UIBC

(Ferrozine Method)

Intended Use

Iron is a reagent kit used for the determination of the unsaturated iron-binding capacity in human serum and heparinized plasma. The reagents are for *in-vitro* diagnostic use.

Principle

Direct determination with FerroZine.

Fe(II) (excess) + transferin $\xrightarrow{\text{Alkaline Buffer}}$ ansferring-Fe(III) + Fe(II)

Fe(III) (excess) + 3 FerroZine Fe (II) $\longrightarrow$ (FerroZine)^3

The color intensity is directly proportional to the unbound excess iron concentration and indirectly proportional to the unsaturated iron-binding capacity.

Reagent provided

1. Reagent 1 - Buffer
2. Reagent 2

Reagent storage and stability

Store all the reagents at 2-8°C.

Specimen collection and preservation

Use fresh serum samples. Samples should preferably be analyzed on the day of collection. Serum is collected by standard procedures. Sample in serum is stable for 4 days at 15-25°C &7days at 2-8°C.

Don't use samples with EDTA, Oxalate, Citrate. Don't use hemolysed samples. To avoid hemolyse; centrifuge and separate samples immediately' after collecting samples. Samples should be taken in morning otherwise results may be decreased by 30% within daytime.

Assay guidelines for Analyzers

Wavelength **578/700 nm**
Method **End Point/Sample Blank**

	Reagent Blank	Adjust the instrument to zero with Reagent Blank	Standard Blank	Standard Tube	Sample Blank	Sample Tube
R1	800 µL		800 µL	800 µL	800 µL	800 µL
CAL	0		10 µL	10 µL	0	0
Sample	0		0	0	10 µL	10 µL
R2	200 µL		Mix for 3 min then take A_{BCAL} and A_{SB} then add			
		R2	0	200 µL	0	200 µL

1. Mix thoroughly and incubate at 37°C for 5 minutes.
2. Read the absorbance against Sample blank at 578/700 nm .

Calculation

$$\text{UIBC (µg/dl)} = \text{Con. Of Std} - \frac{\text{A2 Test} - \text{A 1 Test}}{\text{A2 Std} - \text{A1 Std}} \times \text{Con. of Std.}$$

TIBC (µg/dl) = UIBC + Iron Level

Normal Range

UIBC : 120 – 370 µg/dL
TIBC : 127 – 450 µg/dL
It is recommended that each laboratory establish its own normal range.

Limitation

1. Reaction is linear up to 600µg/dL For higher values, dilute the sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.
2. For in vitro diagnostic use only. Mouth pipetting is prohibited. Avoid contact with skin and mucous membranes.
3. All the calibrators and controls must be considered as human or animal-sourced substances and thus they are potentially infectious; all the protection actions must be applied to avoid any potential biological risk.
4. Exercise the normal precautions required for handling laboratory reagents.
5. Reagents with different lot numbers should not be interchanged or mixed.

Quality Control

To ensure adequate quality control, it is recommended that each batch should include a commercially available control material with established values determined by this method. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, and cleanliness of glassware and accuracy of pipetting.

References

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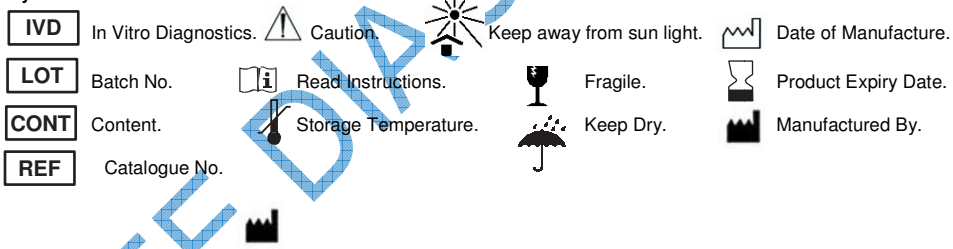
Symbols



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