

MC Reagent Biochemical Assay Series

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 bounded 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 blood loss anemia and infectious anemia.

This product is a colorimetric assay kit without sample deproteinization. Iron bounded with transferrin is dissociated from proteins in weakly acidic region. Fe(III) is reduced to Fe(II), and blue colored complex with Ferrozine [Fe(II)–ferrozine complex] is formed. The concentration of iron in sample is determined by the absorbance of Fe(II)–ferrozine complex at 560 nm.

1. Kit contents

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

- (1) Bring all reagents to room temperature before use.
- (2) Wash test tube and glassware by 1M HNO₃ or 1M HCl, and rinse with distilled water.

4. Sample preparation

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

Urine (24 hour pooled urine)/Tissue homogenate/Cell lysate/Saliva: Add 6M HCl to the sample and adjust pH 1.5-3.0 (e.g. 5-10 µL of 6M HCl/1 mL of sample). Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.

Tissue: Add 5% 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.

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 (540-580 nm). --> OD₁
- (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). --> OD₂

6. Calculation

$$OD = OD_2 - OD_1$$

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

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

$$\text{Iron } (\mu\text{M}) = \Delta OD_{Sample} / \Delta OD_{Standard} \times 35.8$$

(Assay example)

	OD ₁	OD ₂	$\Delta OD (OD_2 - OD_1)$	Iron ($\mu\text{g/L}$)
DW (Blank)	0.030	0.030	-	-
Standard	0.029	0.133	0.104	-
Sample	0.044	0.099	0.055	106

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

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

$$\text{Iron}_{Sample} = \Delta OD_{Sample} / \Delta OD_{Standard} \times 200 = 0.055 / 0.104 \times 200 = 106 \text{ } (\mu\text{g/dL})$$

$$\text{Iron } (\mu\text{M}) = \Delta OD_{Sample} / \Delta OD_{Standard} \times 35.8 = 0.055 / 0.104 \times 35.8 = 19 \text{ } (\mu\text{M})$$

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 assay quality.

9. References

- (1) Yamada M, Tanioka K, Kishi K, Hatanaka U, Ohnishi M. An automated method for measurement of serum iron and unsaturated iron binding capacity using nitroso-PSAP. *Jpn J Lab Auto*, 13, p659-63 (1988).
- (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

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(Supported languages: Japanese and English)



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