Technical Manual

Ferrous Iron Colorimetric Assay Kit

- **Catalogue Code: MAES0206**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

0.4-50 μmol/L

Sensitivity:

0.4 μmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background:

Iron is one of the metal elements in organism and has important physiological functions. Ferrous ion is a key element in heme and hemoglobin and plays an important role in many biochemical reactions. In recent years, with the introduction of the concept of iron death, it has been found that the absorption, transportation, storage and utilization of iron ions and their excessive accumulation in cells have a significant relationship with aging and disease.

3. Intended Use:

This kit can measure ferrous ions (Fe^{2+}) content in serum, cells, animal and plant tissue samples.

4. Detection Principle:

Ferrous ions (Fe^{2+}) in samples can bind with probe to form complexes, which has a maximum absorption peak at 593 nm. The concentration of iron can be calculated by measuring the OD value at 593 nm indirectly.

5. Kit Components & Storage:

Item	Specification	Storage
Buffer Solution	50 mL × 2 vials	2-8°C, 6 months, avoid direct sunlight
Chromogenic Solution	10 mL × 2 vials	2-8°C, 6 months, avoid direct sunlight
Iron Standard (10 mmol/L)	2 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard Diluent	30 mL x 1 vial	2-8°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (590-600 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. Prevent the formation of bubbles when the reagent or sample is transferred into the microplate.

2. Do not use iron appliances to prepare or transfer samples.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **iron standard (100 μmol/L):** Mix 20 μL of 10 mmol/L iron standard with 1980 μL of double distilled water fully. Prepare fresh solution before use.

8. Sample Preparation:

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (4×10^6): Buffer solution (μL) =1: 400. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

4. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of Buffer solution (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.4-50 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
Human serum	1
Mouse serum	1-2
Rat serum	1
10% Mouse liver tissue homogenate	1-3
10% Rat lung tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Rat spleen tissue homogenate	1-3
293T cells	1
10% Epipremnum aureum leaf tissue homogenate	1

Note: The diluent is buffer solution.

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 593 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps:

The preparation of standard curve

Dilute 100 μmol/L iron standard with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 30, 40, 50 μmol/L.

The measurement of samples

For serum and plasma

1. **Standard well:** Take 200 μL of standard solution with different concentrations to the corresponding wells.
   Sample well: Take 200 μL of sample to the corresponding wells.

2. Add 100 μL of chromogenic solution to each well.

3. Mix fully and incubate the tubes at 37°C for 10 min.

4. Measure the OD value of each well with microplate reader at 593 nm.

For tissue and cells

1. **Standard tube:** Take 300 µL of standard solution with different concentrations to the 1.5 mL tubes.
   Sample tube: Take 300 µL of sample to the 1.5 mL tubes.

2. Add 150 µL of chromogenic solution to each tube.

3. Mix fully with vortex mixer and incubate the tubes at 37°C for 10 min.

4. Centrifuge the tubes at 12000 g for 10 min.

5. Take 300 µL of supernatant to the corresponding microplate wells.

6. Measure the OD value of each well with microplate reader at 593 nm.

Operation Table

For serum and plasma

	Standard well	Sample well
Standard solution with different concentrations (µL)	200	
Sample (µL)		200
Chromogenic solution (µL)	100	100
Mix fully and incubate the tubes at 37°C for 10 min. Measure the OD value of each well with microplate reader at 593 nm.		

For tissue and cells

	Standard tube	Sample tube
Standard solution with different concentrations (µL)	300	
Sample (µL)		300
Chromogenic solution (µL)	150	150
Mix fully with vortex mixer and incubate the tubes at 37°C for 10 min. Centrifuge the tubes at 12000 g for 10 min. Take 300 µL of supernatant to the corresponding microplate wells. Measure the OD value of each well with microplate reader at 593 nm.		

11. Calculations:

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

$$Fe^{2+}\ \text{content}\ (\mu mol/L) = \frac{\Delta A - b}{a} \times 4^{*} \times f$$

2. Tissue sample:

$$Fe^{2+}\ \text{content}\ (\mu mol/kg\ \text{wet weight}) = \frac{\Delta A - b}{a} \times f \div \frac{m}{v}$$

3. Cell sample:

$$Fe^{2+}\ \text{content}\ (nmol/10^{6}) = \frac{\Delta A - b}{a} \div \frac{N}{V} \times f$$

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).
x: The concentration of standard.
a: The slope of standard curve .
b: The intercept of standard curve.
ΔA_{593}: OD_{Sample} – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0)
4*: Dilution factor in the preparation step of serum, 4 times .
N: The number of cell sample. For example, the number of cells is 4*106, N is 4.
V: The volume in the preparation step of cell, mL.
f: Dilution factor of sample before test.
m: The wet weight of tissue, g.

12. Performance Characteristics:

Detection Range	0.4-50 μmol/L
Sensitivity	0.4 μmol/L
Average recovery rate (%)	99
Average inter-assay CV (%)	1.5
Average intra-assay CV (%)	1.3

Analysis

For rat liver tissue, take 10% rat liver tissue homogenate and dilute for 2 times, and carry the assay according to the operation table.

The results are as follows:
standard curve: y =0.0187 x - 0.0027, the average OD value of the sample is 0.110, the average OD value of the blank is 0.042, the calculation result is:

$$\textbf{Fe}^{2+}\ \textbf{content}\ \textbf{(μmol/kg wet weight)} = (0.110 - 0.042 + 0.0027) \div 0.0187 \times 2 \div (0.1 \div 0.9)$$

$$= 68.05\ \text{mmol/kg wet weight}$$

Email: info@assaygenie.com **Web:** www.assaygenie.com

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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