UNSATURATED IRON BINDING CAPACITY
UIBC

OSR61205	**4 x 27 mL**	**R1**
	4 x 3 mL	**R1a**
	4 x 6 mL	**R2**
	4 x 2 mL	**R2a**

Intended Use
System reagent for the quantitative determination of Unsaturated Iron Binding Capacity (UIBC) in human serum and plasma on Beckman Coulter AU analyzers.

Summary
Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, hemochromatosis (a disease associated with widespread deposit in the tissues of two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin), and chronic renal disease. Transferrin is the major iron carrying protein in the serum.

Serum iron concentration connotes the Fe (III) bound to serum transferrin and does not include the iron contained in serum as free hemoglobin. Because normally only about one third of the iron binding sites of transferrin are occupied by Fe (III), serum transferrin has considerable reserve Iron Binding Capacity. This is called the serum Unsaturated Iron Binding Capacity (UIBC).[1]

Methodology
In this UIBC procedure intended for use on the Beckman Coulter automated AU analyzers, ferrous-Iron (Fe^{2+}) at alkaline pH, added to serum, binds specifically with transferrin at unsaturated iron binding sites. Remaining unbound ferrous iron reacts with the Nitroso-PSAP [2-Nitroso-5-(N-propyl-N-sulfopropylamino)phenol] to form an intense green complex. The difference between the resulting change in the measured absorbance and the absorbance from the total amount added to serum is equivalent to the quantity bound to transferrin. This is the Unsaturated Iron Binding Capacity (UIBC).

System Information
For AU400/400^{e}/480, AU600/640/640^{e}/680 and AU2700/5400 Beckman Coulter Analyzers**.**

Reagents
Final concentration of reactive ingredients:

Tris buffer pH 8.1 (20°C)	180 mmol/L
Iron	6.9 µmol/L
Nitroso-PSAP	400 µmol/L
Also contains preservatives	

Precautions
1. For in vitro diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. R1a contains hydroxylamine, which may cause sensitization by skin contact.
4. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

Preparation of Reagents
1. UIBC R1: Pour an aliquot of R1 buffer into R1a bottle and mix gently by inversion. Pour entire contents of R1a into R1 bottle and mix gently by inversion.
2. UIBC R2: Pour an aliquot of R2 buffer into R2a bottle and mix gently by inversion. Pour entire contents of R2a into R2 bottle and mix gently by inversion.

Note: It is important that the entire contents of the R2a color reagent be transferred to R2 and thoroughly mixed. Failure to do so will result in high bottle to bottle imprecision of control and patient recovery.

Storage and Stability
The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
Working reagents are stable for 30 days when stored in the refrigerated compartment of the analyzers.

Indications of Deterioration
Visible signs of microbial growth, turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

Specimen Collection and Preparation
Serum or heparinized plasma samples, free from hemolysis, are the recommended specimens. Remove serum from the red cells to minimize hemolysis. Plasma specimens collected with EDTA, oxalate, or citrate are unsatisfactory, since they bind iron, preventing its reaction with the chromogen. Samples should be taken in the morning from patients in a fasting state, since iron values can decrease by 30% during the course of the day.[2]

Unsaturated Iron Binding Capacity (UIBC)

Sample Storage and Stability
Serum iron is stable for 7 days at 2 – 8°C or 4 days at room temperature (15 – 25°C) after the serum is separated from red cells.[2]

Interfering Substances
Results of studies[3] show that the following substances interfere with this Unsaturated Iron Binding Capacity procedure.
The criteria for no significant interference is recovery within 10% of the initial value.
Bilirubin: No significant interference up to 40 mg/dL Bilirubin
Copper: No significant interference up to 1 mg/dL Copper
Hemoglobin: No significant interference up to 200 mg/dL Hemolysate
Lipemia: No significant interference up to 1000 mg/dL Intralipid*

Gross, abnormal high amounts of trace metals may interfere in this assay.

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[4] for a compilation of reported interferences with this test.

Procedure
A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
Unsaturated Iron Binding Capacity reagent

Materials Required But Not Provided
Chemistry Calibrator (Cat # DR0070)

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzers automatically compute every determination at the same interval.

Calibration
The frequency of calibration for the Unsaturated Iron Binding Capacity procedure is every 14 days. Calibration of this Unsaturated Iron Binding Capacity procedure is accomplished by use of Chemistry Calibrator (Cat # DR0070).
Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. A fresh bottle of reagent is used for testing.
3. Major preventative maintenance was performed on the analyzer or a critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, these controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Automatically printed out for each sample in µg/dL at 37°C.

Dynamic Range
The Unsaturated Iron Binding Capacity assay is linear from 55 – 450 µg/dL. Samples exceeding the upper limit of linearity should be diluted and repeated. On the analyzer the sample may be diluted, repeated and multiplied by the dilution factor automatically by utilizing the AUTO REPEAT RUN.

Expected Values
Adults:[5] 155 – 355 µg/dL
Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics
The following data was obtained using the UIBC Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[7]
Estimates of precision, based on CLSI recommendations, are consistent with typical performance. The within run precision is less than 8% CV and total precision is less than 10% CV. Assays of serum pools and control sera were performed and the data reduced following CLSI guidelines above.

N = 80	Within run		Total	
Mean, µg/dL	SD	CV%	SD	CV%
141	2.62	1.86	4.96	3.53
201	2.25	1.12	4.99	2.48
413	1.87	0.45	5.22	1.27

Method Comparison[7]
Patient samples were used to compare this UIBC Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	Method 2
Slope	0.975
Intercept	+6.920
Correlation Coeff. (r)	0.993
No. of Samples (n)	120
Range (µg/dL)	63 – 433

Sensitivity
The typical change in absorbance for 1 µg/dL of UIBC is 0.32 mAbsorbance.

References

1. Burtis, C.A. and Ashwood, E.R., Tietz Textbook of ClinicalChemistry, 2nd Edition, W.B. Saunders Co., 1994.
2. Perrotta, G., Iron and Iron-Binding Capacity, In: Pesce, A.J., Kaplan, L.A. eds., Methods in Clinical Chemistry, C.V. Mosby, St. Louis, 1258 - 1261, 1987.
3. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-A2, 2005.
4. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, Washington, DC, AACC Press, 2000.
5. Beckman Coulter Inc. data on samples collected from 200 blooddonors in North Texas.
6. CLSI/NCCLS Evaluation Protocol EP5 – A2, 2004.
7. Data is on file for specific AU analyzers.

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