MC Reagent Biochemical Assay Series

Iron Assay Kit

Nitroso-PSAP 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 direct colorimetric assay kit without deproteinization of the sample. Dissociated iron from the transferrin-iron complex by weakly acid buffer and reduced by means of reductant (:Ferric→Ferrous). Ferrous ions give a blue colored complex with Nitroso-PSAP (as chromogen). The intensity of the colored complex is proportional to the iron concentration in the sample. The colour intensity is proportional to the amount of iron present in the sample.

1. Kit contents (100 tests)

R-1	Buffer	1 x 16 mL	Ready to use
R-2	Buffer	1 x 7 mL	Ready to use
R-3	Chelate color	1 x 0.7mL	Ready to use
STD	200 µg/dL Fe Standard	1 x 1.5 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: 10-1000 µg/dL

2. Materials required but not provided

(1) Distilled water
(2) Micropipettors and pipette tips
(3) Clear flat-bottom 96-well plate
(4) Micro plate reader with 750 nm capability

3. Assay preparation

(1) Bring all reagents to room temperature before use.
(2) Prepare working reagent: Mix 70 µL of R-2 and 7 µL of R-3 for 1 test.
(e.g.) Preparation for 50 tests
R-2: 70 µL x 50 tests = 3.5 mL
R-3: 7 µL x 50 tests = 350 µL
Mix 3.5 mL of R-2 and 350 µL of R-3 in a vessel.
(3) Wash test tube and glassware with 1M HNO3 or 1M HCl, and rinse with distilled water.

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 1.5-3.0 (e.g. 5-10µL 6M HCl/1mL of lysate). Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.
Tissue: Add 5% TCA solution, voltex 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 15 µL of Distilled water (Blank)/STD(Standard)/Sample into each well.
(2) Add 160 µL of R-1 to each well and incubate for 10 minutes at room temperature.
(3) Add 75 µL of Working reagent to each well and incubate for 5 minutes at room temperature.
(4) Read the absorbance at 750 nm (740-760 nm). ----- OD

6. Calculations

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

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 (750 nm)	Δ OD	Iron (µg/dL)
DW (Blank)	0.048	-	-
Standard	0.107	0.059	-
Sample	0.081	0.033	112

$\Delta OD_{Standard} = OD_{Standard}$ (750 nm) - OD_{Blank} (750 nm) = 0.107 - 0.048 = 0.059
$\Delta OD_{Sample} = OD_{Sample}$ (750 nm) - OD_{Blank} (750nm) =0.081 - 0.048 = 0.033

$Iron_{Sample}$ (µg/dL) = $\Delta OD_{Sample}/\Delta OD_{Standard} \times 200$ = (0.033/ 0.059) x 200 = 112 (µg/dL)
$Iron_{Sample}$ (µM) = $\Delta OD_{Sample}/\Delta OD_{Standard} \times 35.8$ = (0.033/ 0.059) x 35.8 = 20.0 (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 0.1 g/dL and chyle up to 500 FTU.

8. Quality Control

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

9. Reference

(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) 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).
(3) 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).
(4) 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).
(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 HNO3 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 chromogen 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) Sample pH should be between pH 2 and pH 8.
(7) Species of heme-iron cannot be analyzed using this assay kit.

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