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IM-CBC800-150518E
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Catalog # CBC-800

MC Reagent Biochemical Assay Series

COSMO BIO CO., LTD.
Inspiration for Life Science

UIBC Assay Kit

Bathophenanthroline Chromogenic method

Biochemical Significance and Test Summary

Iron is an important element in organism. Many important enzymes use iron as a cofactor. And iron is an indispensable component to generate protein which transports oxygen. Iron is transported in blood by transferrin. 30% of the transferrin is combined with Fe3+ in blood, and the remainder exists as the 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.
MC Reagent Biochemical Assay Series UIBC assay 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+}.
And unbound Fe^{3+} in the buffer is reduced by a reducing agent. Generated Fe^{2+} react with bathophenanthroline and form a pink colored complex. The difference between the resulting change in the measured absorbance and the absorbance from the total amount added to serum is proportional to the quantity bound to transferrin. So UIBC can be calculated by subtract unbound ferric iron from the concentration of the reagent.

1. Kit contents (100 tests)

R-1	Buffer (Iron concentration 80µg/dL)	1 x 20 mL	Ready to use
R-2	Chelate color	1 x 3 mL	Ready to use

*Storage conditions: Store at 2-8°C. **Don't freeze**.
*Expiration: 1 year. After the vials are opened, the kit should be used in one month.
*Measuring range: 10 - 800 µg/dL

2. Materials required but not provided

(1) Distilled water
(2) Micropipettors and pipette tips
(3) Clear flat-bottom 96-well plate
(4) Microplate reader with 546 nm(main) capability

3. Assay preparation

Bring all reagents to room temperature before use.

4. Sample preparation

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

5. Assay protocol

(1) Add 20 µL of distilled water (Blank)/Samples to each well.
(2) Add 200 µL of R-1 to each well and incubate for 5 minutes at room temperature.
(3) Read the absorbance at 546 nm (main) and 600 nm (reference wavelength). ----- OD_1
(4) Add 30 µL of R-2 to each well and incubate for 5 minutes at room temperature.
(5) Read the absorbance at 546 nm and 600 nm (reference wavelength). ----- OD_2

6. Calculation

$OD = OD_2 - OD_1$
$\Delta OD_{Sample} = OD_{Blank} - OD_{Sample}$

UIBC (μg/dL) = $\Delta OD_{Sample}/OD_{Blank}$ x 800
UIBC (μM) = $\Delta OD_{Sample}/OD_{Blank}$ x 143.2

(Assay example)

	OD_1			OD_2			OD	ΔOD	UIBC
	546 nm	600 nm	546 nm - 600 nm	546 nm	600 nm	546 nm - 600 nm			(μg/dL)
DW (Blank)	0.024	0.025	-0.001	0.201	0.046	0.155	0.156	-	-
Sample	0.047	0.039	0.008	0.185	0.052	0.133	0.125	0.031	159.0

(a) Measurement at 546 nm and 600 nm (reference wavelength):
$OD_{Blank} = OD_{2Blank} - OD_{1Blank}$ = (0.201 - 0.046) - (0.024 - 0.025) = 0.155 - (-0.001) = 0.156
$OD_{Sample} = (OD_{2Sample} - OD_{1Sample})$ = (0.185 - 0.052) - (0.047 - 0.039) = 0.133 - 0.008 = 0.125
$\Delta OD_{Sample} = OD_{Blank} - OD_{Sample}$ = 0.156 - 0.125 = 0.031

$UIBC_{Sample} = \Delta OD_{Sample}/\Delta OD_{Blank}$ x 800 = (0.031 / 0.156) x 800 = 159.0 (μg/dL)
UIBC (μM) = $\Delta OD_{Sample}/\Delta OD_{Blank}$ x 143.2 = (0.031 / 0.156) x 143.2 = 28.5 (μM)

(b) Measurement at 546 nm:
$OD_{Blank} = OD_{2Blank} - OD_{1Blank}$ = 0.201 - 0.024 = 0.177
$OD_{Sample} = OD_{2Sample} - OD_{1Sample}$ = 0.185 - 0.047 = 0.138
$\Delta OD_{Sample} = OD_{blank} - OD_{Sample}$ = 0.177 - 0.138 = 0.039

$UIBC_{Sample} = \Delta OD_{Sample}/\Delta OD_{Blank}$ x 800 = (0.039 / 0.177) x 800 = 176.3.0 (μg/dL)
UIBC (μM) = $\Delta OD_{Sample}/\Delta OD_{Blank}$ x 143.2 = (0.039 / 0.177) x 143.2 = 31.6 (μM)

7. Interferences

EDTA inhibits iron to chromogenic system. The test is not affected by presence of bilirubin-F and bilirubin-C up to 40 mg/dL and chyle up to 1,000 FTU.

8. Quality Control

Use of control sera is recommended to monitor the quality of assay results.

9. References

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

10. Technical support & troubleshooting

(1) Unstableness of incubation temperature may result in unstable results.
(2) Use disposable test tube and glassware washed with 1M HNO_3 or 1M HCl, and rinse with distilled water.
(3) Accuracy to the microliter is important to obtain good results. Ensure maximum precision when pipetting.
(4) Temperature for the chromogenic reaction may affect the optical density. It may be necessary to adjust the reaction time depending on the room temperature.

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