



CheKine™ Micro Ferrous Ion Content Assay Kit

Cat #: KTB1115

Size: 48 T/48 S

96 T/96 S

	Micro Ferrous Ion Content Assay Kit		
REF	Cat #: KTB1115	LOT	Lot #: Refer to product label
	Detection range: 1.56-100 nmol/mL		Sensitivity: 1.56 nmol/mL
	Applicable samples: Animal and Plant Tissue, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4°C for 6 months, protected from light		

Assay Principle

Iron is one of the essential trace elements in human body and plays an important role in maintaining the normal physiological function of the body. Ferrous ions are important components of hemoglobin, myoglobin, cytochrome and other enzyme systems, helping to transport oxygen and promote fat oxidation. Lack of iron is likely to cause anemia, metabolic disorder, and affect the body's immune function. CheKine™ Micro Ferrous Ion Content Assay Kit can be used to detect biological samples such as animal and plant tissue, plasma, serum or other liquid samples. In the kit, 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	60 mL	120 mL	4°C
Reagent I	10 mL	15 mL	4°C, protected from light
Reagent II	15 mL	30 mL	4°C, protected from light
Standard	Powder×2 vials	Powder×4 vials	4°C, 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, chloroform
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4°C.

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

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

Standard: Prepared before use; Add 900 µL Reagent II to dissolve fully for each bottle, that is 40 µmol/mL Fe²⁺ Standard ; Standard is freshly prepared.

400 nmol/mL Fe²⁺ standard: Prepare 400 nmol/mL Fe²⁺ Standard by diluting 10 µL 40 µmol/mL Fe²⁺ Standard into 990 µL Reagent II. Using 400 nmol/mL Fe²⁺ 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 400 nmol/mL Standard	600	100
Std.2	400 µL of Std.1 (100 nmol/mL)	400	50
Std.3	400 µL of Std.2 (50 nmol/mL)	400	25
Std.4	400 µL of Std.3 (25 nmol/mL)	400	12.5
Std.5	400 µL of Std.4 (12.5 nmol/mL)	400	6.25
Std.6	400 µL of Std.5 (6.25 nmol/mL)	400	3.125
Std.7	400 µL of Std.6 (3.125 nmol/mL)	400	1.5625
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.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
2. Plasma, Serum or other Liquid samples: Take 125 µL liquid, add 125 µL Extraction Buffer (Amount to 2 times diluted), mix well, and place it on ice to be tested. If the sample is cloudy, centrifuge at 5,000 g, for 5 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

Note: 1. Extraction Buffer of this kit can not be used for protein content determination, if you need to determine protein content, the protein needs to be extracted with deionized water for determination. 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)
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Sample	0	0	200
Standard	0	200	0
Reagent II	200	0	0
Reagent I	100	100	100

Thoroughly mixed, incubated at 37°C 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 performs the following operations:

Chloroform	0	0	100
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Full vortex oscillation 2 min, centrifuge at 10,000 g for 5 min **at room temperature**, take 200 µL the upper inorganic phase 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 $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Blank}}$, $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$.

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 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.6, 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 $\Delta A_{\text{Standard}}$ 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

$$\text{Ferrous ion (nmol/mg prot)} = (V_{\text{Sample}} \times x) \div (V_{\text{Sample}} \times C_{\text{pr}}) = \mathbf{x \div C_{pr}}$$

(2) Calculated by fresh weight of samples

$$\text{Ferrous ion (nmol/g fresh weight)} = (V_{\text{Sample}} \times x) \div (W \times V_{\text{Sample}} \div V_{\text{Total sample}}) = \mathbf{x \div W}$$

(3) Calculated by volume of liquid samples

$$\text{Ferrous ion (nmol/mL)} = F \times x = \mathbf{2x}$$

V_{Sample} : Added the sample volume, 0.1 mL; $V_{\text{Total sample}}$: Added the Extraction Buffer volume, 1 mL; C_{pr} : sample protein concentration, mg/mL; W : Sample weight, g; F : Liquid sample dilution, 2.

Typical Data

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

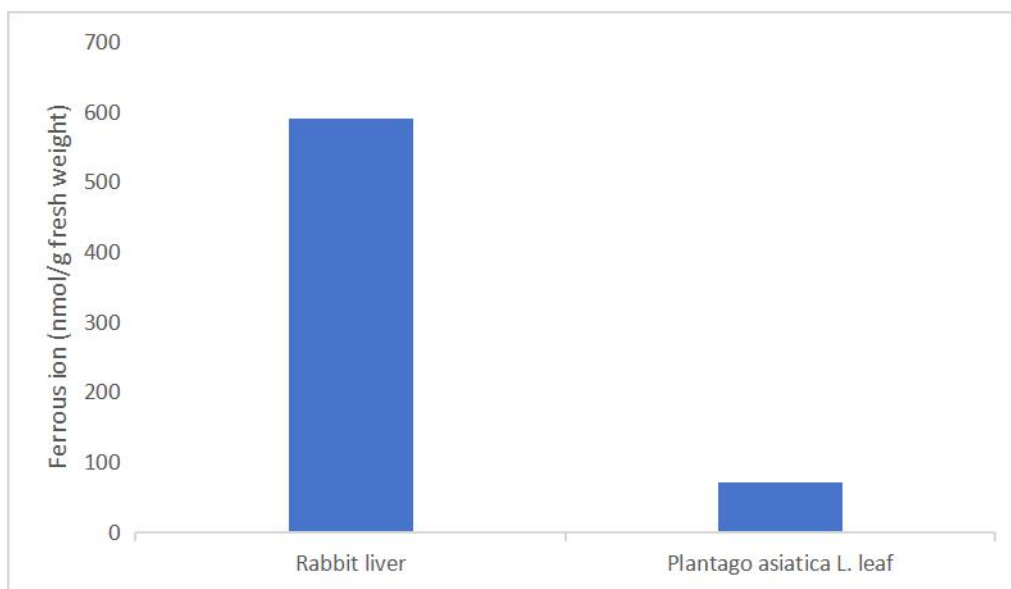


Figure 1. Determination of ferrous ion content in rabbit liver and *Plantago asiatica* L. leaf by this kit.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.