# UIBC

(Ferrozine Method)

**CHEMPAK**

Last update 09-2020

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 anaemia.

## 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$^0$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$^0$ 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 $HNO_3$ 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$^0$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$^0$C.

## REACTION PARAMETERS

**Monochromatic**

| | |
|---|---|
| Type of reaction | : End point (Increase) |
| Wavelength | : 560 nm |
| Temperature | : 37$^0$C |
| Incubation | : 10 min at 37$^0$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$^0$C. Read absorbance of test ($A_1$ & $A_2$) 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$^0$C. Read absorbance of test and standard against distilled water at 560 nm & 630 nm.

## TEST RESULTS

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

Where,

Δ Absorbance = (A2-A1)

80 µmol/L = Concentration of Standard

UIBC (µmol/L) = 80 - excess Iron (µmol/L).

TIBC (µmol/L) = Serum Iron (µmol/L) + UIBC (µmol/L).

To convert (µg/dl) = µmol/L x 5.585

## LIMITATIONS FOR INTERFERENCE

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

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## NORMAL VALUES

UIBC: 28.6 -64.5 µmol/L (160-360 µg/dl)
TIBC: 44.7 -71.6 µmol/L (250-400 µg/dl)

## LINEARITY

This procedure is linear upto 89 µmol/L (500 µg/dl). For sample values higher than 89 µmol/L (500 µg/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.
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