TECHNICAL MANUAL

Cell Ferrous Iron Colorimetric Assay Kit

- **SKU CODE:** MAES0220
- **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
- Incubation
- Measure OD value

2. Intended use

This kit can measure ferrous ion (Fe2+) content in cell samples.

3. Detection principle

Iron is an essential metal element in organisms and plays important physiological roles. Ferrous ion (Fe2+) is a key element in heme and hemoglobin, and also participates in numerous biochemical reactions.

Fe2+ in samples can bind with the probe to form complexes, which have a maximum absorption peak at 593 nm. The concentration of Fe2+ can be calculated by measuring the OD value at 593 nm indirectly.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	45 mL × 1 vial	35 mL × 2 vials	2-8°C, 12 months, shading light
Reagent 2	Control Solution	5 mL × 1 vial	10 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Chromogenic Solution	5 mL × 1 vial	10 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	1 mmol/L Iron Standard	2 mL × 1 vial	2 mL × 1 vial	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:

Centrifuge, Incubator, Microplate reader (590-600 nm, optimum wavelength: 593 nm)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of 100 μmol/L iron standard:** Dissolve 100 μL of 1 mmol/L iron standard with 900 μL of buffer solution, mix well to dissolve. The 100 μmol/L iron standard should be prepared fresh before use.
3. **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 buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 35 μmol/L.

7. Sample preparation

1. **Cell (adherent or suspension) sample preparation:** 1) Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells). 2) Wash cells with PBS (0.01 M, pH 7.4). 3) Lyse 1×10^6 cells with 200 μL buffer solution. Place on ice and mix well every 5 min, lyse for 10 min. 4) Centrifuge at 15,000×g for 10 min, collect supernatant and keep it on ice for detection.
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): HepG2 Cell: 1, molt-4 Cell: 1, Jurkat Cell: 1, HEL Cell: 1. Note: The diluent is buffer solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. To avoid contamination, it is recommended to aliquot the chromogenic solution into smaller quantities before use.
2. Prevent the formation of bubbles when the reagent or sample is transferred into the microplate.
3. Select fresh cell samples for detection.

9. Operating steps

1. Standard well: Take 80 µL of standard solution with different concentrations to the corresponding wells. Sample well: Take 80 µL of sample to the corresponding wells. Control well: Take 80 µL of sample to the corresponding wells.
2. Add 80 µL of control solution to control well.
3. Add 80 µL of chromogenic solution to sample well and standard well.
4. Mix fully and incubate at 37°C for 10 min.
5. Measure the OD value of each well with microplate reader at 593 nm.

10. 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 correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

Cell sample:

Fe2+ content (nmol/10^6) = (ΔA - b)/a ÷ N/V × f

[Note]

ΔA: Absolute OD (OD_{Sample} – $OD_{Control}$).

N: The number of cell sample/10^6.

V: The volume of buffer solution in the preparation step of cell, mL.

f: Dilution factor of sample before test.

11. Appendix

Performance Characteristics

Intra-assay Precision

Three HepG2 cell samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	2.80	162.00	35.00
%CV	1.5	1.3	1.1

Inter-assay Precision

Three HepG2 cell samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	2.80	162.00	35.00
%CV	1.2	1.6	1.7

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration measured 6 times in parallel to obtain an average recovery rate of 99%.

Recovery

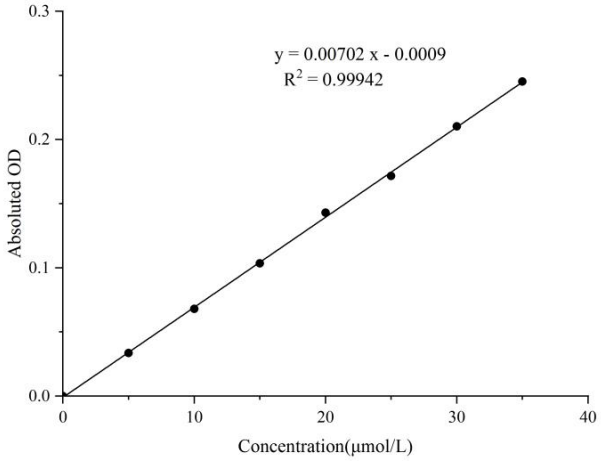
Standard	Expected Conc. (µmol/L)	Observed Conc. (µmol/L)	Recovery rate (%)
Standard 1	6.8	6.9	102
Standard 2	17.5	17.2	98
Standard 3	35.0	35.0	97

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

Concentration (µmol/L)	Average OD	Absolute OD
0	0.044	0.000
5	0.078	0.034
10	0.112	0.068
15	0.148	0.104
20	0.187	0.143
25	0.216	0.172
30	0.254	0.210
35	0.289	0.245

Example Analysis

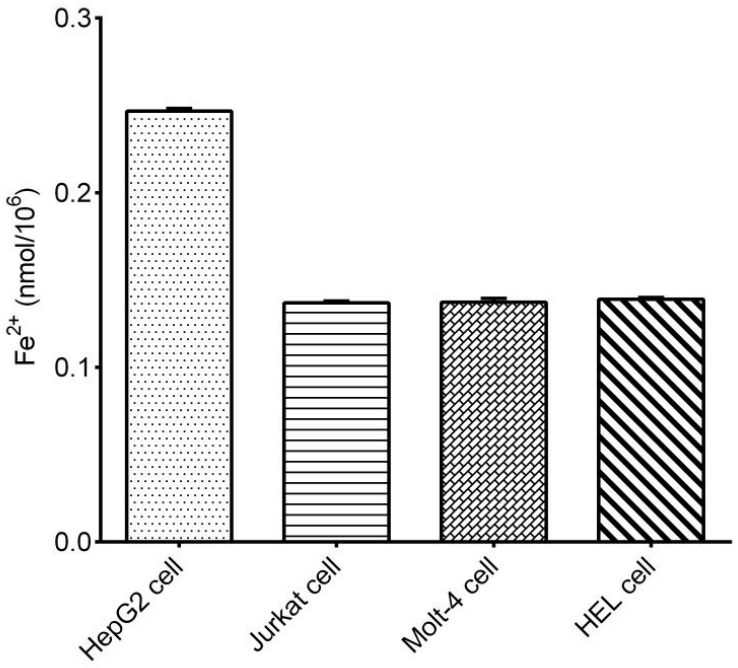
Example analysis:

For HepG2 cell, add homogenization medium at a ratio of cell number (1×10^6): buffer solution (mL) = 1.5: 0.2, take 80 µL of the supernatant, and carry out the assay according to the operation table. The results are as follows:

Standard curve: y = 0.00605x + 0.0009, the average OD value of the sample is 0.055, the average OD value of the Control is 0.043, and the calculation result is:

Fe2+ content (nmol/10^6) = (0.055 - 0.043 - 0.0009) ÷ 0.00605 ÷ 1.5 × 0.2 = 0.24 nmol/10^6

Detect HepG2 cell, Jurkat cell, Molt-4 and 293T cells according to the protocol, the result is as follows:



12. 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 pretest 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.