TECHNICAL MANUAL

Ferrous Iron Colorimetric Assay Kit

- **SKU CODE:** MAES0206
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate at 37°C for 10 minutes
- Measure OD value at 593 nm

2. Intended use

This kit can measure ferrous ion (Fe2+) content in serum, animal and plant tissue samples.

3. Detection principle

Ferrous ions (Fe2+) in samples bind with the probe to form complexes that have a maximum absorption peak at 593 nm. The concentration of iron can be calculated indirectly by measuring the OD value at 593 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	35 mL × 2 vials	60 mL × 2 vials	2-8°C, 12 months, shading light
Reagent 2	Chromogenic Solution	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	10 mmol/L Iron Standard	2 mL × 1 vial	2 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Standard Protectant	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months, shading light
Reagent 5	Extracting Solution	40 mL × 1 vial	40 mL × 2 vials	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Vortex Mixer, Centrifuge, Water bath, Microplate reader (590-600 nm, optimum wavelength: 593 nm)

Reagents:

Double distilled water

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For small volumes of reagents, please centrifuge before use to obtain sufficient amounts of reagents.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of standard protectant:** Dissolve one vial of standard protectant with 20 mL of buffer solution, mix well to dissolve. Store at 2-8°C for 1 month.
3. **Preparation of 100 µmol/L iron standard:** Dilute 20 µL of 10 mmol/L iron standard with 1980 µL of standard protectant, mix well. The 100 µmol/L iron standard should be prepared immediately before use.
4. **The preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 µmol/L iron standard with standard protectant to create a serial dilution. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 30, 40, 50 µmol/L.

7. Standard curve preparation

Item	1	2	3	4	5	6	7	8
Concentration (µmol/L)	0	5	10	15	20	30	40	50
100 µmol/L iron	0	50	100	150	200	300	400	500

Item	1	2	3	4	5	6	7	8
standard (μL)								
Standard protectant (μL)	1000	950	900	850	800	700	600	500

8. Sample preparation

① Sample preparation

Serum and plasma: Add 55 μL of sample and 165 μL of buffer solution, mix well and keep on ice for detection. If the sample is turbid, centrifuge at 5000×g for 5 min, then take the supernatant for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 50 mg tissue in 450 μL extracting solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 minutes at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection.

② Dilution of sample

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 - 2-3

10% Rat lung tissue homogenate - 1

10% Mouse heart tissue homogenate - 1

10% Rat spleen tissue homogenate - 2-3

10% Epipremnum aureum leaf tissue homogenate - 1

Note: The diluent for tissue samples is extracting solution. The diluent for serum samples is buffer solution. The serum sample has been diluted 4 times during sample processing.

For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

9. The key points of the assay

- Avoid bubbles when adding samples.
- Do not use iron appliances to prepare or transfer samples.

10. Operating steps

For serum and plasma

① Standard well: Add 200 µL of standard solution with different concentrations to the corresponding wells

Sample well: Add 200 µL of sample to the corresponding wells.

② Add 100 µL of chromogenic solution to each well.

③ Mix thoroughly and incubate at 37°C for 10 min.

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

For tissue

① Standard well: Add 300 µL of standard solution with different concentrations to the 1.5 mL tubes

Sample well: Add 300 µL of sample to the 1.5 mL tubes.

② Add 150 µL of chromogenic solution to each tube.

③ Mix thoroughly with vortex mixer and incubate at 37°C for 10 min.

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

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

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

11. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using the absolute OD value of standards and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Fe2+ content (μmol/L) = (ΔA593 - b) ÷ a × 4* × f

2. Tissue sample:

Fe2+ content (μmol/kg wet weight) = (ΔA593 - b) ÷ a × f ÷ (V/m)

[Note]

ΔA593: 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.

V: The volume of homogenate, mL.

f: Dilution factor of sample before test.

m: The wet weight of tissue, g.

12. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	8.40	22.60	43.50
%CV	1.6	1.2	1.1

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	8.40	22.60	43.50
%CV	1.3	1.7	1.5

Recovery

Three samples of high, medium, and low concentrations were tested six times in parallel for each concentration to obtain an average recovery rate of 99%.

Recovery Results

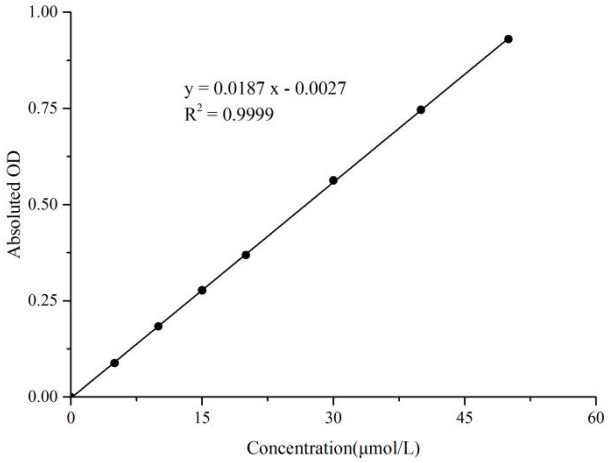
Parameters	standard 1	standard 2	standard 3
Expected Conc. (µmol/L)	9	16.5	33
Observed Conc. (µmol/L)	9.1	16.3	32.0
recovery rate(%)	101	99	97

Sensitivity

The analytical sensitivity of the assay is 0.4 µmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (μmol/L)	Average OD	Absolute OD
0	0.048	0.000
5	0.136	0.088
10	0.232	0.184
15	0.326	0.278
20	0.417	0.369
30	0.611	0.563
40	0.795	0.747
50	0.978	0.930

13. Appendix II Example Analysis

Example analysis:

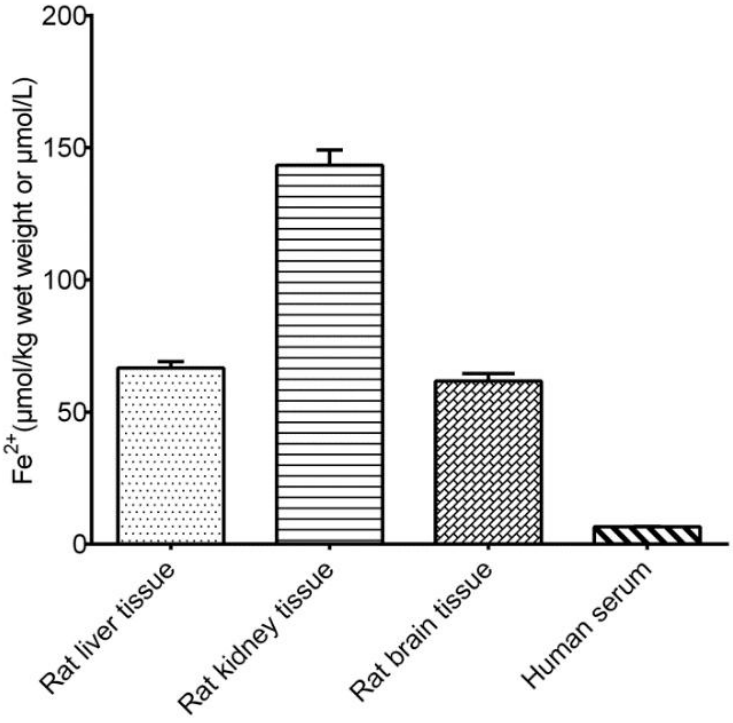
For rat liver tissue, take 10% rat liver tissue homogenate and dilute 2 times, then carry out the assay according to the operating procedure. The results are as follows:

Standard curve: y = 0.0187x - 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:

Fe2+ content (μmol/kg wet weight) = (0.110 - 0.042 + 0.0027) ÷ 0.0187 × 2 ÷ (0.1 ÷ 0.9)

= 68.05 µmol/kg wet weight

Detect 10% rat liver tissue homogenate (diluted 2 times), 10% rat kidney tissue homogenate (diluted 2 times), 10% rat brain tissue homogenate (diluted 2 times) and human serum according to the protocol, the result is as follows:



14. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.