Iron Content Assay Kit (Ferrozine, Micro Method)

Cat. No.: I1505775 | Pack size: 100T | Storage: 2-8 °C, protected from light; RT as indicated

Overview

Iron is an essential trace element in the human body, with a total content of approximately 3270 mg. It is widely distributed: 67.6% of iron is found in hemoglobin as a prosthetic group, involved in oxygen transport; 2.59% and 4.15% are present in bone and myoglobin, respectively; and storage iron accounts for about 25.37%. Serum iron is bound to transferrin in the form of ferric ions (Fe^{3+}). Therefore, measuring serum iron requires first dissociating Fe^{3+} from transferrin.

Assay Principle

This assay utilizes a microplate method with Ferrozine as the chromogen. In an acidic medium, serum iron bound to transferrin is released. Iron in other samples is also liberated under acidic conditions. The released iron is then reduced to ferrous ions (Fe^{2+}) by a reducing agent (present in the assay buffer). The Fe^{2+} subsequently reacts with Ferrozine to form a magenta-colored complex. The absorbance at 562 nm is measured using a microplate reader. This method is suitable for detecting iron content in samples such as serum, plasma, and tissues. As this direct detection method is used, a serum blank should be included to correct for the sample's inherent color. The iron content is calculated according to the formula. This kit shows a good linear relationship up to 140 µmol/L. Triglycerides ≤ 3.39 mmol/L and bilirubin ≤ 171 µmol/L basically do not interfere with this method. This kit is for research use only and not suitable for clinical diagnosis or other purposes.

Product Information

Item	Specification
Grade	BioReagent
Application	Cell Metabolism
Stability and Storage	Each component has a shelf life of 1 year under corresponding storage conditions.
Storage	Store at 2-8 °C, protected from light; room temperature for specified components.
Shipped In	Wet ice. This product requires cold chain shipping. Ground and other economy services are not available.
Sensitivity	Light-sensitive

Kit Contents & Storage

Cat. No.	Component	Size	Storage
I1505775A	Iron Standard (100 μg/mL)	1 mL	2-8 °C. Store in the dark.
I1505775B	Iron Standard Diluent	2 mL	RT
I1505775C	Fe Assay Buffer	25 mL	2-8 °C
I1505775D	Ferrozine Chromogenic Solution	1 mL	2-8 °C. Store in the dark.
I1505775E	ddH_2O	10 mL	RT

Shelf life: Each component has a shelf life of 1 year under corresponding storage conditions.

Materials Required But Not Supplied

Centrifuge tubes or test tubes, microplate reader, and 96-well plate.

Experimental Procedure

1. (Optional) Sample Preparation

1.1 Plasma/Serum Samples

Prepare plasma or serum by conventional methods.

Store at -20 °C for Fe detection.

1.2 Cell or Tissue Samples

Homogenize an appropriate amount of cells or tissue.

Centrifuge at low speed and collect the supernatant.

Store at -20 °C for Fe detection.

1.3 High-Concentration Samples

If the sample contains a high concentration of Fe, dilute it with ddH_2O. Do not use regular distilled water for dilution.

Note: (Optional) After preparation, determine the protein concentration using a BCA Protein Assay Kit to calculate Fe content per mg protein.

2. Preparation of Iron Standard Working Solution

Dilute the Iron Standard (100 μg/mL) with Iron Standard Diluent at a 1:49 ratio to prepare the Iron Standard Working Solution (2 μg/mL).

Store at 4 °C protected from light; stable for 3 months.

3. Fe Assay Setup

Use a 96-well plate cleaned with dilute hydrochloric acid and rinsed with deionized water.

Set up Blank, Standard, and Test wells according to the table below.

Add reagents in the specified order, avoiding bubbles.

If the sample's iron concentration is too high, reduce the sample volume or dilute appropriately before assaying. Duplicate wells are recommended.

Reagent (µL)	Blank Well	Standard Well	Test Well
ddH_2O	75	—	—
Iron Standard Working Solution (2 µg/mL)	—	75	—
Sample	—	—	75
Fe Assay Buffer	200	200	200
Mix well. Read absorbance at 562 nm, zeroed with the Blank well. Record for Test well (this is the Serum Blank, $A_{serum\ blank}$).			
Ferrozine Chromogenic Solution	8.5	8.5	8.5

4. Fe Measurement

Mix well.

Incubate at room temperature for 15 minutes or at 37 °C for 10 minutes.

Zero the instrument with the Blank well.

Measure the absorbance of the Standard and Test wells at 562 nm (recorded as $A_{standard}$ and A_{test}).

Complete the colorimetric measurement within 1 hour.

5. Calculation

5.1 Plasma Iron, Serum Iron

Fe (µmol/L) = { [A_{test} - ($A_{serum\ blank}$ × 0.97)] / $A_{standard}$ } × 35.8

Fe (mg/L) = { [A_{test} - ($A_{serum\ blank}$ × 0.97)] / $A_{standard}$ } × 2

5.2 Tissue Iron, Cell Iron

Fe (µmol/g prot) = { [A_{test} - ($A_{serum\ blank}$ × 0.97)] / $A_{standard}$ } × 35.8 / Pr

Fe (mg/g prot) = { [A_{test} - ($A_{serum\ blank}$ × 0.97)] / $A_{standard}$ } × 2 / Pr

Fe (µmol/N cells) = { [A_{test} - ($A_{serum\ blank}$ × 0.97)] / $A_{standard}$ } × 35.8 / (N × V_{sample} / $V_{total\ extract}$)

Fe (mg/N cells) = { [A_{test} - ($A_{serum\ blank}$ × 0.97)] / $A_{standard}$ } × 2 / (N × V_{sample} / $V_{total\ extract}$)

Parameter Definitions:

A_{test}: Absorbance of the Test well after adding Ferrozine Chromogenic Solution.

$A_{serum\ blank}$: Absorbance of the Test well before adding Ferrozine Chromogenic Solution.

$A_{standard}$: Absorbance of the Standard well.

Pr: Protein concentration of the test sample (g/L).

N: Total number of cells or bacteria.

V_{sample}: Volume of sample added to the reaction system (L).

$V_{total\ extract}$: Total volume of the sample extraction buffer (L).

Unit Conversions:

Iron Standard (2 μg/mL) = Iron Standard (2 mg/L) = Iron Standard (35.8 μmol/L)

μg/dL = μmol/L / 0.179

μmol/L = 0.179 × μg/dL = 17.9 × μg/mL

Reference Interval:

Healthy Adults Serum Iron:

Male: 11-30 μmol/L (60-170 μg/dL)

Female: 9-27 μmol/L (50-150 μg/dL)

6. Standard Curve Reference

Standard curve graph and data typically show absorbance versus concentration for standards such as 0, 0.5, 1, 2, 4, 6, 8, and 10 μg/mL, measured at 562 nm.

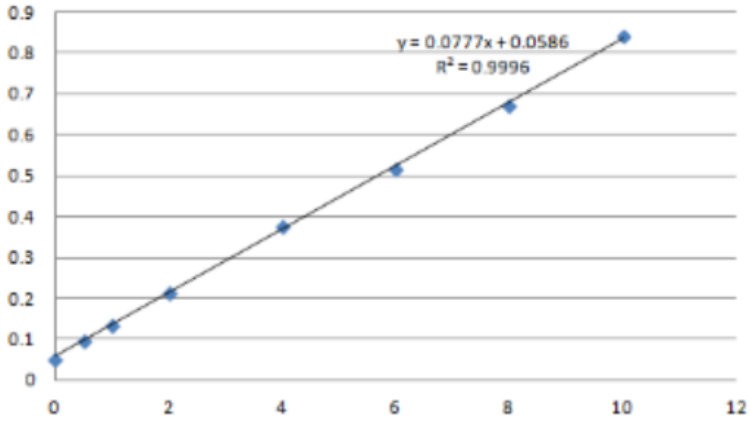

Figure 1. Reference standard curve measured at 562 nm.

Note: Standard curves may vary due to differences in instruments and operational techniques. Empirical data suggests deviations in the standard curve at iron standard concentrations below 0.1 μg/mL and above 40 μg/mL.

Precautions

Hemolyzed samples interfere with the assay; avoid using them if possible.

If the sample concentration is too high, dilute with distilled water and re-assay, multiplying the result by the dilution factor.

Use high-purity deionized water during the experiment; do not use ordinary distilled water.

Glassware should be soaked in 10% hydrochloric acid for 24 hours and rinsed with deionized water before use.

Avoid contact with iron objects to prevent iron contamination.

The standard color is stable for 24 hours. The serum color is stable for 30 minutes; the color intensifies slowly over time, so measurement must be completed within 1 hour.

The value 0.97 is a volume correction factor.

The within-run coefficient of variation (CV) for this method is ≤ 3.1%; the between-run CV is ≤ 2.6%.

Use reagents promptly after opening to avoid affecting subsequent experimental results.

For your safety and health, wear lab coats and disposable gloves during operation.

Quality Control

QC Item	Method	Acceptable Range / Reference
Component completeness	Visual inspection on receipt	All components and counts match the kit contents table; lot number and expiry date are legible.
Standard curve and linearity	Prepare Iron Standard Working Solution and measure absorbance at 562 nm	Good linear relationship up to 140 µmol/L; standard curves should be generated under the same instrument and operating conditions.
Precision	Within-run and between-run repeatability evaluation	Within-run CV ≤ 3.1%; between-run CV ≤ 2.6%.
Interference assessment	Sample status and interference check	Avoid hemolyzed samples. Triglycerides ≤ 3.39 mmol/L and bilirubin ≤ 171 µmol/L basically do not interfere with this method.
Storage compliance	Visual inspection and storage review	Protect light-sensitive components from light; use reagents promptly after opening.

Troubleshooting

Issue	Possible Causes	Corrective Action
High background or abnormal serum blank	• Hemolyzed sample • Strong inherent sample color • Serum blank not recorded before chromogenic solution	• Avoid hemolyzed samples if possible • Include a serum blank for each sample • Record the Test well absorbance before adding Ferrozine Chromogenic Solution
Unexpectedly high iron value	• Iron contamination from glassware, water, or iron objects • Sample concentration above the suitable range	• Soak glassware in 10% hydrochloric acid for 24 hours and rinse with deionized water • Use high-purity deionized water and avoid iron objects • Dilute the sample and multiply by the dilution factor
Low or nonlinear signal	• Reagent deterioration after opening • Incubation time or temperature not consistent • Standard concentration outside the recommended range	• Use reagents promptly after opening • Keep incubation at room temperature for 15 minutes or 37 °C for 10 minutes • Prepare a fresh standard working solution and standard curve
Poor repeatability	• Bubbles in wells • Inconsistent reagent addition order or timing • Plate or reader inconsistency	• Add reagents in the specified order and avoid bubbles • Run duplicate wells • Zero with the Blank well and complete measurement within 1 hour

Recommended Applications

Cell Metabolism · Iron content detection in serum, plasma, tissues, cells, and other compatible biological samples · Research on iron metabolism and related cellular processes

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office for your region; for general inquiries, the North American office is the corporate primary.

NORTH AMERICAN SALES, SUPPORT & GENERAL INQUIRIES

Aladdin Scientific Corporation

14078 Meridian Parkway, Riverside, CA 92518, USA

Phone: 1-833-552-7181

Sales: sales@aladdinsci.com

Customer Service: custserv@aladdinsci.com

EU SALES, LOGISTICS & LOCALIZED SUPPORT

Aladdin Biochem Deutschland GmbH

Westring 2, 33142 Büren, Germany

Phone: +02951 9383958

Support: support.eu@aladdinsci.com

Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.
- This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals for research and development purposes.
- Where any kit component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, and disposal information.
- Performance depends on sample type, sample condition, handling, and operator technique. Users are responsible for validating the kit for their specific application.
- Aladdin product labels, SDS, COA, and approved specifications take precedence over this document. If product formulation, label, SDS, storage conditions, pack size, or quality specifications change, this document would be reviewed and reissued.