CheKine™ Micro Total Iron Ion Content Assay Kit

Cat #: KTB1113 Size: 48 T/48 S 96 T/96 S

	Micro Total Iron Ion Content Assay Kit		
REF	**Cat #:** KTB1113	LOT	**Lot #:** Refer to product label
	Detection range: 5-100 nmol/mL		**Sensitivity:** 5 nmol/mL
	Applicable samples: Animal and Plant Tissue, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Iron is one of the essential trace elements in the human body, it is the main component of hemoglobin, myoglobin, cytochrome and other enzyme systems, helps to transport oxygen, promote fat oxidation. Lack of iron is likely to cause anemia, metabolic disorder, and affect the body's immune function. CheKine™ Micro Total Iron 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, sodium sulfite reduces Fe^{3+} to produce Fe^{2+}, Fe^{2+} forms a blue complex with tripyridyl triazine under acidic conditions, and has an absorption peak at 593 nm. The content of total iron ion 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 Ⅰ	60 mL	120 mL	4℃, protected from light
Extraction Buffer Ⅱ	10 mL	20 mL	4℃, protected from light
Reagent Ⅰ	10 mL	15 mL	4℃, protected from light
Reagent Ⅱ	20 mL	30 mL	4℃, protected from light
Standard	1 mL	1 mL	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, chloroform
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer Ⅰ : Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.
Extraction Buffer Ⅱ: 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: Ready to use as supplied; 10 μmol/mL Fe^{3+} standard solution; Equilibrate to room temperature before use; Store at 4℃, protected from light. Using 10 μmol/mL Fe^{3+} standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Reagent Ⅱ (μL)	Concentration (nmol/mL)
Std.1	20 μL of 10 μmol/mL Standard	1980	100
Std.2	375 μL of Std.1 (100 nmol/mL)	125	75
Std.3	250 μL of Std.1 (100 nmol/mL)	250	50
Std.4	150 μL of Std.1 (100 nmol/mL)	350	30
Std.5	100 μL of Std.1 (100 nmol/mL)	400	20
Std.6	50 μL of Std.1 (100 nmol/mL)	450	10
Std.7	25 μL of Std.1 (100 nmol/mL)	475	5
Blank	0	500	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. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer Ⅰ and homogenize or mortar on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plasma, Serum or other Liquid samples: Take 55 μL liquid, add 165 μL Extraction Buffer Ⅱ (Amount to 4 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℃. 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)

Sample	0	0	200
Standard	0	200	0
Reagent II	200	0	0
Reagent I	100	100	100
Thoroughly mixed, incubated at 37℃ for 40 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
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 $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

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 0.02, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.6, the sample can be appropriately diluted with corresponding 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 total iron ion content

(1) Calculated by protein concentration

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

(2) Calculated by fresh weight of samples

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

(3) Calculated by volume of liquid samples

Total iron ion (nmol/mL)=$F\times x$**$=4x$**

V_{Sample}: Added the sample volume, 0.1 mL; $V_{Total\ sample}$: Added the Extraction Buffer I volume, 1 mL; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; F: Liquid sample dilution, 4.

Typical Data

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

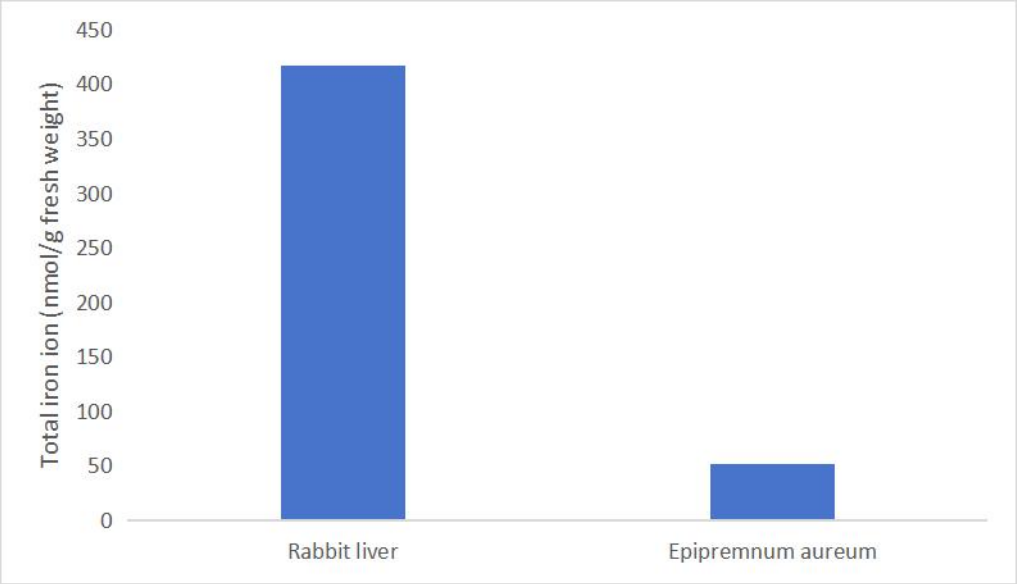

Figure 1. Determination of total iron ion content in rabbit liver and *Epipremnum aureum* leaf by this kit.

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.