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MC Reagent Biochemical Assay Series

COSMO BIO CO., LTD.
Inspiration for Life Science

Iron Assay Kit

Ferrozine Chromogenic method

Biochemical Significance and Test Summary

Iron is one of the most important elements, which function as enzyme cofactor. Iron in the blood is bound with transferrin and transported throughout the body to synthesize globin proteins such as myoglobin and hemoglobin. Iron is crucial for synthesis of oxygen-transport protein. Its deficiency causes iron deficiency anemia, chronic hemorrhagic anemia and infectious anemia.
This product is a colorimetric assay kit without sample deproteinization. Iron bound with transferrin is dissociated from proteins in weakly acidic region and ferric iron is reduced to ferrous iron [Fe(II)–ferrozine complex]. Fe(II)–ferrozine complex causes purple color. Sample iron concentration is determined by comparing the 560nm absorbance of sample wells to the absorbance of known standard.

1. Kit contents (100 tests)

R-1	Buffer	1 x 20 mL	Ready to use
R-2	Chelate color	1 x 0.8 mL	Ready to use
STD	200 µg/dL Fe Standard	1 x 4.0 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: 5-1000 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 560 nm 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.
Urine (24 hour pooled urine)/ Biological fluid: Add 6M HCl to the sample and adjust pH 2.0-3.0 (e.g. 5-10 µL of 6M HCl/1mL of lysate). Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.
Tissue: Add 3% TCA solution, vortex for 1 minute and incubate for 30 minutes at 4-8°C. Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.

Note: Sample pH should be between pH 2 and pH 8.

5. Assay protocol

(1) Add 40 µL of distilled water (Blank)/STD(Standards)/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 560 nm. ----- OD_1
(4) Add 8 µL of R-2 to each well and incubate for 5 minutes at room temperature.
(5) Read the absorbance at 560 nm (540-580 nm). ----- OD_2

6. Calculation

$OD = OD_2 - OD_1$

$\Delta OD_{Standard} = OD_{Standard} - OD_{Blank}$, $\Delta OD_{Sample} = OD_{Sample} - OD_{Blank}$

Iron (μg/dL) = $\Delta OD_{Sample} / \Delta OD_{Standard} \times 200$

Iron (μM) = $\Delta OD_{Sample} / \Delta OD_{Standard} \times 35.8$

(Assay example)

	OD_1	OD_2	ΔOD (OD_2-OD_1)	Iron (μg/dL)
DW (Blank)	0.030	0.030	-	-
Standard	0.029	0.133	0.104	-
Sample	0.044	0.099	0.055	106

$\Delta OD_{Standard} = (OD_{2Standard} - OD_{1Standard}) - (OD_{2Blank} - OD_{1Blank}) = (0.133 - 0.029) - (0.030 - 0.030) = 0.104$

$\Delta OD_{Sample} = (OD_{2Sample} - OD_{1Sample}) - (OD_{2Blank} - OD_{1Blank}) = (0.099 - 0.044) - (0.030 - 0.030) = 0.055$

$Iron_{Sample} = \Delta OD_{Sample} / \Delta OD_{Standard} \times 200 = (0.055 / 0.104) \times 200 = 106$ (μg/dL)

Iron (μM) = $\Delta OD_{Sample} / \Delta OD_{Standard} \times 35.8 = (0.055 / 0.104) \times 35.8 = 19$ (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, hemoglobin up to 0.2 g/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) Lawrence L. Stookey , *Analytical chemistry*, VOL. 42, NO. 7,(1970)
(2) Ikeda. Y, Tajima. S, Izawa-Ishizawa. Y, Kihira. Y, Ishizawa. K, Tomita. S, Tsuchiya. K, Tamaki. T: Estrogen regulates hepcidin expression via GPR30-BMP6-dependent signaling in hepatocytes, *PLoS One*, 7(7) (2012).
(3) Tsugawa. H, Suzuki. H, Matsuzaki. J, Hirata. K, Hibi. T: FecA1, a bacterial iron transporter, determines the survival of Helicobacter pylori in the stomach, *Free Radic Biol Med*. 15, 52(6), p1003-10 (2012).
(4) Nakaya. M, Tajima. M, Kosako. H, Nakaya. T, Hashimoto. A, Watari. K, Nishihara. H, Ohba. M, Komiya. S, Tani. N, Nishida. M, Taniguchi. H, Sato. Y, Matsumoto. M, Tsuda. M, Kuroda. M, Inoue. K, Kurose. H: GRK6 deficiency in mice causes autoimmune disease due to impaired apoptotic cell clearance, *Nature Commun*, 4, p1532 (2013).
(5) Hayashi. K, Nakamura. M, Sakamoto. W, Yogo. T, Miki. H, Ozaki. S, Abe. M, Matsumoto. T, Ishimura. K: Superparamagnetic nanoparticle clusters for cancer theranostics combining magnetic resonance imaging and hyperthermia treatment, *Theranostics*. 23, 3(6), p366-76 (2013).

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.
(5) High concentration of proteins or lipid in cell lysate or in tissue extract may affect the observed value. Please remove them by ultrafiltration or centrifugation.
(6) Species of heme-iron cannot be analyzed using this assay kit.

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