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

Catalog No：E-BC-K304-S

Specification：50 Assays(48 samples)/ 100 Assays(96 samples)

Measuring instrument：Spectrophotometry（520 nm）

Detection range：0.08-20 mg/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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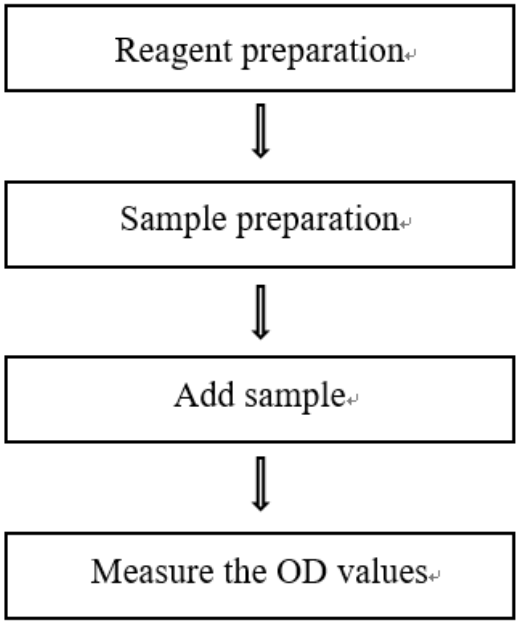
Assay summary



Intended use

This kit can be used to measure the concentration of ferrous iron in serum, plasma and tissue samples.

Detection principle

Under the action of acidic solution and reductant, ferric ions can be separated from transferrin in serum, and reduced into ferrous ions (Fe^{2+}). The latter then bind to bipyridine and form pink complexes. The concentration of iron can be calculated by measuring the OD value at 520 nm indirectly.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	$FeSO_4 \cdot 7H_2O$ Power	Powder × 1 vial	Powder × 2 vials	2-8 ℃, 12 month shading light
Reagent 2	Chromogenic Agent A	Powder × 2 vials	Powder × 4 vials	2-8 ℃, 12 month shading light
Reagent 3	Chromogenic Agent B	50 mL × 2 vials	50 mL × 4 vials	2-8 ℃, 12 months

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:

Spectrophotometer (520 nm), Test tubes, Vortex Mixer, Centrifuge, Water bath, Test tubes, Vortex Mixer, Centrifuge, Water bath

Reagents:

Deionized water, PBS (0.01 M, pH7~7.4)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of 100 mg/L standard stock solution：
Dissolve a vial of $FeSO_4 \cdot 7H_2O$ power with deionized water to a final volume of 20 mL. Store at 2~8 ℃ for 1 h.

③ The preparation of 2 mg/L standard working solution：
For each tube, prepare 500 μL of 2 mg/L standard working solution (mix well 10 μL of 100 mg/L standard stock solution and 490 μL of deionized water). The 2 mg/L standard working solution should be prepared on spot.

④ The preparation of chromogenic agent：
Dissolve a vial of chromogenic agent A with 50 mL chromogenic agent B and mix well. Store at 2~8 ℃ for 1 month protected from light.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

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 20 mg tissue in 180 μL deionized water with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human plasma	1
Human serum	1
10% Rat liver tissue homogenate	1
10% Epipremnum aureum homogenate	1

Note: The diluent is deionized water. For the dilution of other sample types, please do pretest to confirm the dilution factor

Operating steps

① Blank tube: Add 0.5 mL of deionized water into a 5 mL centrifuge tube.
Standard tube: Add 0.5 mL of 2 mg/mL standard working solution into a 5 mL centrifuge tube.
Sample tube: Add 0.5 mL of sample into a 5 mL centrifuge tube.

② Add 1.5 mL of chromogenic agent, mix fully with vortex mixer, then incubate in 100℃ water bath for 5 min. (Blank tube and standard tube can be treated without 100℃ water bath.).

③ Cool the tubes with running water, centrifuge the tubes at 2300 g for 10 min. (If the supernatant is still turbid, take the turbid supernatant into another centrifuge tube and centrifuge again.).

④ Take 1.0 mL of supernatant. Set to zero with deionized water, and measure the OD value of each tube with spectrophotometer at 520 nm with 0.5 cm optical path quartz cuvette.

Calculation

The sample:

1. Serum (plasma) sample:

$$\text{Ferrous iron content (mg/L)} = \frac{\Delta A_1}{\Delta A_2} \times c_1 \times f$$

or

$$\text{Ferrous iron content (μmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c_2 \times f$$

2. Tissue sample:

$$\text{Ferrous iron content (mg/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times c_1 \times f \div C_{pr}$$

or

$$\text{Ferrous iron content (μmol/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times c_2 \times f \div C_{pr}$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c_1: The concentration of standard, 2 mg/L

c_2: The concentration of standard, 35.8 μmol/L

2 mg/L standard = 2000 μg/L ÷ Molecular weight of Iron (55.847) = 35.8 μmol/L

f: Dilution factor of sample before test.

C_{pr}: The concentration of protein in sample, gprot/L.

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 (mg/L)	1.50	9.70	13.20
%CV	3.4	2.9	2.7

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/L)	1.50	9.70	13.20
%CV	2.9	3.3	3.4

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 97%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/L)	2.5	11.6	17.4
Observed Conc. (mg/L)	2.5	11.0	16.9
Recovery rate (%)	99	95	97

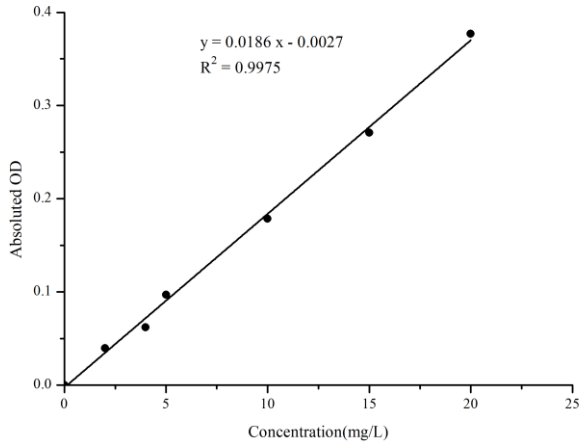
Sensitivity

The analytical sensitivity of the assay is 0.08 mg/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 (mg/L)	**0**	**2**	**4**	**5**	**10**	**15**	**20**
Average OD	0.000	0.040	0.062	0.097	0.179	0.271	0.377
Absoluted OD	0.000	0.040	0.062	0.097	0.179	0.271	0.377



Appendix II Example Analysis

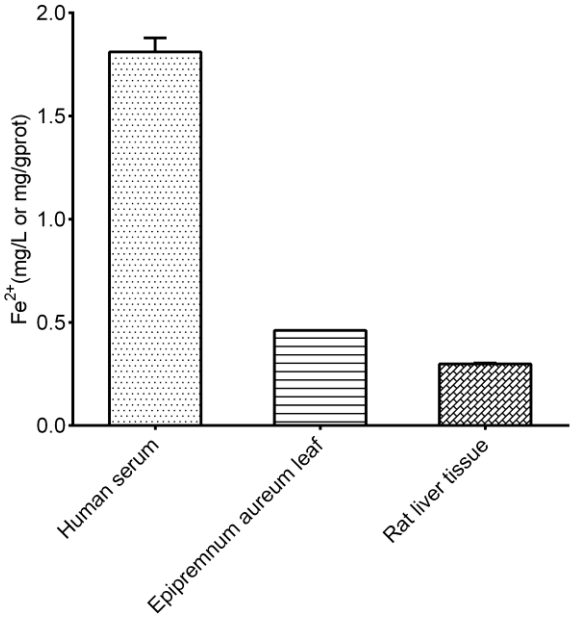
Example analysis:

Take 0.5 mL of human serum, and carry the assay according to the operation steps. The results are as follows:

the OD value of the standard tube is 0.034, the OD value of the blank tube is 0.004, the OD value of the sample tube is 0.030, and the calculation result is:

$$\text{Ferrous iron content (mg/L)} = \frac{0.030 - 0.004}{0.034 - 0.004} \times 2 = 1.73 \text{ mg/L}$$

Detect human serum, human plasma, 10% epipremnum aureum homogenate (the concentration of protein is 2.29 gprot/L), 10% rat liver homogenate (the concentration of protein is 12.26 gprot/L) 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.