Instruction manual

* FOR RESEARCH USE ONLY
* STORE AT 4℃ UPON ARRIVAL

UIBC Assay Kit

（Bathophenanthroline Chromogenic method）

Background.

Iron is an essential element in mammals. It is a cofactor for many enzymes involved in oxidation–reduction reactions and is indispensable for oxygen transport as a constituent of hemoglobin and myoglobin. In healthy individuals, about 30% of serum transferrin is saturated with ferric iron (Fe^{3+}). Total iron-binding capacity (TIBC) equals the sum of serum iron and unsaturated iron-binding capacity (UIBC). TIBC and UIBC change in various clinical conditions: UIBC typically increases in iron deficiency, whereas decreased values are observed in infections, malignancy, nephrotic syndrome, and hypoproteinemia.

Assay principle.

This UIBC assay uses the chromogen bathophenanthroline, which forms a colored complex with ferrous iron (Fe^{2+}). First, serum transferrin is saturated by adding a buffer containing a known amount of Fe^{3+}. Unbound Fe^{3+} is then reduced to Fe^{2+}, which reacts with bathophenanthroline to yield a stable colored complex. The absorbance, read on a 96-well plate reader or spectrophotometer, is proportional to UIBC.

Kit contents

200 tests （Catalog # : UIB02E）

R-A Buffer （Fe concentration 80μg/dL）●	40 mL×1
R-R Chelate color ●	6 mL×1

Note

A) Instability in incubation temperature may result in unstable results.
B) Use disposable test tube and glassware washed with 1M HNO_3 or 1M HCl solution and distilled water
C) Accuracy in pipetting the volumes for samples and reagents may affect the quality of assay. Please note that samples, standards and Working Reagent must be dispensed accurately at the microliter level.
D) The temperature during the chromogen reaction may affect optical density.
E) Samples containing chelating agents (such as EDTA or citrate) are not suitable for this assay.
F) Hemolyzed samples cannot be measured.

Operation

1. Sample preparation

◇Serum or Plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation.

2. Assay preparation

Bring all reagents and samples to room temperature before use.

3. Assay procedure.

Procedure using microplate reader.

(Volume per assay sample: 250 µL)

○Assay

(1) Add 200 µL of R-A Buffer to each well.

(2) Add 20 µL of Distilled water (Blank) / STD (Standard)/ sample into each well and incubate at room temperature for 5 min.

(3) Read the absorbance at 546 nm (main) and 600 nm (sub). --> OD1

(4) Add 30 µL of R-R Chelate color to each well and incubate at room temperature for 5 min.

(5) Read the absorbance at 546 nm(main) and 600 nm (sub). --> OD2

* Select the filter: 540-550 nm at 546nm(main).

		Assay Sample	
Add	(µL)	Blank OD_{Bl}	Sample OD_S
1	R-A Buffer	200	200
2	Distilled water	20	-
	Assay sample	-	20
↓			
Mix and incubate for 5 minutes at room temperature. Read the absorbance at 546 nm(main) and 600 nm (sub).			
3	R-R Chelate colr	30	30
↓			
Mix and incubate for 5 minutes at room temperature. Read the absorbance at 546 nm(main) and 600 nm (sub).			

○Calculations

$OD_{Bl} = OD2_{Bl} – OD1_{Bl}$

$\Delta OD_S = OD_{Bl} – (OD2_S – OD1_S)$

UIBC (µg/dL) = $\Delta OD_S / OD_{Bl}$ X 800

UIBC (µM) = $\Delta OD_S / OD_{Bl}$ X 143.2

(Assay example)

	Wavelength (nm)	OD1	OD2	OD	UIBC (µg/dL)
Blank	546 nm	0.026	0.202	-	-
	600 nm	0.027	0.045	-	-
	546nm-600 nm	-0.001	0.157	0.158	-
Sample	546 nm	0.047	0.185	-	-
	600 nm	0.039	0.052	-	-
	546nm-600nm	0.008	0.133	0.125	167.09

***Observed 546 nm with 600 nm**

[OD = OD(546 nm) - OD(600 nm)]

OD_{Bl} = (0.202-0.045) – (0.026-0.027) = 0.158

OD_S = (0.185-0.052) – (0.047 – 0.039) = 0.125

ΔOD_S = 0.158 – 0.125 = 0.033

$UIBC_{Sample}$ (µg/dL) = $\Delta OD_S / OD_{Bl}$ x 800

= 0.033 / 0.158 x 800 = 167.09 (µg/dL)

$UIBC_{Sample}$ (µM) = $\Delta OD_S / \Delta OD_{Std}$ x 143.2

= 0.033 / 0.158 x 143.2 = 29.9 (µM)

***Observed 546 nm only**

[OD = OD(546 nm)]

OD_{Bl} = 0.202 – 0.026 = 0.176

OD_S = 0.185 – 0.047 = 0.138

ΔOD_S = 0.176 – 0.138= 0.038

$UIBC_{Sample}$ (µg/dL) = $\Delta OD_S / OD_{Bl}$ x 800

= 0.038 / 0.176 x 800 = 173 (µg/dL)

$UIBC_{Sample}$ (µM) = $\Delta OD_S / \Delta OD_{Std}$ x 143.2

= 0.038 / 0.176 x 143.2 = 30.9 (µM)

*In diluted sample of seminal fluid, multiply the result by dilution-factor.

Performance

Measuring range 10 – 800µg/dL

Imprecision Imprecision was evaluated using commercially available quality control serum.

Within run

	Mean µg/dL	S.D	C.V %
Level 1	146	3.6	2.4
Level 2	232	2.6	1.1

Interferences No interference by the note of substances were observed.
Conjugated bilirubin and unconjugated bilirubin 40 mg/dL
Chyle 1,000 FTU

Expiration date and preservation conditions

Storage conditions: Store at 2-8°C. Don't freeze. Keep away from light.

Expiration: 1 year from the date of manufacture. After the bottles are opened, the kit should be used in 1 month.

Reference

Ramsay,W.N.M.:Chin.Chim.Acta,2.221-226(1957)

Manufacturing-and-selling contractor

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*Metallogenics ™ is the name of reagent kit from Cellspect Co., Ltd.