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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: 0.78-200 nmol/mL		**Sensitivity:** 0.78 nmol/mL
	Applicable samples: Animal and Plant Tissue, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 12 months		

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 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 the probe under acidic conditions, and has an absorption peak at 593 nm. The ferrous ion concentration can be calculated by measuring the absorbance at this wavelength.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	RT
Reagent Ⅰ	6 mL	12 mL	4℃
Reagent Ⅱ	6 mL	12 mL	4℃
Standard	Powder×2 vials	Powder×2 vials	4℃

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
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; If the solution appears white and cloudy before first use, heat it to 37℃ until it becomes clear.

Reagent Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

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

Standard: Add 1 mL of deionized water and dissolve thoroughly before use; 20 μmol/mL standard solution; Store in aliquots at -20℃ after dissolution.

50 nmol/mL Standard: 20 μmol/mL standard solution can be diluted 400-fold with deionized water; for example, add 5 μL of the 20 μmol/mL standard solution to 1995 μL of deionized water. Always prepare fresh standard 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. To avoid iron contamination, do not use iron utensils during any sample handling or transfer operations.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar at room temperature. Centrifuge at 12,000 g for 10 min at room temperature. Use supernatant for assay.(It is recommended that the supernatant can be diluted 5-fold with Extraction Buffer before testing, and the result should be multiplied by the dilution factor)
2. Plasma, Serum or other Liquid samples: Take 500 μL sample, add 500 μL Extraction Buffer, and mix thoroughly (dilute 2-fold). Centrifuge at 12,000 g at room temperature for 10 minutes, then use the supernatant for assay.

Note: 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.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 593 nm.
2. Ferrous ion 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	100
50 nmol/mL Standard	0	100	0
Deionized water	100	0	0
Reagent Ⅰ	100	100	100
Reagent Ⅱ	100	100	100
Thoroughly mixed, incubated at 37℃ for 30 min, centrifuge at 12,000 g for 5 min **at room temperature**, take 200 μL supernatant into a 96-well plate, 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 1.4, the sample can be appropriately diluted with Extraction Buffer, the

calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. 2. If an oxidizing agent is introduced into the sample or during the testing process, it may result in an underestimation of ferrous iron levels, while having a minimal effect on the measured total iron levels. If a reducing agent is introduced into the sample or during the testing process, it may result in an overestimation of ferrous iron levels, while having a minimal effect on the measured total iron levels. For example, animal tissue samples may contain certain antioxidants, causing the detectable total iron to be primarily in the ferrous form; in such cases, try diluting the sample 5-fold before testing.

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. Calculation of the ferrous ion content

(1) Calculated by protein concentration

Ferrous ion (nmol/mg prot)=$\Delta A_{Test} \div \Delta A_{Standard} \times C_{Standard} \div Cpr \times n$**=$50 \times \Delta A_{Test} \div \Delta A_{Standard} \div Cpr \times n$**

(2) Calculated by fresh weight of samples

Ferrous ion (nmol/g fresh weight)=$\Delta A_{Test} \div \Delta A_{Standard} \times C_{Standard} \div W \times V_{Total\ sample} \times n$**=$50 \times \Delta A_{Test} \div \Delta A_{Standard} \div W \times n$**

(3) Calculated by volume of liquid samples

Ferrous ion (nmol/mL)=$\Delta A_{Test} \div \Delta A_{Standard} \times C_{Standard} \times F$**=$100 \times \Delta A_{Test} \div \Delta A_{Standard}$**

$C_{Standard}$: 50 nmol/mL; Cpr: sample protein concentration, mg/mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 1 mL; n: tissue sample dilution; W: Sample weight, g; F: Liquid sample dilution, 2.

Typical Data

Take 0.1 g of rabbit liver and follow the steps described above. $\Delta A_{Test} = A_{Test} - A_{Blank}$ = 0.151-0.035 = 0.116; $\Delta A_{Standard} = A_{Standard} - A_{Blank}$= 0.338-0.035 = 0.303. Calculating the content based on the fresh weight of the sample: Ferrous ions (nmol/g) =$50 \times \Delta A_{Test} \div \Delta A_{Standard} \div W \times n$= 191.4 nmol/g.

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.