

Ref. CC1-UIB.55M, 20 Test

INTENDED USE

Reagent kit for quantitative estimation of UIBC/TIBC in serum/plasma.

DIAGNOSTIC SIGNIFICANCE

Measurement of Unsaturated Iron-binding Capacity (UIBC) are used to assist in the diagnosis and treatment of anemia.

PRINCIPLE

Excess ferrous ions bind specifically with available iron-binding sites of transferrin and saturating the molecules with iron in alkaline medium.

Ferrozine reacts with the remaining unbound iron to form a strongly purple coloured complex which is measured at 560 nm. The difference between the known excess amount of iron added and the remaining unbound iron is equivalent to unsaturated iron-binding capacity (UIBC). Total iron binding capacity (TIBC) is calculated as serum iron plus UIBC.

PRESENTATION

Store all reagents at 2-8°C	No. of Bottles
	20 Test
• 1 UIBC (Buffer Reagent)	2
• 2 UIBC (Colour Reagent)	1
• 3 UIBC (Enhancer)	2
• UIBC standard (80 µmol/L)	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Buffer	100 mmol/L
• Detergent	5 mmol/L
• Ferrozine	4 mmol/L
• Hydroxylamine Hydrochloride	150 mmol/L
• Enhancer	10 mmol/L

pH 4.5 ± 0.1 at 25°C

UIBC Standard (80 µmol/L)

Also contains non-reactive fillers and Stabilizers.

PRECAUTION

It is essential that all the glasswares used for assay should be Iron-free. Glasswares should be soaked in 0.1N HNO₃ or 1N HCl & rinsed thoroughly with iron-free deionized water.

PREPARATION OF WORKING REAGENT

Carefully transfer the content of one vial of 3 UIBC to one bottle of 1 UIBC. Mix to dissolve. Wait for 15 minutes before use.

REAGENT STORAGE AND STABILITY

All the reagents included in the kit are stable at 2-8°C until the expiry date indicated on the label, when protected from light. The working reagent is stable for 60 days at 2-8°C.

SPECIMEN COLLECTION

Fresh clear serum or plasma with no hemolysis should be used. Specimens are stable for one day at room temperature or one week at 2-8°C.

REACTION PARAMETERS

Monochromatic

Type of reaction	: End point (Increase)
Wavelength	: 560 nm
Temperature	: 37°C
Incubation	: 10 min at 37°C
Std. Concentration	: 80 µmol/L (446 µg/dL)
Std./Sample Volume	: 200 µl (0.200 ml)
Reagent 1	: 1.0 ml
Reagent 2	: 0.050 ml (50 µl)
Light path	: 1.0 cm
Zero setting with	: Reagent blank

Bichromatic

Other parameters as above	
Wavelength	: 560 nm and 630 nm
Sample Blank	: No
Zero setting with	: Distilled water
Set the instrument using above system parameters	

TEST PROCEDURE

A) Monochromatic Method

Pipette into test tubes	Blank	Std.	Test	
			Sample Blank (A1)	Sample Test (A2)
Working Reagent (ml)	1.0	1.0	1.0	1.0
Sample (ml)	-	-	0.2	0.2
Standard (ml)	-	0.2	0.2	0.2
Dist. Water (ml)	0.4	0.2	-	-
Rgt.-2 (ml)	0.05	0.05	-	0.05

Mix and allow to stand for 10 minutes at 37°C. Read absorbance of test (A₁ & A₂) and standard against reagent blank at 560 nm.

B) Bichromatic Method

Pipette into Test tubes	Standard	Test
Working Reagent (ml)	1.0	1.0
Sample (ml)	-	0.2
Standard (ml)	0.2	0.2
Dist. Water (ml)	0.2	-
Reagent-2 (ml)	0.05	0.05

Mix and allow to stand for 10 minutes at 37°C. Read absorbance of test and standard against distilled water at 560 nm & 630 nm.

TEST RESULTS

$$\text{Excess Iron } (\mu\text{mol/L}) = \frac{\text{Abs or } \Delta \text{ Absorbance of test}}{\text{Absorbance of Std.}} \times 80 (\mu\text{mol/L})$$

Where,

$$\Delta \text{ Absorbance} = (A_2 - A_1)$$

80 µmol/L = Concentration of Standard

$$\text{UIBC } (\mu\text{mol/L}) = 80 - \text{excess Iron } (\mu\text{mol/L}).$$

$$\text{TIBC } (\mu\text{mol/L}) = \text{Serum Iron } (\mu\text{mol/L}) + \text{UIBC } (\mu\text{mol/L}).$$

$$\text{To convert } (\mu\text{g/dl}) = \mu\text{mol/L} \times 5.585$$

LIMITATIONS FOR INTERFERENCE

- Hemolysis causes falsely elevated results.
- Iron medications (oral, intravenous or intravascular) affect serum levels.

NORMAL VALUES

UIBC: 28.6 - 64.5 $\mu\text{mol/L}$ (160-360 $\mu\text{g/dl}$)

TIBC: 44.7 - 71.6 $\mu\text{mol/L}$ (250-400 $\mu\text{g/dl}$)

LINEARITY

This procedure is linear upto 89 $\mu\text{mol/L}$ (500 $\mu\text{g/dl}$). For sample values higher than 89 $\mu\text{mol/L}$ (500 $\mu\text{g/dl}$), dilute the sample suitably with 0.9% saline and repeat the assay. Apply dilution factor to obtain test results.

REFERENCES

1. Tietz NW "Text book of clinical chemistry 2nd Edition" Tetz NW (Ed) WB Saunders company Philadelphia 1994; 2059.
2. CaO G.and Prior R.L. Chemistry Anthocyanins and iron metabolism in human serum 1999b; 574-76.
3. National committee for Clinical Laboratory Standards. User evaluation of precision performance of clinical chemistry Devices. NCCLS, 1984 NCCLS Publication EP5-T.

