UIBC

UNSATURATED IRON BINDING CAPACITY

Method: Nitrozo-PSAP
Product code: 141G-0281
Packaging: 2 x 50 mL (R1) + 1 x 7,5 mL (R2) + 1 x 20 mL (Standard)
Store at 2 – 8°C
For *in vitro* use

CLINICAL SIGNIFICANCE

The determined concentration of iron in serum is usually Fe(III) bound to serum transferrin and does not include the iron contained in serum as free hemoglobin. As only about one third of transferrin iron binding sites are taken up by Fe(III), normally, serum transferrin has a high iron binding capacity. That is named unsaturated, or dormant, iron binding capacity. UIBC measurement can be used in combination with the concentration of serum iron for the determination of total iron binding capacity (TIBC), that is, the maximum concentration of iron that serum proteins—mainly transferrin—can bind.
TIBC is reduced in chronic infections, malignancy, iron poisoning, kidney disease, Kwashiorok type pellagra, and thalassemia. Common causes of TIBC increase include iron deficiency anemia, advanced pregnancy, oral contraception and infectious hepatitis.

METHOD PRINCIPLE

The Nitrozo-PSAP method is used. Unsaturated Iron Binding Capacity (UIBC) is determined by adding at alkaline pH an excess of iron to the sample, in order to saturate the iron binding sites of transferrin. After reduction, the unbound iron reacts with Nitroso-PSAP to form a colored complex. The difference between the amount of iron added and the amount of iron measured represents the Unsaturated Iron Binding Capacity.

REAGENT COMPOSITION

UIBC Buffer (Reagent 1)	
Tris Buffer (pH 8.4)	500 mM
Surfactant	
Preservative	
Chromogen (Reagent 2)	
Nitroso – PSAP	1.6mM
Standard Solution	
Fe(II) Salt Solution in Hydroxylamine Hydrochloride	500µg/dL

WARNINGS – PRECAUTIONS

- The reagent has been manufactured for in vitro diagnostics use only. In vitro diagnostics may be dangerous. Use according to the proper laboratory techniques; that is, avoid inhalation and contact with the eyes and skin.
- Samples should be considered as potentially infectious. Handle according to the universal precautions and good laboratory practices.
- Dispose of all waste according to national laws.
- MSDS is available by MEDICON upon request.

PREPARATION

Reagents are liquid and ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contamination is prevented during their use. Do not use reagents over the expiration date.

Standard sollution: Stable until the expiration date on the label when stored tightly closed at 2-8°C.

SAMPLE

Non hemolyzed serum or heparinized plasma. Do not use plasma with EDTA, oxalates, or citrates as they strongly chelate iron ions. Morning samples should be used from patients in a fasting state, since iron values can decrease by 30% due to the course of the day. Samples are stable for 4 days at room temperature.

PROCEDURE / CALCULATIONS

Assay conditions:

Wavelength	Temperature	Cuvette light path
750 nm	37°C	1 cm

1. Adjust the instrument to zero with distilled water.
2. Pipette into appropriate tubes named Reagent Blank, Standard and Test, 1mL UIBC Buffer:
3. Add 0.4ml of iron-free water to the tube named, Reagent Blank. Mix.
4. Add 0.2ml of iron-free water and 0.2 ml of Standard to the tube named Standard. Mix.
5. Add 0.2ml of Standard and 0.2 ml of sample to all tubes named Test. Mix.
6. Read the absorbance (A_1) of all tubes against Reagent Blank.
7. Add 75 µL Chromogen (Reagent 2) to all tubes. Mix.
8. Incubate for 10min at 37°C.
9. Read the absorbance (A_2) all tubes against Reagent Blank.
10. The Unsaturated Iron Binding Capacity (UIBC) of the sample is given from the formula:

$$\text{Conc. of Std.} - \frac{(A_{2\,\text{Test}} - A_{1\,\text{Test}})}{(A_{2\,\text{Std}} - A_{1\,\text{Std}})} \times \text{Conc. Of Std} = \text{UIBC (µg/dL) of sample}$$

QUALITY CONTROL

MEDICON suggests the MEDICON Clinical Chemistry Control Lev.1 & 2 (1578-0901-12 & 1578-0902-12) for Quality Control.
Control recovery should lie within the acceptable range. Results outside the acceptable range even after recalibration could be due to reagent deterioration, unsuitable storage conditions, control deterioration or error during test procedure. Target values for UIBC should be verified with the corresponding working protocol.

EXPECTED VALUES

Serum: 155 – 300 µg/dL
Each laboratory should determine its own expected values as dictated by good laboratory practice.

WASTE DISPOSAL

This product contains sodium azide (NaN_3), which forms sensitive explosive compounds with copper or lead. Flush waste pipes with water after the disposal of undiluted reagent in order to avoid azide build up in the drain pipes.

SPECIFIC PERFORMANCE CHARACTERISTICS*

Linearity
The assay is linear within measuring range 21 – 400 µg/dL. When values exceed this range samples should be diluted accordingly.
Sensitivity
The lowest detectable level of UIBC is estimated at 21 µg/dL.
The lowest detection limit (LDL) is defined as the lowest concentration of analyte that is distinguishable from zero. A sample free of analyte is assayed 20 times within the assay and the LDL is calculated as the absolute mean plus three standard deviations.
Precision

Level (µg/dL)	Within Run CV%	Total CV%
158.5	3.90	5.18
234.5	1.58	3.64

Precision is estimated on two concentration levels of analyte according to CLSI protocol EP-5T (20 consecutive days, 2 runs per day, 2 repeats per run).
Interference

Lipemic	Insignificant up to 1000 mg/dl Intralipid®
Hemoglobin	Insignificant up to 500 mg/dL
Non Conj. Bilirubin	Insignificant up to 20 mg/dL
Conj. Bilirubin	Insignificant up to 20 mg/dL
Ascorbic Acid	Insignificant up to 3 mg/dL

Refer to Young[6] for further information on interfering substances.

Method Comparison
A comparison was performed between this reagent and another commercially available product. The results were as follows:
Y = 1.0969X – 0.9324 R=0.9802 N=49 Sample range: 76 – 310 µg/dL
*Performance characteristics are analyzer-specific

BIBLIOGRAPHY

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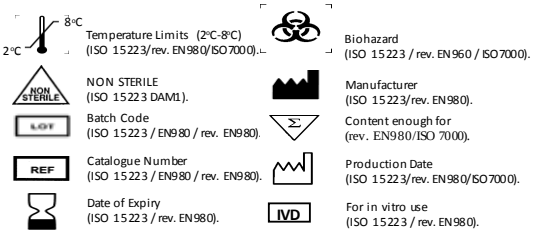
SYMBOLS



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