K-ASSAY®

UIBC

For the Quantitative Determination of Unsaturated Iron Binding Capacity (UIBC) in Serum

Cat. No. KAI-300

INTENDED USE

For the quantitative determination of Unsaturated Iron Binding Capacity (UIBC) in serum. Iron binding capacity measurements are used in the diagnosis and treatment of anemia. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

Unsaturated Iron Binding Capacity (UIBC) represents the amount of binding sites on Transferrin that are unbound to iron. UIBC is often run in tandem with other iron capacity assays such as Transferrin. This can be useful in assessment of such conditions as anemia or hereditary hemochromatosis.

PRINCIPLE OF TEST

The **K-ASSAY®** UIBC quantifies the unsaturated iron binding capacity in the patient's serum.

Ferric ammonium sulfate (R-1) is first added to saturate free transferrin in the sample. Next, Nitroso-PSAP (R-2) is added to allow measurement of the amount of unbound iron. The excess iron value is then used to determine the unsaturated iron binding capacity.

The **K-ASSAY®** UIBC assay can be run using a two-reagent clinical chemistry analyzer when paired with the **K-ASSAY®** UIBC Calibrator (Cat. No. KAI-302C). A 2 point calibration is made by using saline and the calibrator. This calibration is then used to quantify the UIBC of the patient's serum sample.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Ferric ammonium sulfate, 90 µg/dL Fe

R2: 2-Nitroso-5-(N-propyl-N-sulfopropylamino) Phenol (Nitroso-PSAP), 0.8 mg/mL

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution.

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used until the expiration date on package and bottle labels.

REAGENT STABILITY

Opened reagents can be used for 8 weeks if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of obvious precipitation in solution is cause to discard.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at approximately 750 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and / or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum test samples must be collected in the manner routinely used for clinical laboratory tests. Freshly drawn serum is preferred and should be used within the day of collection. Samples may also be stored refrigerated (2-8°C) for up to 1 week.[1]

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a two point calibration method.

PROCEDURE

Materials Supplied

KAI-300, UIBC

Reagent 1 (R-1) Ferric Ammonium Sulfate	1 x 40 mL
Reagent 2 (R-2) Nitroso-PSAP	1 x 20 mL

Materials Required But Not Supplied

Calibrator: **K-ASSAY®** UIBC Calibrator, Cat. No. KAI-302C.

Isotonic saline or DI water for use as 0 calibrator.

Two-Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings ~ 750 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

Assay Procedure

Note: Mix all reagents gently before using.

An example of automated application (Hitachi 917):

Sample	18.8 µL
↓	
• ←R1 (Ferric Ammonium Sulfate)	200 µL
↓ 37 °C, 5 min.	
• ←R2 (Nitroso-PSAP)	100 µL
↓ 37 °C, 5 min.	

2-point endpoint, 750 nm (primary) / 600 nm (secondary)

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 917
TEMPERATURE	37°C
TEST	(UIBC)
ASSAY CODE	(2 POINT END)(10) (16)(34)(0)(0)
WAVELENGTH	(600) (750) (secondary) (primary)
SAMPLE VOLUME	(18.8) (0.0) (0)
REAGENT VOL (R1)	(200) (0)
REAGENT VOL (R2)	(0) (0)
REAGENT VOL (R3)	(100) (0)
REAGENT VOL (R4)	(0) (0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-32000)(34)(LOWER)
CALIB. TYPE	(LINEAR)
POINT	(2)
SPAN POINT	(2)
SD LIMIT	(100)
DUPLICATE LIMIT	(1000)
SENSITIVITY LIMIT	(0)
S1ABS RANGE	(-32000) (32000)
INSTRUMENT FACTOR	a=(1.0) b=(0.0)
UNIT	(ug/dL)
STD.(1) Conc.-POS.	(0) - (1)
STD.(2) Conc.-POS.	(*2) - (2)
STD.(3) Conc.-POS.	( ) - ( )
STD.(4) Conc.-POS.	( ) - ( )
STD.(5) Conc.-POS.	( ) - ( )
STD.(6) Conc.-POS.	( ) - ( )

Use isotonic saline as Standard 1 and UIBC Calibrator as Standard 2.

*2: Input concentration of calibrator.

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that UIBC levels be determined using a 2 point calibration curve prepared using the **K-ASSAY®** UIBC Calibrator. On the Roche / Hitachi 917, calibration curves were found to be stable for up to one month. However, it is recommended that the user determine calibration curve frequency as this depends on the instrument and type/number of other assays being performed.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the ranges established by each lab for the particular lot of controls. The validity of the assay is in question if the value for the controls generated by the assay's calibration curve does not fall within the stated range. Recalibrate if the value determined for the controls falls outside the established recovery range.

RESULTS / CALCULATIONS

UIBC levels are determined using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

Use of hemolyzed samples is not recommended as is known to cause errors.

The measuring range for UIBC is between 14 and 700 µg/dL.

If the UIBC concentration of a patient sample is greater than 700 µg/dL, dilute with isotonic saline and reassay. If the result is within the measuring range, multiply results by the dilution factor.

PERFORMANCE

Precision

Within-run (Intra-assay) CV is less than 5% (N=10)

Accuracy

Control serum recovers within 10% of the assigned value.

Correlation

A comparison of the **K-*ASSAY*®** UIBC and another company's UIBC assay was performed with the following results:

y = 0.997x - 0.50
r = 0.998
n = 50
x = another company's UIBC assay
y = **K-*ASSAY*®** UIBC

Assay Range

14 - 700 µg/dL

INTERFERENCE

Ascorbic Acid	No interference up to 50 mg/dL
Bilirubin	No interference up to 20 mg/dL

Use of hemolyzed samples is not recommended as is known to cause errors.

EXPECTED VALUE

The expected value for healthy people as per the literature is between 191 and 269 µg/dL.[2] Due to population differences, each laboratory should establish its own expected values using this kit.

REFERENCES

1. Weissman, N., Pileggi, V.J., Clinical Chemistry: Principles and Techniques, 2nd ed, 692-693 (1974).
2. Japanese Laboratory Reference Manual, 29th ed. (Rinshou kensa hou teiyou kaitei dai 29 ban), 268(1981).

LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostics Use
2-8 °C	Temperature Limitation. Store between 2 and 8 degrees C
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

Advena Ltd.
Tower Business Centre, 2nd Flr.,
Tower Street, Swatar, BKR 4013 Malta

ORDERING / PRICING / TECHINCAL INFORMATION

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive
Seattle, WA 98168 USA
TEL: (206) 575-8068 / (800) 526-4925
FAX: (206) 575-8094