Instruction manual

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

UIBC Assay kit LS

(Bathophenanthroline Chromogenic method)

Description

Iron is an essential element in mammalian. Iron is contained in various enzymes and is involved in oxidation reaction. Iron is essential to a transport of oxygen as composition element of hemoglobin or myoglobin. 30% of the transferrin has associated with Fe^{3+} in blood. Transferrin which iron has not associated is free transferrin. TIBC (Total iron binding capacity) = UIBC (Unsaturated iron binding capacity) + Serum iron. TIBC level changes in blood disorder, hepatic disease, tumor, and inflammation. UIBC level is increased in patients with iron deficiency. Decreased levels are seen in patients with infection disease, malignant, nephrosis syndrome, and low proteinosis. This UIBC Assay reagent kit utilizes the chromogen bathophenanthroline to bind Fe^{2+}. Transferrin in serum is saturated by the addition of buffer which known concentration of Fe^{3+}. Unbound Fe^{3+} is then reduced to Fe^{2+} by a reducing agent. The Fe^{2+} react with bathophenanthroline to form a colored complex which is measured 96-well reader or spectrophotometer and is proportional to the amount of UIBC present.

Kit contents

200 tests （Catalog # : UIB01E ）

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

Note

A) Unstableness of 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 volume for samples and reagents may affect the quality of assay. Please note that samples, R-A Buffer and Working R-R Chelate color must be poured accurately μL level.
D) Temperature for chromogen reaction may affect optical density. Please try to extend or shorten chromogen reaction time depending on room temperature.
E) In the cell lysate or the tissue extract use as specimen, high concentration of proteins or lipid, may affect observed value. Please remove its by ultrafiltration or centrifugation.

Operation

1. Sample preparation

◇Serum or Plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma cannot be used.

2. Assay preparation

Bring all reagents to room temperature before use.

3. Assay procedure.

Procedure using microplate reader.

(1 assay sample 250µL)

○Assay

(1) Add 200 µL of R-A 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 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_{BI}	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	30	30
↓ Mix and incubate for 5 minutes at room temperature. Read the absorbance at 546 nm(main) and 600 nm (sub).			

○Calculations

$OD_{BI} = OD2_{BI} – OD1_{BI}$

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

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

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

(Assay example)

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

***Observed 546 nm with 600 nm**

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

OD_{BI} = (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) = $\Delta OD_S/ OD_{BI}$ 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(546nm)]

OD_{BI} = 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) = $\Delta OD_S/ OD_{BI}$ 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.

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)

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