NEO CHEM
TIBC
(MODIFIED COLORIMETRIC METHOD)

KIT NAME	KIT SIZE
NEO CHEM - TIBC	2X20 ML(40 Test)

INTRODUCTION
Total iron binding capacity (TIBC) is the measure of the maximum concentration of iron that the serum proteins can bind. Together with the total serum iron concentration, the TIBC is used in the diagnosis and treatment of iron deficiency anaemia, other disorders of iron metabolism, and chronic inflammatory disorders. As an index of nutritional status, TIBC reflects the degree of transferrin saturation by serum iron. Serum TIBC is increased in iron deficiency, and decreased in anaemia that is due to chronic disease

PRINCIPLE
Step 1: An acidic buffer containing an iron binding dye and ferric chloride, is added to the serum sample. The low pH of R1 release iron from transferrin.

Step 2: The iron then forms a coloured complex with the dye present in R2. The coloured complex at the end of this step represents both the serum iron and excess iron. The neutral buffer in R2 shifts the pH and resulting in a large increase in affinity of transferrin for iron. The serum transferrin rapidly binds to the iron by forming a dye-iron complex. The observed increase in absorbance of the coloured dye- iron complex is directly proportional to the total iron binding capacity of the sample.

KIT CONTENTS

Reagent Name	Pack Size-80T
R1: TIBCBuffer reagent	2 X 20 ml
R2: TIBC Dye reagent	2 X 4 ml
R3: TIBC Calibrator	1 X 0.5 ml

Calibrator concentration mentioned on vial label.

WORKING REAGENT PREPARATION AND STABILITY

All the reagents R1 and R2 are supplied ready to use and stable until expiration date stated on label when stored refrigerated at 2– 8°C

R3-Reconstitute with 0.5ml of distilled water

SAMPLE COLLECTION AND STORAGE
1. Serum is the specimen of choice. DO NOT USE PLASMA.
2. Samples should be separated from the red cells and analyzed promptly.
3. If the samples cannot analyzed promptly or is being transported to a reference laboratory, the serum must be separated from the cells immediately after collection.
4. Once separated from cells, serum may be stored at either 2-8°C or at -20°C for up to one month. Serum may also be stored at room temperature (22-28°C) for two weeks.

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PROCEDURE
Wavelength / Filter : 630 nm
Temperature : 37°C
Cuvette : 1 cm light path.

	B	S	T
R1Buffer reagent	**500µl**	**500µl**	**500µl**
R3-TIBC Calibrator	-----	**10µl**	-----
Sample	-----	-----	**10µl**

Mix carefully and incubate at 37°C for 4 min & then add

R2-Dye reagent	**100µl**	**100µl**	**100µl**

Mix well and incubate at 37°C for 7 min and read absorbance of Calibrator and sample against Reagent blank at 630 nm

CALCULATION:
$$\text{TIBC (µg/dl)} = \frac{\text{Abs. sample}}{\text{Abs. Calibrator}} \times \text{Calibrator conc.}$$

UIBC (µg/dl) = TIBC (µg/dl) – Iron (µg/dl)

SI units

(µg/dl) x 0.1791 = µmol/l

REFERENCE VALUES:
TIBC = 250 - 450 µg/dl

PERFORMANCE CHARACTERISTICS:
LINEARITY:
The reaction is linear up to iron concentration of 600 µg/dl, Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay.

SPECIFICITY / INTERFERENCES:
SERUM
1. Using normal sera (average TIBC: approx.350 µg/dl), several substances were tested for possible interference. The following did not interfere as demonstrated by less than 5% bias to the limits shown:

Bilirubin	up to at least	32mg/dl
Copper	up to at least	3 mg/dl
Zinc	up to at least	250 µg/dl
Nickel	up to at least	500 µg/dl
Chromium	up to at least	5 µg/dl
Cuprimine	up to at least	250 µg/dl
Iron Dextran	up to at least	1430 µg/dl
Hemoglobin	up to at least	500 mg/dl
Triglycerides	up to at least	1300 mg/dl

2. Ascorbate demonstrated less than 5% bias up to 10 mg/dl and less than 10% bias up to 20 mg/dl. Greater than 20mg/dl of ascorbic acid causes significantly decreased TIBC results.

3. Desferal demonstrated less than 5% bias upto 11.5 µg/ml and less than 10% positive bias upto at least 20 µg/ml. Greater than 250 µg/ml Desferal causes significantly increased TIBC results.

4. Greater than 460 µg/dl of iron (ferrous sulfate) causes significantly decreased TIBC results.

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.

SYSTEM PARAMETERS

TIBC REAGENT – Modified Colorimetric Method

PARAMETER	**FOR SEMI-AUTO**
Mode of reaction	End point method
Wavelength / Filter	630 nm
Slope of reaction	Increasing
Temperature	37°C
Units	µg/dl
Blank	Reagent Blank
Calibrator conc.	Concentration mentioned on vial label
Delay Time	05 sec
Read Time	4 min + 7 min
Sample volume	10 µl
Reagent volume R1	500 µl
Reagent volume R2	100 ul
Linearity	600 µg/dl