PRODUCT INFORMATION & MANUAL

Cell Ferrous Iron (Fe2+) Assay Kit (Colorimetric)

NBP3-25915

For research use only.
Not for diagnostic or therapeutic procedures.

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Cell Ferrous Iron (Fe2+) Assay Kit (Colorimetric)

Catalog No: NBP3-25915

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.4 μmol/L

Detection range: 0.4-50 μmol/L

Average intra-assay CV (%): 1.3

Average inter-assay CV (%): 1.5

Verage recovery rate (%): 99

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure errous ions (Fe^{2+}) content in cell sample.

▲Detection principle

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. 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 ferrous ions can be calculated by measuring the OD value at 593 nm indirectly.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	35 mL × 2 vials	2-8℃ , 12 months, shading light
Reagent 2	Control Solution	10 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 3	Chromogenic Solution	10 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 4	10 mmol/L Iron Standard	2 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 5	Standard Protectant	Powder × 1 vial	2-8℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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.			

▲Materials prepared by users

Instruments

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

Reagents:

Double distilled water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ The key points of the assay

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

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of standard protectant:
   Dissolve a vial of reagent 5 with 15 mL of reagent 1 and mix fully. The prepared solution can be stored at 2-8°C for 1 month.
3. Preparation of 100 μmol/L iron standard:
   Mix 10 μL of reagent 4 with 990 μL of standard protectant fully. Prepare fresh needed amount solution before use.

▲ Sample preparation

Cell sample:

Collect about 1×10^6 cells, mix with 400 μL 0.9%NaCl, centrifuge at 300 g at 4°C 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): reagent 1 (mL) =1: 0.2. Place on the ice box and crack for 10 min. Centrifuge at 15000 g for 10 min, then take the supernatant and preserve it on ice for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do 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).

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 reagent 1.

Assay protocol

▲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.

▲Detailed operating steps

1. The preparation of standard curve

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.

Reference is as follows:

Number	Standard concentrations (μmol/L)	100 μmol/L Standard (μL)	Standard protectant (μL)
A	0	0	500
B	5	25	475
C	10	50	450
D	15	75	425
E	20	100	400
F	30	150	350
G	40	200	300
H	50	250	250

2. The measurement of samples

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

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

(2) Add 80 μL of reagent 2 to Control well.

(3) Add 80 μL of reagent 3 to Sample well、Standard well.

(4) Mix fully and incubate at 37℃ for 10 min.

(5) Measure the OD value of each well with microplate reader at 593 nm.

▲Summary operation table

	Standard well	Control well	Sample well
Standard of different concentrations (μL)	80		
Sample (μL)		80	80
Reagent 2 (μL)		80	
Reagent 3 (μL)	80		80
Mix fully and incubate the tubes at 37℃ for 10 min. Measure the OD value at 593 nm.			

▲Calculation

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.

Cell sample:

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

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: Absoluted 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 tested.

Appendix I Data

▲Example analysis

For HepG2 cell, Add homogenization medium at a ratio of cell number (1×10^6): reagent 1 (mL) =1.5: 0.2., take 80 μL of the supernatant, and carry the assay according to the operation table.

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:

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

$$= 0.24 \text{ nmol}/10^6$$