台塑生醫科技股份有限公司
FORMOSA BIOMEDICAL TECHNOLOGY CORP.

IRON/TIBC TEST IRON

Ferrozine Colorimetric Method

INTENDED USE:

This reagent is for in vitro diagnostic quantitative determination of Iron in serum and Total Iron binding Capacity measurement.

CLINICAL SIGNIFICANCE

The measurement of Iron concentration in serum is always performed together with total iron binding capacity measuring, Iron in serum is bound with iron transfer protein and only a portion of Iron transfer protein is saturated with iron, but the unsaturated portion of iron transfer protein has the capacity of iron binding (UIBC), for measurement of total iron binding capacity (TIBC), first, exceed iron was added in order to saturate the iron transfer protein then remove the unbound iron by adding Magnesium Carbonate powder and measure the total iron concentration in serum.

The iron in serum increasing indicates that the destruction ratio of erythrocyte is increased too, as the disease of Hemolytic anemia, obstacle to regeneration or mature of erythrocyte; giant erythrocyte anemia, iron concentration decrease in serum can be observed in iron deficiency anemia, chronic blood deficiency, malignant tumor; TIBC increases can be caused by iron deficiency anemia and peracute Hepatitis; TIBC decrease observed in cirrhosis of liver, nephropathy, and uremia.

PRINCIPLE

Iron – Iron transfer protein (Fe^{3+}) + Acidic buffer solution ⟶ Iron transfer protein + 2 Fe^{2+}

Fe^{2+} + Ferrozine ⟶ Purple red conjugate substance

SPECIMEN COLLECTION AND PREPARATION

Freshly drawn serum is the specimen of choice, Avoid from iron pollution, no hemolytic sample, all the glass container should be dipped with diluted Hydrochloric acid.

REAGENT

1. Package:

R1: 2×40 ml,	R2: 2×10 ml	Common
R1: 4×60 ml,	R2: 4×15 ml	Hitachi 917 / Olympus
R1: 4×80 ml,	R2:4×20 ml	Hitachi 7060 / Shimadzu
R1: 8×40 ml,	R2: 2×40 ml	Hitachi 912
R1: 4×50 ml,	R2: 2×25 ml	Toshiba

2. Components:

	Ingredient	Concentration
R1:	Acetic acid buffer	(pH = 4.3)
	Citric acid	0.1 mol/L
	Thioglycolic acid	20 mmol/L
R2:	Ferrozine	0.4 mmol/L

Ferrous Chloride and Magnesium Carbonate 1 bottle each (For TIBC measurement)

Iron standard solution 35.8 μmol/L

Reagent stability:

Store all the reagents at 2~8℃, will stable until the expiration date on the label .

PRECAUTIONS

1. For in vitro diagnostic use only.
2. Since all specimens are potentially infectious, they should be handled with appropriate precautions and practices in accordance with Biosafety level 2 as recommended by USA NIH manual Biosafety inMicrobiological and Biomedical Laboratories, and in accordance with National or local regulations related to the safety precautions of such materials.
3. Each laboratory has to perform the quality control test to assure the results being reliable before running the specimen tests.

PROCEDURE

A. serum Iron measurement

	Blank	Standard	Test
R1 ml	0.8	0.8	0.8
R2 ml	0.2	0.2	0.2
DI Water ml	0.1	---	---
Standard ml	---	0.1	---
Serum ml	---	---	0.1
Mix well, let stand for10 minutes at 37℃, then measure the absorbance at 560 nm against blank.			

***** The Hitachi reagents are used directly on Hitachi analyzers in accordant with Hitachi parameters.

RESULT CALCULATION

$$\text{serum Iron (μmol/L)} = \frac{\text{Abs. of test}}{\text{Abs. of standard}} \times \text{concentration of standard (35.8 μmol/L)}$$

B. Serum TIBC

a) Take 0.25 ml serum in test tube and add 0.5 ml of Ferrous Chloride solution, mix well, stand in room temperature for 5 – 7 minutes.

b) Add one spoon (≈50 mg) of Magnesium Carbonate powder, mix well, stand in room temperature for 10 minutes, centrifuged at 3000 rpm for 10 minutes, pipette the upper layer solution for test.

	Blank	Standard	Test
R1 ml	0.8	0.8	0.8
R2 ml	0.2	0.2	0.2
DI Water ml	0.1	---	---
Standard ml	---	0.1	---
Upper solution ml	---	---	0.1
Mix well, let stand for10 minutes at 37℃ temperature, then measure the absorbance at 560 nm against blank.			

RESULT CALCULATION

$$\text{TIBC (μmol/L)} = \frac{\text{Abs. of test}}{\text{Abs. of standard}} \times \text{concentration of standard (35.8 μmol/L)} \times 3 \text{ (Dilution fold)}$$

$$\text{UIBC (μmol/L)} = \text{TIBC} - \text{Serum Iron}$$

$$\text{Iron saturation percentage} = \frac{\text{Serum Iron}}{\text{TIBC}} \times 100\ \%$$

EXPECTED VALUES

	Male Adult	Female Adult
Serum Iron	11 – 30μmol/L	9 – 27μmol/L
TIBC	50 – 77μmol/L	54 – 77μmol/L
Iron saturation percentage	20 – 55 %	

PROCEDURAL LIMITATIONS`

1. This reagent can be use on semi-auto/auto biochemistries analyzer and manual method for determination of serum iron and TIBC, please set the parameters respect to equipment request.
2. Avoid iron pollution from operating procedures, all the container must be absolute clean for the test.
3. When sample concentration exceed 90μmol/L, dilute serum with non-ferrous DI water in the ratio of 1 : 1, the result must be multiple with 2.
4. The reagent contains Sodium Azide, do not swallow and avoid contact with skin or mucosa, if contacted, flush with plenty of water.
5. For Beckman analyzer, please use bar code reading to follow the Beckman CX4 parameters and procedures.