CheKine™ Micro Cell Ferrous Ion Content Assay Kit

Cat #: KTB1116 Size: 48 T/48 S 96 T/96 S

	Micro Ferrous Cell Ion Content Assay Kit		
REF	**Cat #:** KTB1116	LOT	**Lot #:** Refer to product label
	Detection range: 0.78-50 nmol/mL		**Sensitivity:** 0.78 nmol/mL
	Applicable samples: Cells		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Iron is one of the essential trace elements in human body and has important physiological effects. Ferrous ions are key elements in heme and hemoglobin, and also play an important role in many biochemical reactions. CheKine™ Micro Cell Ferrous Ion Content Assay Kit can be used to detect biological samples such as cells. In the kit, after cells is cracked, Fe^{2+} forms a blue complex with tripyridyl triazine under acidic conditions, and has an absorption peak at 593 nm. The content of Fe^{2+} can be calculated by measuring the absorbance of this wavelength.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	30 mL	60 mL	4℃, protected from light
Reagent Ⅰ	7 mL	14 mL	4℃, protected from light
Reagent Ⅱ	20 mL	40 mL	4℃, protected from light
Standard	Powder×2 vials	Powder×4 vials	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 593 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water, PBS, chloroform

Reagent Preparation

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Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Reagent Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light. To avoid contamination, it is recommended to use Reagent Ⅰ after packaging.

Reagent Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Standard: Prepared before use; Add 1,020 μL Reagent Ⅱ to dissolve fully for each bottle, that is 10 μmol/mL Fe^{2+} Standard ; Standard is be used up on the prepared day.

100 nmol/mL Fe^{2+} standard: Prepare 400 nmol/mL Fe^{2+} Standard by diluting 10 μL 10 μmol/mL Fe^{2+} Standard into 990 μL Reagent Ⅱ. Using 100 nmol/mL Fe^{2+} standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Reagent Ⅱ (μL)	Concentration (nmol/mL)
Std.1	200 μL of 100 nmol/mL Standard	400	50
Std.2	400 μL of Std.1 (50 nmol/mL)	400	25
Std.3	400 μL of Std.2 (25 nmol/mL)	400	12.5
Std.4	400 μL of Std.3 (12.5 nmol/mL)	400	6.25
Std.5	400 μL of Std.4 (6.25 nmol/mL)	400	3.13
Std.6	400 μL of Std.5 (3.13 nmol/mL)	400	1.56
Blank	0	400	0

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. Ferrous ion is easily oxidized, and long sample placement time or repeated freezing and thawing may result in inaccurate results.

Cells: Collect 1×10^7 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 0.5 mL Extraction Buffer and the cells were split on ice for 10 min, mixed upside down once every 2 min, and then placed on ice to be tested.

Note: 1. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

2. To avoid iron contamination, do not use iron utensils for all sample handling and transfer operations. If necessary, 1% diluted hydrochloric acid can be used to soak the equipment for 4 h.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 593 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Sample	0	0	200
Standard	0	200	0
Reagent Ⅱ	200	0	0
Reagent Ⅰ	100	100	100
Thoroughly mixed, incubated at 37℃ for 10 min, cooled to room temperature with running water, 200 μL was taken from the Blank Tube and the Standard Tube into 96-well plates or microglass cuvette, detect the absorbance at 593 nm. The Test tube			

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performs the following operations:			
Chloroform	0	0	100
Full vortex oscillation 2 min, centrifuge at 10,000 g for 5 min **at room temperature**, take 200 μL the upper inorganic phase from the Test Tube carefully into a 96-well plate or microglass cuvette, detect the absorbance at 593 nm.			

3. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: 1. The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.005, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.3, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. 2. Each test should not exceed three samples. After the reaction is completed, the absorption value should be detected immediately to avoid experimental errors. 3. Chloroform will corrodes the 96-well plate, so be careful not to absorb the lower chloroform when absorbing the upper inorganic phase.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is brought into the equation to get x (nmol/mL).

2. Calculation of the ferrous ion content

(1) Calculated by protein concentration

Ferrous ion (nmol/mg prot)=$(V_{Sample}\times x)\div(V_{Sample}\times Cpr)$**=x÷Cpr**

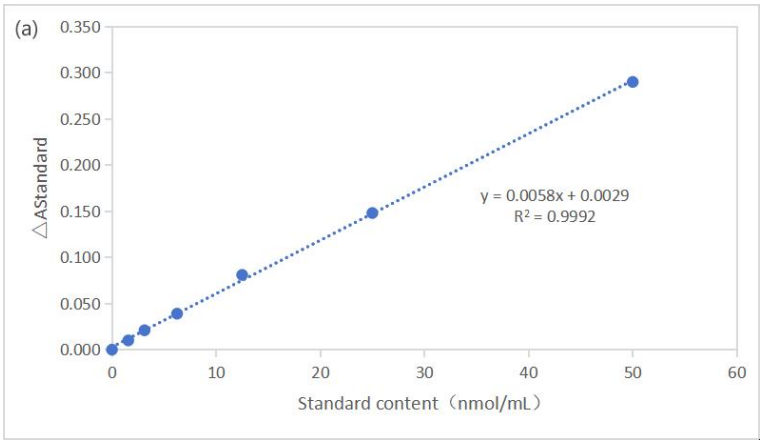
(2) Calculated by number of cells

Ferrous ion (nmol/10^6 cell)=$(V_{Sample}\times x)\div(n\times V_{Sample}\div V_{Total\ sample})$**=0.25x÷n**

V_{Sample}: Added the sample volume, 0.1 mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 0.25 mL; Cpr: Sample protein concentration, mg/mL; n: Number of cells, calculated in units of one million.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.



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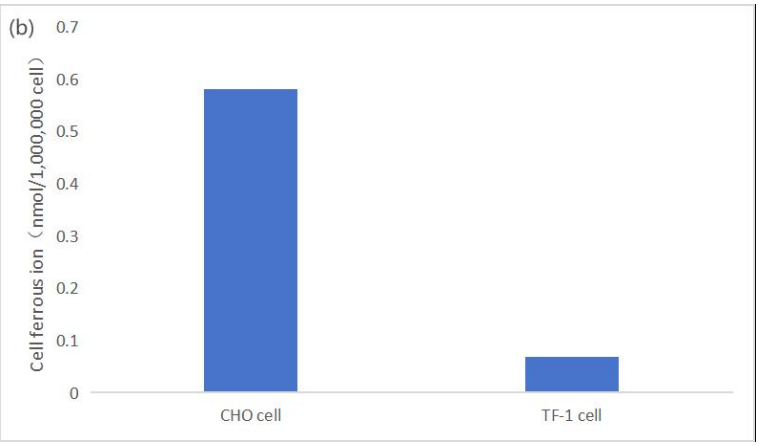

Figure 1. (a) Standard curve of cell ferrous ion content. (b) Determination of ferrous ion content in CHO cell and TF-1 cell by this kit.

Recommended Products

Catalog No.	Product Name
KTB1113	CheKine™ Mirco Total Iron Ion Content Assay Kit
KTB1114	CheKine™ Mirco Cell Total Iron Content Assay Kit
KTB1115	CheKine™ Mirco Ferrous Ion Content Assay Kit

Disclaimer

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