Iron Assay Kit

Iron Assay Kit is a detection kit for the quantification of Iron ions Fe3+ or Fe2+ in serum and plasma.

Catalog number: ARG81386

Package: 250 tests

For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

This kit is designed to measure total free iron (Fe^{2+} and Fe^{3+}) directly in serum and plasma without any pretreatment. Fe^{3+} in the sample is reduced to Fe^{2+}, and Fe^{2+} was complexed with a chromogen to form a blue colored complex. Therefore, this improved method assay kit allowing the assay for total free iron (Fe^{2+} and Fe^{3+}) concentration. The intensity of the color is measured at a wavelength of 590 nm. The concentration of total free iron in the sample is then determined by comparing the O.D. of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Reagent A	50 ml	RT
Reagent B	4 ml	4°C
Reagent C	4 ml	4°C
Standard (10 mg/dL $Fe2^{+}$)	1 ml	4°C

The kit is shipped at room temperature. Store the Reagent A at room temperature and all other reagents at 4°C. Shelf life of 12 months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 590 nm
- Flat bottomed 96-well microplate
- Pipettes and pipette tips
- Heat block or hot water bath or oven
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times, the Reagent A can be stored at room temperature.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant (EDTA is a kind of iron chelators, and it interferes with this assay and should be avoided to use in sample preparation). Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Samples should be clear and free of precipitates or turbidity. If not, centrifuge or filter to clarify samples prior to assay

REAGENT PREPARATION

- **Standards**: Add **40 µl** of **10 mg/dL stock** standard into **360 µl** of **distilled water** to generate a standard #S1 with **1000 µg/dL** of Fe^{2+}. Dilute the standards with **distilled water** and distilled water serves as zero standard (0 µg/dL). The example of the standards dilution table is as below:

Standard #	Standard Fe^{2+} (µg/dL)	Volume of 1000 µg/dL Fe^{2+} (µl)	Volume of Distilled water (µl)
S1	1000	100	0
S2	800	80	20
S3	600	60	40
S4	400	40	60
S5	300	30	70
S6	200	20	80
S7	100	10	90
S8	0	0	100

- **Working Reagent T (For total Fe)**:

  Prepare immediately before use, mix **20 volumes** of Reagent A, **1 volume** of Reagent B and **1 volume** of Reagent C and mix well to generate the **Working Reagent T** for total free Fe measurement. (E.g. mix 200 µl of Reagent A, 10 µl of Reagent B and 10 µl of Reagent C for each well). Equilibrate to room temperature before assay.

- **Fe^{2+} Working Reagent (For Fe^{2+} measurement only, optional) :**

  This kit can also be applied to measure Fe^{2+} content only if needed. To assay **Fe^{2+}** content, prepare **Fe^{2+} Working Reagent** by mixing 20 volumes of Reagent A, 1 volume **distilled water** and 1 volume Reagent C and mix well to generate the Fe^{2+} Working Reagent for Fe^{2+} measurement. Equilibrate to room temperature before assay. This Fe^{2+} Working

Reagent does not include reductant and Fe^{3+} was not reduced to Fe^{2+}.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

For total Fe:

1. Add **50 µl** per well of **standards and samples** in duplicates into appropriate wells of a clear flat bottom 96-well plate.
2. *For serum and plasma samples, it is recommended add 50 µl of one of the sample in two of separate wells as **sample blank.***
3. Add **200 µl** of **Working Reagent T** to Standards and Samples wells.
4. Add **200 µl** of **Reagent A** to sample blank wells.
5. Gently tap plate to mix thoroughly.
6. Incubate the plate for **40 min at room temperature**
7. Read O.D. with a microplate reader at **590 nm (510-630 nm)** immediately.

For Fe^{2+} measurement (optional if needed):

1. Add **50 µl** per well of **standards and samples** in duplicates into appropriate wells of a clear flat bottom 96-well plate.
2. *For serum and plasma samples, it is recommended add 50 µl of one of the sample in two of separate wells as **sample blank**.*
3. Add **200 µl** of **Fe^{2+} Working Reagent** to Standards and Samples wells.
4. Add **200 µl** of **Reagent A** to sample blank wells.
5. Gently tap plate to mix thoroughly.
6. Incubate the plate for **40 min at room temperature**
7. Read O.D. with a microplate reader at **590 nm (510-630 nm)** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a linear regression fitting. Determine the slope using linear regression fitting. The Iron concentration of Sample is calculated as

$$\text{Iron } (\mu g/dL) = (OD_{sample} - OD_{Blank}) / \text{Slope}$$

For serum/plasma samples,

OD_{Blank} = OD values of the Sample Blank (add reagent A only)

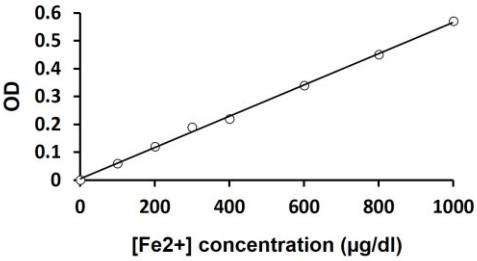
For other samples,

OD_{Blank} = OD values of the water blank (Standard #S8)

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

6. Typical serum iron values: 70-180 µg/dL.

EXAMPLE OF TYPICAL STANDARD CURVE



QUALITY ASSURANCE

Sensitivity

The standard ranged: 0-1000 µg/dL.

Linear detection range: 27 µg/dL (4.8 µM) to 1000 µg/dL (179 µM)

Sensitivity: 27 µg/dL (4.8 µM)