TECHNICAL MANUAL

Iron Colorimetric Assay Kit

- **SKU CODE:** MAES0113
- **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
- Measure the OD value

2. Intended use

This kit can measure iron content in serum and tissue samples.

3. Detection principle

Under the action of acidic solution and reductant, ferric ions can be separated from transferrin in serum and reduced to ferrous ions (Fe2+). The ferrous ions then bind to bipyridine and form pink complexes. The concentration of iron can be calculated by measuring the OD value at 520 nm indirectly.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	10 mg/L Iron Standard	1 mL × 1 vial	1 mL × 2 vials	2-8°C, 12 months
Reagent 2	Chromogenic Agent A	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months, shading light
Reagent 3	Chromogenic Agent B	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months, shading light
Reagent 4	Chromogenic Agent C	20 mL × 1 vial	20 mL × 2 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (510-530 nm, optimum wavelength: 520 nm), Micropipettor, Centrifuge, Water bath, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of iron chromogenic agent:** Dissolve one vial of chromogenic agent A and one vial of chromogenic agent B with 20 mL of chromogenic agent C, mix well to dissolve. Store at 2-8°C for 1 month protected from light.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 10 mg/L iron standard stock solution with double distilled water to create a serial dilution. The recommended dilution gradient is as follows: 0, 1, 2, 4, 5, 6, 8, 10 mg/L.

7. Standard dilution table

Item	1	2	3	4	5	6	7	8
Concentration (mg/L)	0	1	2	4	5	6	8	10
10 mg/L standard (µL)	0	20	40	80	100	120	160	200
Double distilled water (µL)	200	180	160	120	100	80	40	0

8. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for one month.

Tissue samples:

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 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

• Human serum: 1

• Mouse serum: 1

• 10% Mouse liver tissue homogenate: 1

• 10% Rat kidney tissue homogenate: 1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

9. Key points of the assay

1. The supernatant after centrifugation must be clear, and if there is turbidity, it must be centrifuged again.
2. During the experiment, the experimental vessel must be clean to avoid iron contamination which may affect the experimental results.
3. Iron chromogenic agent should be prepared in advance because chromogenic agent A powder and chromogenic agent B powder are difficult to dissolve.

10. Operating steps

1. Standard tube: Add 75 µL of standard solution with different concentrations to the tubes. Sample tube: Add 75 µL of sample to the tubes.
2. Add 300 µL of iron chromogenic agent, mix fully with vortex mixer.
3. Incubate the tubes in 100°C water bath for 5 min.
4. Cool the tubes with running water, centrifuge the tubes at 3,000×g for 10 min.
5. Take 200 µL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 520 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 by using absolute OD value of standard 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) samples:

Fe content (mg/L) = (ΔA_520 - b) ÷ a × f

2. Tissue samples:

Fe content (mg/gprot) = (ΔA_520 - b) ÷ a × f ÷ C_{pr}

[Note]

f: Dilution factor of sample before tested.

ΔA_520: OD_{Sample} – OD_{Blank}.

C_{pr}: Concentration of protein in sample, gprot/L.

12. 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 (mg/L)	1.30	3.70	7.50
%CV	1.5	1.2	0.9

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 (mg/L)	1.30	3.70	7.50
%CV	3.0	2.7	2.7

Recovery

Three samples of high concentration, middle concentration, and low concentration were tested with each concentration performed 6 times in parallel to obtain an average recovery rate of 100%.

Recovery Results

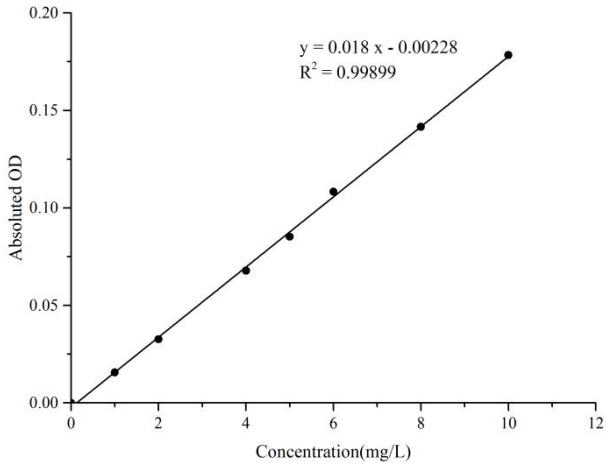
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/L)	1.5	4.5	7
Observed Conc. (mg/L)	1.4	4.4	6.7
Recovery rate (%)	95	97	96

Sensitivity

The analytical sensitivity of the assay is 0.08 mg/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 (mg/L)	0	1	2	4	5	6	8	10
OD value	0.045	0.058	0.075	0.109	0.126	0.149	0.183	0.221
	0.038	0.056	0.073	0.109	0.126	0.150	0.182	0.218
Average OD	0.041	0.057	0.074	0.109	0.126	0.149	0.183	0.220
Absolute OD	0.000	0.016	0.033	0.068	0.085	0.108	0.142	0.178

13. Example Analysis

Example analysis:

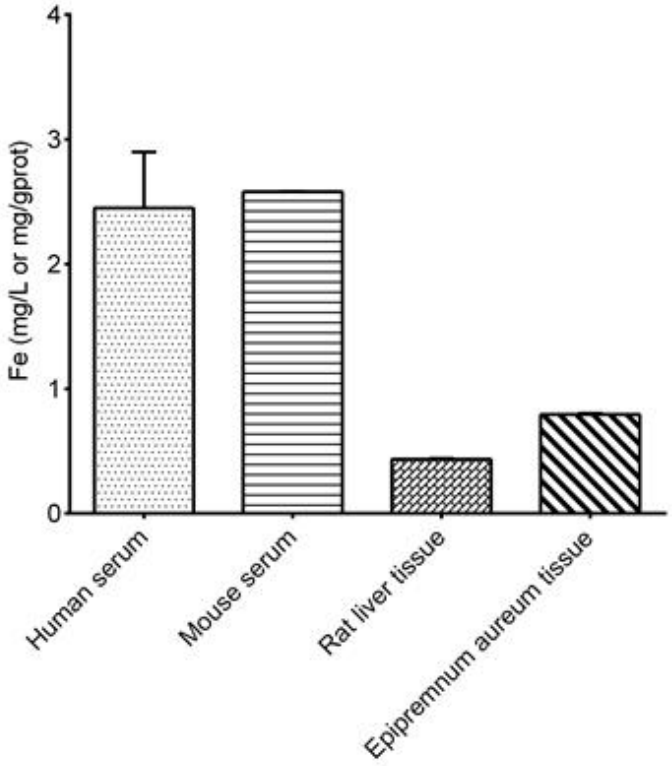
Take 75 µL of human serum sample and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.01427x + 0.0008, the average OD value of the sample is 0.078, the average OD value of the blank is 0.038, and the calculation result is:

Fe content (mg/L) = (0.078 - 0.038 - 0.0008) ÷ 0.01427 = 2.75 mg/L

Detect human serum, mouse serum, 10% rat liver tissue homogenate (the concentration of protein in sample is 9.47 gprot/L) and 10% epipremnum aureum tissue homogenate

(the concentration of protein in sample is 3.08 gprot/L) according to the protocol, the results are 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.