Version 19-11/24

User's Manual

Iron Assay Kit

REF **DEIA-NS2408-6**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

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.

Key Features:

Sensitive and accurate: Linear detection range 27 µg/dL (4.8 µM) to 1,000 µg/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.

General 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.

Principles of Testing

The 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 590 nm, is directly proportional to the iron concentration in the sample.

Reagents And Materials Provided

1. Reagent A, 50 mL
2. Reagent B, 4 mL
3. Reagent C, 4 mL

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

Materials Required But Not Supplied

1. Pipeting devices and accessories.
2. Procedure using 96-well plate: Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.
3. Procedure using cuvette: Cuvets and spectrophotometer for measuring OD at 510-630 nm.

Storage

Store Reagent A at room temperature and other reagents at 4°C. Shelf life: 12 months after receipt.

Assay Procedure

Note: (1).Iron chelators (e.g. EDTA) interfere with this assay and should be avoided in sample preparation. (2). Serum or plasma samples should be clear and free of precipitates or turbidity. If not, centrifuge or filter to clarify samples prior to assay. (3). This kit can be applied to measure Fe^{2+} (vs. total iron) content. Prepare Working Reagent by mixing 20 volumes of Reagent A, 1 volume of water and 1 volume of Reagent C (no reductant in the Working Reagent). The procedure is the same as described for the total iron assay.

Procedure using 96-well plate:

1. Standards. Prepare 400 µL 1000 µg/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 (µg/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

   Transfer 50 µL diluted standards and 50 µL sample into wells of a clear flat bottom 96-well plate. For serum/plasma samples, it is recommended to run a sample blank (i.e. a 50 µL sample in a separate well).

2. 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. Add 200 µ L Working Reagent to Standards and Samples wells. (For serum/plasma samples which require a Sample Blank Control, add 200 µL Reagent A to the Sample Blank wells). Tap plate to mix.
3. Incubate 40 min at room temperature and read optical density at 510 nm-630 nm (peak absorbance at 590 nm)

Procedure using cuvette:

1. Prepare standards as in 96-well assay. Set up centrifuge tubes labeled Standards and Samples. Transfer 250 µL Standards and Samples to tubes.
2. Add 1000 µL Working Reagent to all tubes. Mix by vortexing. Incubate 40 min at room temperature.
3. Transfer to cuvettes and read OD at 590 nm (510nm-630nm).

Calculation

Subtract OD of "0 µg/dL Fe" from all other 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

[Iron] (µg/dL)= (OD_{SAMPLE} - OD_{BLANK}) / Slope

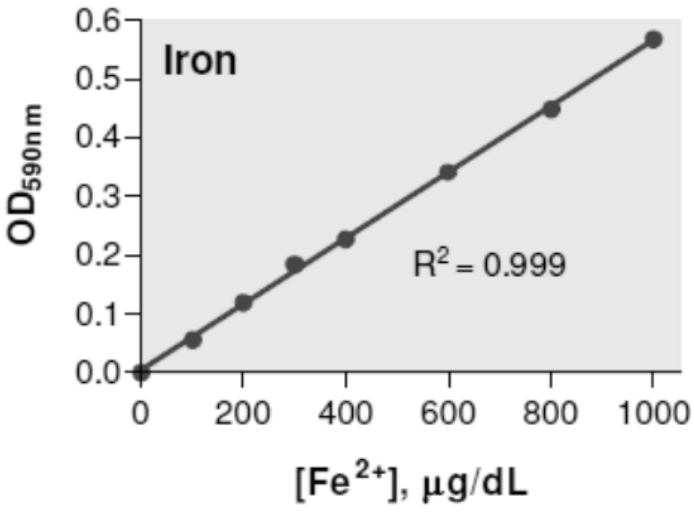
Where OD_{BLANK} is OD values of the water blank (Standard #8), or Sample Blank, if a sample blank is used (e.g. serum or plasma). Typical serum iron values: 70-180 µg/dL.

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

Typical Standard Curve

Examples:

Mouse 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 µg/dL (n = 4), respectively. Coefficient of variance < 2%.



Standard Curve in 96-well plate assay

Detection Range

27 ug/dL (4.8 uM) to 1,000 ug/dL (179 uM) in 96-well plate assay.

Precautions

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

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