

QuantiChrom™ Iron Assay Kit (DIFE-250)

Quantitative Colorimetric Iron Determination at 590nm

DESCRIPTION

Iron level in blood is a reliable diagnostic indicator of various disease states. Increased levels of iron concentration in blood are associated with blood loss, increased destruction of red blood cells (e.g. hemorrhage) or decreased blood cell survival, acute hepatitis, certain sideroachrestic anemias, ingestion of iron-rich diets, defects in iron storage (e.g. pernicious anemia). Decreased levels of blood iron may result from insufficient iron ingestion from diets, chronic blood loss pathologies, or increased demand on iron storage as during normal pregnancy.

Simple, direct and automation-ready procedures for measuring iron concentrations find wide applications in research, drug discovery and environmental monitoring. BioAssay Systems' iron assay kit is designed to measure total iron directly in serum without any pretreatment. The improved method utilizes a chromogen that forms a blue colored complex specifically with Fe^{2+} . Fe^{3+} in the sample is reduced to Fe^{2+} , thus allowing the assay for total iron concentration. The intensity of the color, measured at 590nm, is directly proportional to the iron concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

KEY FEATURES

Sensitive and accurate. Linear detection range 27 $\mu\text{g/dL}$ (4.8 μM) to 1,000 $\mu\text{g/dL}$ (179 μM) iron in 96-well plate assay.

Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 40 min. Can be readily automated as a high-throughput assay for thousands of samples per day.

Improved reagent stability and versatility. The optimized formulation has greatly enhanced reagent and signal stability. Cuvet or 96-well plate assay.

Low interference in biological samples. No pretreatments are needed. Assays can be directly performed on serum samples.

APPLICATIONS:

Direct Assays: iron in biological samples (e.g. serum).

Drug Discovery/Pharmacology: effects of drugs on iron metabolism.

Environmental Monitoring: iron in soil extracts, mineralized samples.

KIT CONTENTS (250 tests in 96-well plates)

Reagent A: 50 mL Reagent B: 4 mL Reagent C: 4 mL

Iron Standard: 1 mL 10 mg/dL Fe^{2+}

Storage conditions. Reagent A can be stored at room temperature or 4 °C. Other components should be stored at 4 °C. Shelf life: 12 months.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Reagent Preparation:

Prepare enough Working Reagent by mixing 20 volumes of Reagent A, 1 volume Reagent B and 1 volume Reagent C. Fresh reconstitution is recommended. Equilibrate to room temperature before assay.

Procedure using 96-well plate:

- Standards. Prepare 400 μL 1000 $\mu\text{g/dL}$ Premix by mixing 40 μL 10 mg/dL standard and 360 μL distilled water. Dilute standards as follows.

No	Premix + H_2O	Vol (μL)	Fe ($\mu\text{g/dL}$)
1	100 μL + 0 μL	100	1000
2	80 μL + 20 μL	100	800
3	60 μL + 40 μL	100	600
4	40 μL + 60 μL	100	400
5	30 μL + 70 μL	100	300
6	20 μL + 80 μL	100	200
7	10 μL + 90 μL	100	100
8	0 μL + 100 μL	100	0

- Set up standards and samples. Transfer 50 μL diluted Standards and 50 μL samples into wells of a clear bottom 96-well plate. As a sample

blank control, add 200 μL Reagent A to sample "blank" wells. Add 200 μL Working Reagent to all other wells. Tap plate to mix. Store diluted standards at 4 °C for future use.

- Incubate 40 min at room temperature and read optical density at 510-630nm (peak absorbance at 590nm). Signal is stable for > 60 min.

Procedure using cuvette:

- Prepare standards as in 96-well assay. Set up centrifuge tubes labeled Standards and Samples ("Sample" and sample "Blank"). Transfer 250 μL Standards and Samples to tubes.
- Add 1000 μL Reagent A to "Blank" and 1000 μL Working Reagent to all other tubes. Mix by vortexing. Incubate 40 min at room temperature.
- Transfer to cuvetts and read OD at 590nm (510nm-630nm).

CALCULATION

Subtract blank OD (water, #8) from the standard OD values and plot the OD against standard iron concentrations. Determine the slope using linear regression fitting. Iron concentration of the sample is calculated as

$$= \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope}} \quad (\mu\text{g/dL})$$

$\text{OD}_{\text{SAMPLE}}$ and OD_{BLANK} are $\text{OD}_{590\text{nm}}$ values of sample and sample "Blank". Typical serum iron values: 70-180 $\mu\text{g/dL}$.

Conversions: 1 mg/dL Fe equals 179 μM , 0.001% or 10 ppm.

Note: this kit can be applied to measure Fe^{2+} (vs. total iron) content. Prepare Working Reagent by mixing 20 volumes of Reagent A and 1 volume Reagent C (no reductant in the Working Reagent). The procedure is the same as described for the total iron assay.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories.

Procedure using 96-well plate:

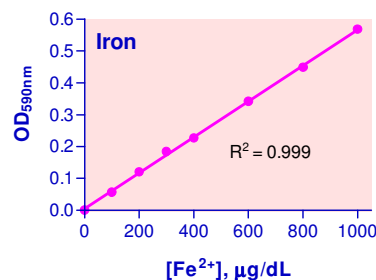
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Cuvetts and spectrophotometer for measuring OD at 510-630nm.

EXAMPLES:

Mice serum, fetal bovine serum (Invitrogen), and goat serum (Invitrogen) were assayed using the 96-well plate assay protocol. The iron concentrations were 173 ± 2 (n = 4), 149 ± 1 (n = 4), 88 ± 2 $\mu\text{g/dL}$ (n = 4), respectively. Coefficient of variance < 2%.



Calibration curve in 96-well plate

LITERATURE

- Velez LI et al (2004). Iron bezoar retained in colon despite 3 days of whole bowel irrigation. J Toxicol Clin Toxicol. 42(5):653-6.
- Harvey JW et al (1987). Potential effects of glucocorticoids on serum iron concentration in dogs. Vet Clin Pathol. 16(2):46-50.
- Hoppe M et al (2003). Serum iron concentration as a tool to measure relative iron absorption from elemental iron powders in man. Scand J Clin Lab Invest. 63(7-8):489-96.