Cell Ferrous Iron Colorimetric Assay Kit

Catalog Number EEA008 (96 tests)

Rev 1.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit can measure ferrous ions (Fe^{2+}) content in cell samples. Iron plays an important role in various physiological functions. The ferrous ion is a key element in heme and hemoglobin and is present in many biochemical reactions. Ferrous ions (Fe^{2+}) in samples can bind with probes in solution to form a complex which has a maximum absorption peak at 593 nm. The concentration of ferrous ions can be calculated by measuring the OD value at 593 nm indirectly.

Contents and storage

Kit and components are shipped at 2-8 ℃. An unopened kit can be stored at 2-8 ℃ for 12 months.

Components	Quantity (96 tests)
Buffer Solution	35 ml ×2 vials
Control Solution	10 ml
Chromogenic Solution	10 mL
10 mmol/L Iron Standard	2 mL
Standard Protectant	Powder
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 593 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37 ℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

- Select fresh cell samples for detection

Cells:

- Collect about 1×10^6 cells, mix with 400 μL 0.9% NaCl, centrifuge at 300 g at 4 ℃ for 10 min and then discard the supernatant and keep the cell sediment
- Add homogenization medium at a ratio of cell number (1×10^6): buffer solution (mL) =1: 0.2.
- Place on the ice box and wait for 10 min. Centrifuge at 15000 g for 10 min, then take the supernatant and preserve it on ice for detection.

If not detected on the same day, the cells sample (without homogenization) can be stored at -80 ℃ for 1 month.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.4 -50 μmol/L).

Note: Use all samples within 2 hours of dilution

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

Sample type	Dilution factor
HepG2 Cell	1
Molt-4 Cell	1
Jurkat Cell	1
HEL Cell	1

Note: the diluent is the buffer solution

Preparation of standard protectant

Dissolve a vial of standard protectant with 15 mL of buffer solution and mix fully. The prepared solution can be stored at 2-8 ℃ for 1 month.

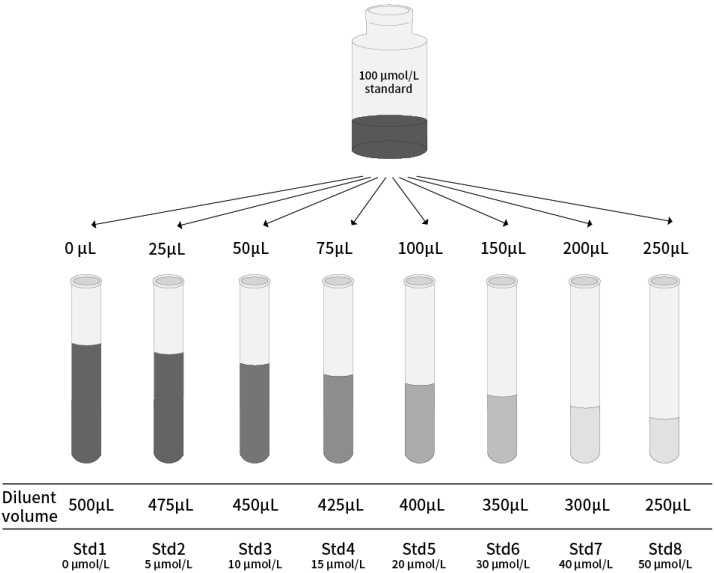
Preparation of 100 μmol/L iron standard

Mix 10 μL of 10 mmol/L iron standard with 990 μL of standard protectant fully. Prepare fresh needed amount solution before use.

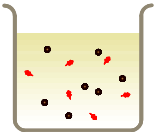
Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

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



Assay Protocol

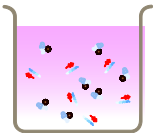


1. Add standard, sample and control

a. Standard well: Take 80 μL of standard solution with different concentrations to the corresponding wells.

b. Sample well: Take 80 μL of sample to the corresponding wells.

c. Control well: Take 80 μL of sample to the corresponding wells.



2. Add substrate

a. Add 80 μL of control solution to control well.

b. Add 80 μL of chromogenic solution to sample wells and standard wells.

c. Mix fully and incubate at 37 ℃ for 10 min.

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

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted 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).

Cell sample:

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

[Note]

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

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

V: The volume of regent 1 in the preparation step of cell, mL.

f: Dilution factor of sample before test.

To easy calculate the test results, refer to the calculation file available on the webpage.

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 the assay according to the operation steps. The results are as follows:

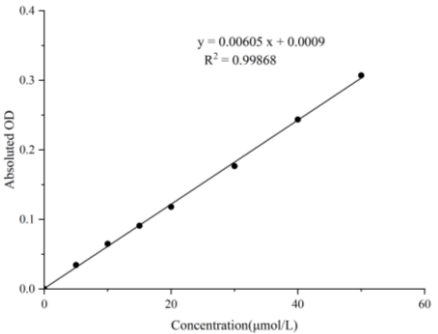
standard curve: y = 0.00605 x + 0.0009, the average OD value of the sample is 0.055, the average OD value of the control is 0.043, and the calculation result is:

$$Fe^{2+} \text{ content (nmol/}10^6\text{)} = (0.055 - 0.043 - 0.0009) \div 0.00605 \div 1.5 \times 0.2 = 0.24 \text{ nmol/}10^6$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–50 µmol/L standard.

Concentration (µmol/L)	OD at 593 nm	Standard Curve
50.00	0.352	
40.00	0.288	
30.00	0.221	
20.00	0.163	
15.00	0.136	
10.00	0.110	
5.00	0.079	
0.00	0.045	

- Inter-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	7.50	25.00	35.00
%CV	1.5	1.5	1.6

CV = Coefficient of Variation

- Intra-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	7.50	25.00	35.00
%CV	1.3	1.3	1.1

CV = Coefficient of Variation

- Expected values

This assay was tested with cell samples without dilutions.

Sample Type	Range (**nmol/10^6**)	Average (**nmol/10^6**)
NCTC 1469 (2.6×10^6)	0.35-0.625	0.464
RBC-2H3 (2.6×10^6)	0.625-0.934	0.797

- Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc. (μmol/L)	7.50	25.00	35.00
Observed Conc. (μmol/L)	7.28	24.75	34.65
Recovery rate (%)	97	99	99

- Recommended 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.												

- 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.

Limited product warranty

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