Product Information & ELISA Manual

Cell Ferrous Iron (Fe2+) Assay Kit (Colorimetric)
NBP3-25915

Enzyme-linked Immunosorbent Assay for quantitative detection.

Contact

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Novus kits are guaranteed for 6 months from date of receipt.

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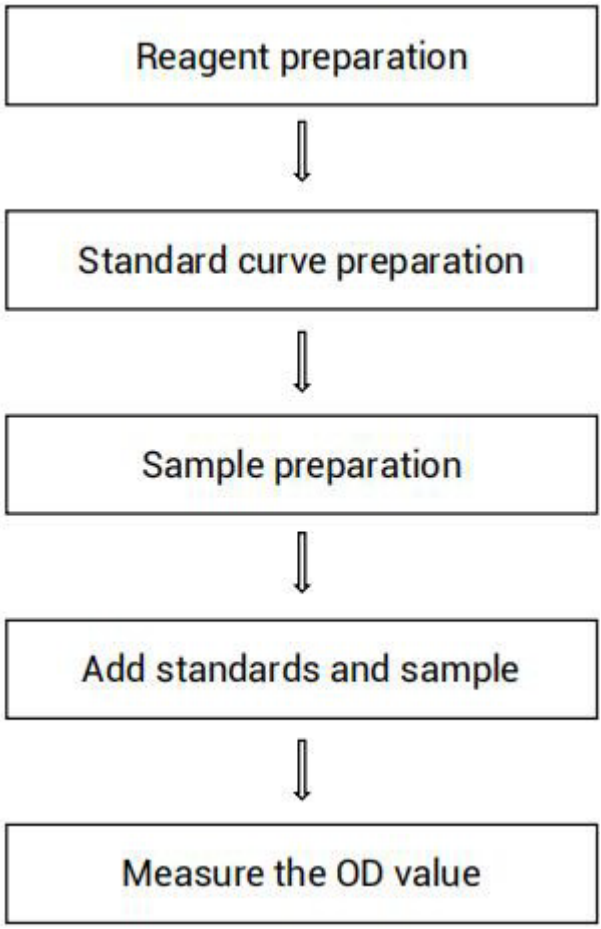
Assay summary



Intended use

This kit can measure ferrous 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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	45 mL × 1 vial	35 mL × 2 vials	2-8℃, 12 months, shading light
Reagent 2	Control Solution	5 mL × 1 vial	10 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 3	Chromogenic Solution	5 mL × 1 vial	10 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 4	1 mmol/L Iron Standard	2 mL ×1 vial	2 mL ×1 vial	2-8℃, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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 a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

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 on spot.

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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**35**
100 μmol/L iron standard (μL)	0	25	50	75	100	125	150	175
Buffer solution (μL)	500	475	450	425	400	375	350	325

Sample preparation

① Sample preparation

Cell (adherent or suspension) samples:

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 the ice box and mix well every 5 min, lyse for 10 min.

④ Centrifuge at 15000×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):

Sample type	Dilution factor
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 do pretest to confirm the dilution factor

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 formulation of bubbles when the reagent or sample is transferred into the microplate.

③ Select fresh cell samples for detection.

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℃ for 10 min.
⑤ 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 (Standard # ①) 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).

The sample:

Cell sample:

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

[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.

Appendix I Performance Characteristics

1. **Parameter:**

Intra-assay Precision

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

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.

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

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 99%.

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

Sensitivity

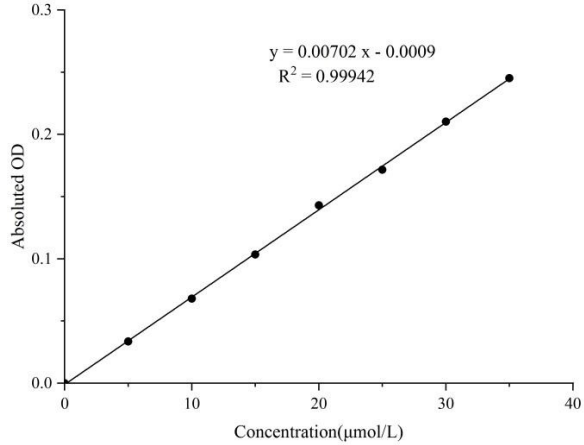
The analytical sensitivity of the assay is 0.4 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding

concentration.

2. 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), so the standard curve and data are provided as below for reference only :

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



Detection range : 0.4-35 μmol/L

Appendix Π Example Analysis

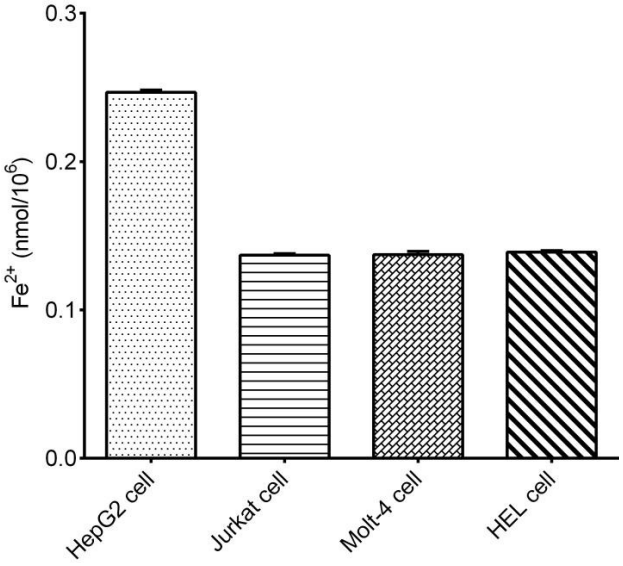
Example analysis :

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



Appendix III Publications

1. Liu Z, Liu C, Fan C, et al. E3 ubiquitin ligase DTX2 fosters ferroptosis resistance via suppressing NCOA4-mediated ferritinophagy in non-small cell lung cancer[J]. Drug Resistance Updates, 2024, 77: 101154.
2. Li J, Zhang Q, Yang H, et al. Sequential dual-locking strategy using photoactivated Pt (IV)-based metallo-nano prodrug for enhanced chemotherapy and photodynamic efficacy by triggering ferroptosis and macrophage polarization[J]. Acta Pharmaceutica Sinica B, 2024, 14(7): 3251-3265.
3. Liu J, Luo Y, Chen S, et al. Deubiquitylase USP52 Promotes Bladder Cancer Progression by Modulating Ferroptosis through Stabilizing SLC7A11/xCT[J]. Advanced Science, 2024, 11(45): 2403995.
4. Zhong Z, Zhou S, Liang Y, et al. Natural flavonoids disrupt bacterial iron homeostasis to potentiate colistin efficacy[J]. Science Advances, 2023, 9(23): eadg4205.
5. Wu Z, Chen K, Mo W, et al. Multimodal enhancement of ferroptosis for synergistic cascade colorectal cancer therapy[J]. Chemical Engineering Journal, 2024, 498: 155048.
6. Li X, Peng X, Zhou X, et al. Small extracellular vesicles delivering lncRNA WAC-AS1 aggravate renal allograft ischemia–reperfusion injury by inducing ferroptosis propagation[J]. Cell Death & Differentiation, 2023, 30(9): 2167-2186.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Novus Biologicals will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.