Direct TIBC Reagent

PRODUCT SUMMARY

Stability	:	Until expiry at 2-8°C
Linear Range	:	70-700 µg/dL TIBC
Specimen Type	:	Serum
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use

SYMBOLS IN PRODUCT LABELLING

IVD	*In Vitro* Diagnostic Medical Device		Temperature Limitation
LOT	Lot Number		Use By
REF	Catalogue Number		Manufacturer
	Consult Instructions for Use		

INTENDED USE

For use in the determination of total iron-binding capacity in serum. For in vitro diagnostic use only.

SUMMARY AND EXPLANATION

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 anemia, 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 anemia that is due to chronic disease.

PRINCIPLE OF PROCEDURE

Step 1: Reagent 1 (R1), an acidic buffer containing an iron-binding dye and ferric chloride, is added to the serum sample. The low pH of R1 releases iron from transferrin. The iron then forms a colored complex with the dye. The colored complex at the end of this first step represents both the serum iron and excess iron already present in R1.

Step 2: Reagent 2 (R2), a neutral buffer is then added, shifting the pH and resulting in a large increase in affinity of transferrin for iron. The serum transferrin rapidly binds the iron by abstracting it from the dye-iron complex. The observed decrease in absorbance of the colored dye-iron complex is directly proportional to the total iron-binding capacity of the serum sample.

METHODOLOGY: Endpoint colorimetric

REAGENTS

Reagent 1 (R1) contains: Chromazurol B, Cetrimide, Ferric chloride, acetate buffer, stabilizers, and preservatives

Reagent 2 (R2) contains: Sodium Bicarbonate, buffer, stabilizers, and preservatives

PREPARATION

The Direct TIBC Reagents (dTIBC), R1 and R2 are ready to use as supplied.

STORAGE AND STABILITY

The reagent is stable until the expiration date shown on the label when stored at 2-8°C. After opening and use, close cap tightly when storing.

SPECIMEN COLLECTION & 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 sample cannot be 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 the cells, serum may be stored at either 2-8°, or at -20°C for up to one month. Serum may also be stored at room temperature (22-28°C) for two weeks.

REQUIRED, BUT NOT PROVIDED

- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 660 nm.
- Analyzer specific consumables, eg: sample cups.
- Normal and Abnormal control material.

CALIBRATION

The Direct TIBC Calibrator (Cat. No. TS3000) is required for calibration; refer to the Calibrator package insert for directions. Follow the instrument manufacturer's guidelines for calibration performance and frequency, using quality control samples with each run to verify satisfactory calibration.

SYSTEM PARAMETERS

Wavelength:	660 nm
Temperature:	37ºC
Mode	Endpoint
Direction:	Decreases
Reaction time step 1	5 min
Reaction time step 2	7.5 min
Sample: Reagent 1: Reagent 2 Ratio	4:50 (R1) : 15 (R2)
eg: Sample volume	16 µL
Reagent 1 (R1) volume	200 µL
Reagent 2 (R2) volume	60 µL

The assay can be performed on a variety of automated chemistry analyzers.
All performance data included here were obtained using a COBAS Fara II® analyzer.

CALCULATION OF RESULTS

The instrument automatically calculates the results.
[Results expressed in µg/dL may be converted to µmol/L by multiplying by 0.179]

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal controls with assayed values should be run as unknown samples:

- At least once per day or as established by the laboratory.
- When a new bottle reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling above the upper limit or below the lower limit of the established range indicates the assay may be out of

control. The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the controls.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the controls.
- If results are still out of control perform a calibration with fresh reagent, then repeat the controls.
- If results are still out of range, contact Technical Services or your local distributor.

LINEARITY

The Direct TIBC method demonstrated linearity between 70 and 700 µg/dL TIBC. Samples above 700 µg/dL should be diluted with 0.9% w/v laboratory saline.

ACCURACY

A total of 125 serum samples having TIBC concentrations ranging from 95 – 554 µg/dL were assayed with the Direct TIBC assay and a commercially available magnetic separation based TIBC method. Regression analysis of the results yielded y = 1.05 (x) - 5.4, where y = the Direct TIBC method and x = magnetic method, and a correlation coefficient (r) of 0.987.

PRECISION

Two levels of TIBC were tested, using Bio-Rad Multiqual® quality control material. Within-run and run-to-run precision (seven day) studies yielded the following:

Within-Run Precision (N=25)		
	Level 1	Level 2
Mean (µg/dL)	250	446
S.D (µg/dL)	9.0	8.2
c.v. (%)	3.6	1.8

Within-Run Precision (N=25)		
	Level 1	Level 2
Mean (µg/dL)	247	451
S.D (µg/dL)	9.5	10.4
c.v. (%)	3.8	2.3

EXPECTED VALUES

250 - 450 µg/dL

Since these ranges vary with different populations, it is recommended that each laboratory establish its own expected range.

PRECAUTIONS

The Direct TIBC Reagent is for in vitro diagnostic use. Normal precautions for handling laboratory reagents should be taken.

- Do not ingest, do not pipette by mouth. Prevent contact with skin and eyes.
- Do not mix reagents of different lot numbers.
- All specimens and controls being tested should be considered potentially infectious. Universal precautions, as they apply to your facility, should be used for handling and disposal of materials during and after testing.

LIMITATIONS

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	32 mg/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 (Imferon)	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 20 mg/dL of ascorbic acid causes significantly decreased TIBC results.

3. Desferal demonstrated less than 5% bias up to 11.5 µg/mL and less than 10% positive bias up to at least 23 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.

5. Serum is the preferred sample. Do Not Use Plasma.

REFERENCES

1. Tietz NW (ed). Textbook of Clinical Chemistry, ed. 3. Philadelphia, PA: WB Saunders; 1701-1703; 1999.

2. NCCLS. Determination of Serum Iron and Total Iron Binding Capacity; Proposed Standard, NCCLS Document H17-P. Wayne, PA: NCCLS, Vol. 10, No. 4; 1990.

3. Gambino R., et al. The Relation Between Chemically Measured Total Iron-Binding Capacity Concentrations and Immunologically Measured Transferrin Concentrations in Human Serum. Clin. Chem. 43: 2408-2412, 1997.

Order Information	
Cat No.	**Content**
TS3500	**R1 2 x 30 mL** **R2 2 x 15 mL**

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