（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K773-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（590-600 nm）

Detection range：0.4-50 μmol/L

Elabscience®Ferrous Iron Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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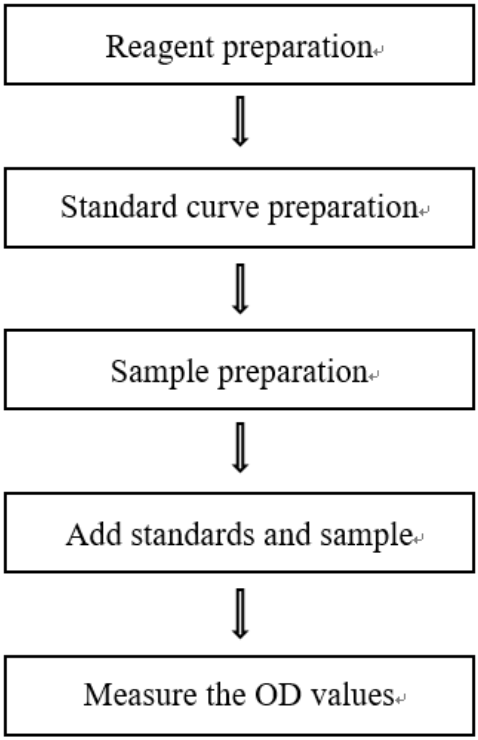
Assay summary



Intended use

This kit can measure ferrous ions (Fe^{2+}) content in serum, animal and plant tissue samples.

Detection principle

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 iron 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	35 mL ×2 vials	60 mL ×2 vials	2-8℃, 12 months, shading light
Reagent 2	Chromogenic Solution	10 mL ×1 vial	20 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 3	10 mmol/L Iron Standard	2 mL ×1 vial	2 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 4	Standard Protectant	Powder ×1 vial	Powder ×1 vial	2-8℃, 12 months, shading light
Reagent 5	Extracting Solution	40 mL ×1 vial	40 mL ×2 vials	2-8℃, 12 months, shading light
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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:

Vortex Mixer, Centrifuge, Water bath, Microplate reader (590-600 nm, optimum wavelength: 593 nm）

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of standard protectant：

Dissolve one vial of standard protectant with 20 mL of buffer solution, mix well to dissolve. Store at 2-8℃ for 1 month.

③ Preparation of 100 μmol/L iron standard：

Dilute 20 μL of 10 mmol/L iron standard with 1980 μL of standard protectant, mix well. The 100 μmol/L iron standard should be prepared on spot.

④ 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 standard protectant to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 30, 40, 50 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**50**
100 μmol/L iron standard (μL)	0	50	100	150	200	300	400	500
Standard protectant (μL)	1000	950	900	850	800	700	600	500

Sample preparation

① Sample preparation

Serum and plasma: Add 55 μL of sample and 165 μL of buffer solution, mix well and keep it on ice for detection. If the sample is turbidity, centrifuge at 5000×g for 5 min, then take the supernatant for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 50 mg tissue in 450 μL extracting solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. 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
Human serum	1
Mouse serum	1-2
Rat serum	1
10% Mouse liver tissue homogenate	2-3
10% Rat lung tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Rat spleen tissue homogenate	2-3
10% Epipremnum aureum leaf tissue homogenate	1

Note: The diluent of tissue sample is extracting solution. The diluent of serum sample is buffer solution. The serum sample has been diluted 4 times during sample processing. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Avoid bubbles when adding samples.

② Do not use iron appliances to prepare or transfer samples..

Operating steps

For serum and plasma

① Standard well: Take 200 μL of standard solution with different concentrations to the corresponding wells

Sample well: Take 200 μL of sample to the corresponding wells.

② Add 100 μL of chromogenic solution to each well.

③ Mix fully and incubate the tubes at 37℃ for 10 min.

④ Measure the OD value of each well with microplate reader at 593 nm.

For tissue

① Standard well: Take 300 μL of standard solution with different concentrations to the 1.5 mL tubes

Sample well: Take 300 μL of sample to the 1.5 mL tubes.

② Add 150 μL of chromogenic solution to each tube.

③ Mix fully with vortex mixer and incubate the tubes at 37℃ for 10 min.

④ Centrifuge the tubes at 12000×g for 10 min.

⑤ Take 300 μL of supernatant to the corresponding microplate wells.

⑥ 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:

1. Serum (plasma) sample:

$$Fe^{2+} \text{ content } (\mu mol/L) = (\Delta A_{593} - b) \div a \times 4^* \times f$$

2. Tissue sample:

$$Fe^{2+} \text{ content } (\mu mol/kg \text{ wet weight}) = (\Delta A_{593} - b) \div a \times f \div \frac{m}{V}$$

[Note]

ΔA_{593}: $OD_{Sample} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

4*: Dilution factor in the preparation step of serum, 4 times.

V: The volume of homogenate, mL.

f: Dilution factor of sample before test.

m: The wet weight of tissue, g.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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)	8.40	22.60	43.50
%CV	1.6	1.2	1.1

Inter-assay Precision

Three human serum 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)	8.40	22.60	43.50
%CV	1.3	1.7	1.5

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）	9	16.5	33
Observed Conc. （μmol/L）	9.1	16.3	32.0
recovery rate(%)	101	99	97

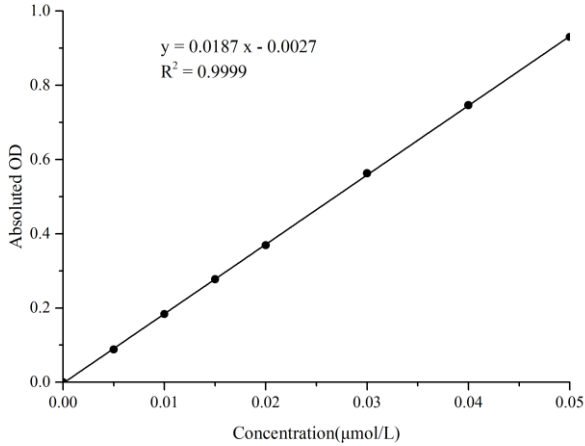
Sensitivity

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**	**30**	**40**	**50**
Average OD	0.048	0.136	0.232	0.326	0.417	0.611	0.795	0.978
Absoluted OD	0.000	0.088	0.184	0.278	0.369	0.563	0.747	0.930



Appendix II Example Analysis

Example analysis：

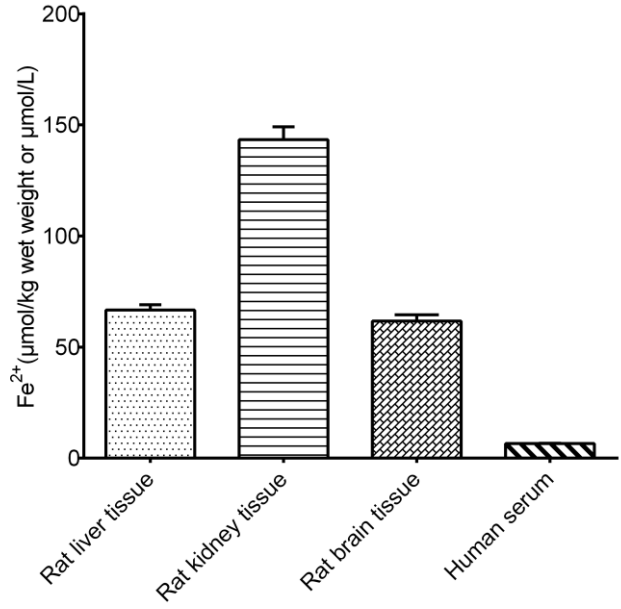
For rat liver tissue, take 10% rat liver tissue homogenate and dilute for 2 times, and carry the assay according to the operation table. The results are as follows:

standard curve: y =0.0187 x - 0.0027, the average OD value of the sample is 0.110, the average OD value of the blank is 0.042, the calculation result is:

Fe^{2+} content (μmol/kg wet weight) = （0.110 - 0.042 + 0.0027）÷0.0187 ×2 ÷(0.1 ÷0.9)

= 68.05 μmol/kg wet weight

Detect 10% rat liver tissue homogenate (dilute for 2 times), 10% rat kidney tissue homogenate (dilute for 2 times), 10% rat brain tissue homogenate (dilute for 2 times) and human serum according to the protocol, the result is as follows：



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. Elabscience 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.