	40 ml
PR : TIBC Precipitating Reagent	2 gms	4 gms

COMPOSITION

Acetate Buffer 100 mmol; Sodium Hydroxyl Ammonium Chloride 225 mmol; Ferrozine 15 mmol; Detergents and Preservatives.
Iron Solution 50 µg/L; Magnesium Carbonate.

STORAGE / STABILITY

Contents are stable at 2-8°C till the expiry mentioned on the labels.

REAGENT PREPARATION

Reagents are ready to use.

SAMPLE MATERIAL

Serum, free from haemolysis.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.
This product requires the handling of human specimens. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.
Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

PROCEDURE

Wavelength/filter	:	570 nm (Hg 578 nm) / Yellow
Temperature	:	R.T.
Light path	:	1 cm

MATERIALS REQUIRED BUT NOT PROVIDED

General laboratory instrumentation like Spectrophotometer/Analyzer, Thermostatic Cuvette holder, Cuvettes, Micropipettes, Test tubes, Waterbath, Stopwatch/Timer.

Iron Assay:
Pipette into clean dry test tubes labeled as Blank (B), Standard (S), Sample Blank (SB) and Test (T):

Addition Sequence	B (ml)	S (ml)	SB (ml)	T (ml)
Iron Buffer Reagent (L1)	1.0	1.0	1.05	1.0
Distilled Water	0.2	--	--	--
Iron Standard (S)	--	0.2	--	--
Sample	--	--	0.2	0.2
Iron Colour Reagent (L2)	0.05	0.05	--	0.05

Mix well and incubate at R.T. for 5 minutes. Measure the absorbances of the Blank (Abs.B), Standard (Abs.S), Sample Blank (Abs.SB) and Test Sample (Abs.T) against D.W.

TIBC Assay:
Pipette into a clean dry test tube

Serum TIBC Saturating Reagent (SR)	0.5 ml 1.0 ml
Mix well and allow to stand at R.T. for 10 min. and add	
TIBC Precipitating Reagent (PR)	Approx. 50 mg.

Mix well and allow to stand at R.T for 10 minutes. Centrifuge at 2500-3000 rpm for 10 minutes to obtain a clear supernatant. Determine the Iron content in the supernatant as mentioned in the iron assay.

CALCULATIONS

$$\text{Iron in µg/dl} = \frac{\text{Abs.T} - (\text{Abs. SB} + \text{Abs.B})}{\text{Abs. S} - \text{Abs. B}} \times 100$$

$$\text{TIBC in µg/dl} = \frac{\text{Abs.T} - (\text{Abs. SB} + \text{Abs.B})}{\text{Abs. S} - \text{Abs. B}} \times 300$$

UBIC in µg/dl = TIBC in µg/dl - Iron in µg/dl

QUALITY CONTROL

The following process is recommended for QC during the assay of Iron/TIBC. *Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, recalibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity:
This procedure is linear upto 1000 µg/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using an appropriate dilution factor.

Limit of detection:
The limit of detection for Iron / TIBC is 4 / 12 µg/dl .

Interferences:
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Precision:
Precision studies were performed with two controls using NCCLS protocol EP5-A. The results of the precision studies are shown below:

Sample	Within-run		Between-run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control 1	121.05	2.92	121.35	2.89	242.4	5.81
Control 2	174.92	1.96	165.17	2.37	340.09	4.33

Method comparison:
Comparative studies were done to compare our reagent with another commercial Iron/TIBC Assay. No significant differences were observed. Details of the comparative studies are available on request.

REFERENCE RANGE

Serum Iron	(Males)	:	60 - 160 µg/dl
	(Females)	:	35 - 145 µg/dl
	(Neonates)	:	150 - 220 µg/dl
TIBC		:	250 - 400 µg/dl
UBIC		:	160 - 360 µg/dl

It is recommended that each laboratory establish its own normal range representing its patient population.

NOTE

Iron is reported to be stable in serum for 7 days at 2-8°C. Hemolysis interferes with the test as the hemoglobin present in the RBC's have a very high iron content.
All glassware being used for the test should first be rinsed with 1% or 0.1 N HCl and then with good quality deionised water before use. The reagent may be used in several automated analyzers. Instructions are available on request. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

1. Siedel, J., et. al. (1984) Clin Chem. 30 : 975.
2. Clinical Chemistry, Principles, Procedures, Correlations, Michael L. Bishop, et.al., 5th Edition.

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EC REP

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