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

Catalog No：E-BC-K139-M

Specification：48T(32 samples)/96T(80 samples)/96T×5(464 samples)

Measuring instrument：Microplate reader（510-530 nm）

Detection range：0.29-10 mg/L

Elabscience® 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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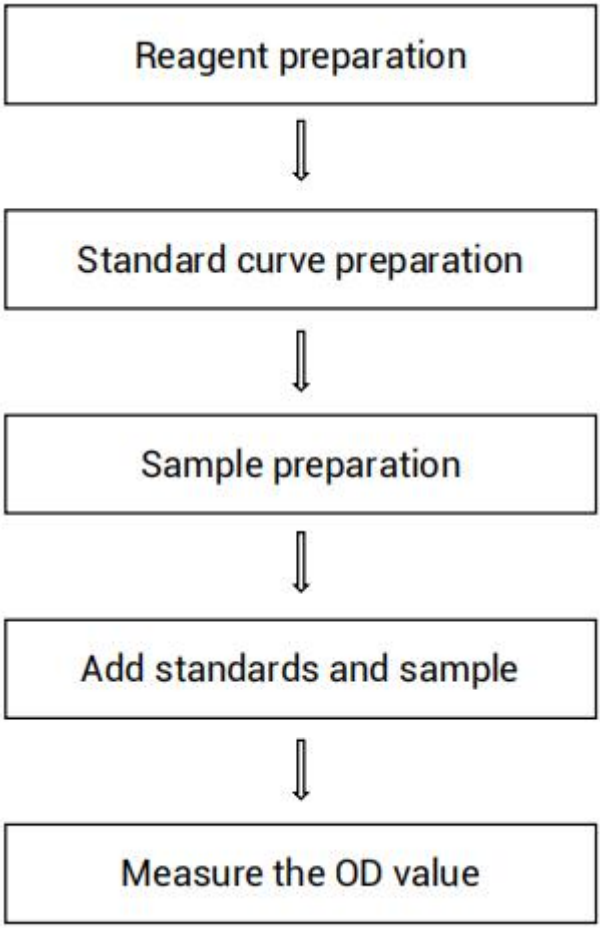
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Assay summary



Intended use

This kit can measure Iron content in serum, 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(48T)	Size 2(96T)	Size 3(96T×5)	Storage
Reagent 1	10 mg/L Iron Standard	1 mL × 1 vial	1 mL × 2 vials	1 mL × 10 vials	2-8°C, 12 months
Reagent 2	Chromogenic Agent A	Powder × 1 vial	Powder × 2 vials	Powder × 10 vials	2-8°C, 12 months, shading light
Reagent 3	Chromogenic Agent B	Powder × 1 vial	Powder × 2 vials	Powder × 10 vials	2-8°C, 12 months, shading light
Reagent 4	Chromogenic Agent C	20 mL × 1 vial	20 mL × 2 vials	20 mL × 10 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	96 wells×5	No requirement
	Plate Sealer	2 pieces		7 pieces	
	Sample Layout Sheet	1 piece		7 piece	

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:

Microplate reader (510-530 nm, optimum wavelength: 520 nm),

Micropipettor, Centrifuge, Water bath, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of iron chromogenic agent：

Dissolve one vial of chromogenic agent A and one vial of chromogenic agent B with 20 mL of chromogenic agent C, mix well to dissolve. Store at 2-8°C for 1 month protected from light.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 10 mg/L iron standard stock solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 4, 5, 6, 8, 10 mg/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/L)	**0**	**1**	**2**	**4**	**5**	**6**	**8**	**10**
10 mg/L standard (μL)	0	20	40	80	100	120	160	200
Double distilled water (μL)	200	180	160	120	100	80	40	0

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°C for a month.

Tissue samples:

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 normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 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 serum	1
Mouse serum	1
10% Mouse liver tissue homogenate	1
10% Rat kidney tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The supernatant after centrifugation must be clarified, and if there is turbidity, it must be centrifuged again.

② During the experiment, the experimental vessel must be clean to avoid iron contamination which may affect the result of the experiment.

③ Iron chromogenic agent should be prepared in advance because the chromogenic agent A powder and chromogenic agent B powder are difficult to dissolved.

Operating steps

① Standard tube: Add 75 μL of standard solution with different concentrations to the tubes.
Sample tube: Add 75 μL of sample to the tubes.

② Add 300 μL of iron chromogenic agent, mix fully with vortex mixer.

③ Incubate the tubes in 100°C water bath for 5 min.

④ Cool the tubes with running water, centrifuge the tubes at 3000×g for 10 min.

⑤ Take 200 μL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 520 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) samples:

$$\text{Fe content (mg/L)} = (\Delta A_{520} - b) \div a \times f$$

2. Tissue samples:

$$\text{Fe content (mg/gprot)} = (\Delta A_{520} - b) \div a \times f \div C_{pr}$$

[Note]

f: Dilution factor of sample before tested.

ΔA_{520}: $OD_{Sample} - OD_{Blank}$.

C_{pr}: 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)	1.30	3.70	7.50
%CV	1.5	1.2	0.9

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 (mg/L)	1.30	3.70	7.50
%CV	3.0	2.7	2.7

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 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/L)	1.5	4.5	7
Observed Conc. (mg/L)	1.4	4.4	6.7
Recovery rate (%)	95	97	96

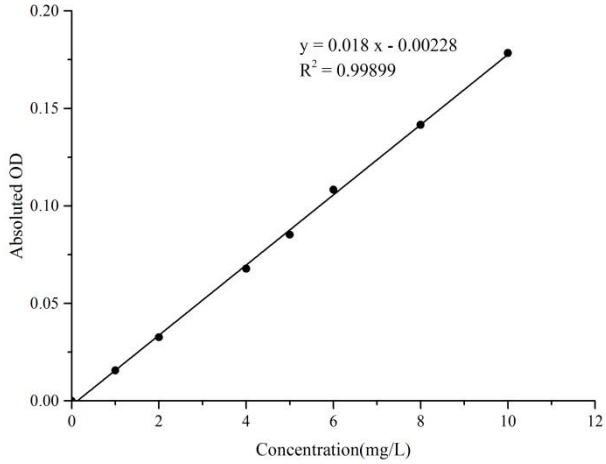
Sensitivity

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	1	2	4	5	6	8	10
OD value	0.045	0.058	0.075	0.109	0.126	0.149	0.183	0.221
	0.038	0.056	0.073	0.109	0.126	0.150	0.182	0.218
Average OD	0.041	0.057	0.074	0.109	0.126	0.149	0.183	0.220
Absoluted OD	0.000	0.016	0.033	0.068	0.085	0.108	0.142	0.178



Appendix Π Example Analysis

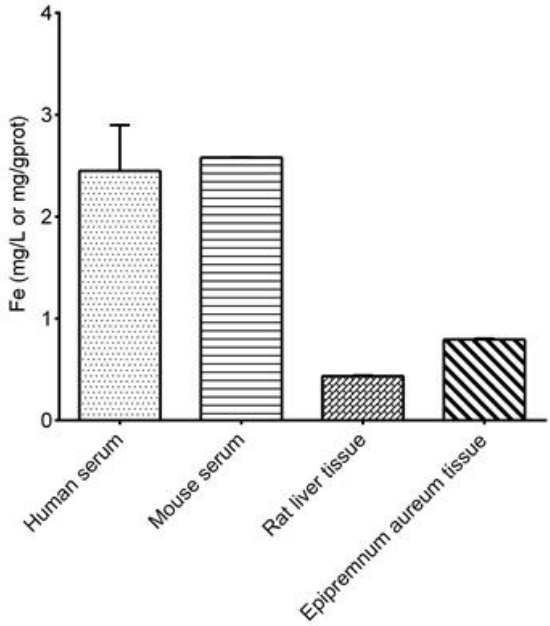
Example analysis：

Take 75 μL of human serum sample and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.01427 x + 0.0008, the average OD value of the sample is 0.078, the average OD value of the blank is 0.038, and the calculation result is:

$$\text{Fe content (mg/L)} = (0.078 - 0.038 - 0.0008) \div 0.01427 = 2.75 \text{ mg/L}$$

Detect human serum, mouse serum, 10% rat liver tissue homogenate (the concentration of protein in sample is 9.47 gprot/L) and 10% epipremnum aureum tissue homogenate (the concentration of protein in sample is 3.08 gprot/L) according to the protocol, the result is as follows：



Appendix III Publications

1. RNA m6A Modification Alteration by Black Phosphorus Quantum Dots Regulates Cell Ferroptosis: Implications for Nanotoxicological Assessment[J].Small Methods, 2021, 5(3).DOI:10.1002/smtd.202001045.
2. Jiang T, Zhou J, et al. Arbutin alleviates fatty liver by inhibiting ferroptosis via FTO/SLC7A11 pathway[J]. Redox biology, 2023, 68: 102963.
3. Liu Y, Cheng D, Wang Y, et al. UHRF1-mediated ferroptosis promotes pulmonary fibrosis via epigenetic repression of GPX4 and FSP1 genes[J]. Cell Death & Disease, 2022, 13(12): 1070.
4. Yang Y, Ma Y, Li Q, et al. STAT6 inhibits ferroptosis and alleviates acute lung injury via regulating P53/SLC7A11 pathway[J]. Cell death & disease, 2022, 13(6): 530.
5. Hao W , Zhang H , Hong P ,et al.Critical role of VHL/BICD2/STAT1 axis in crystal-associated kidney disease[J].Cell death & disease, 14(10):680[2025-03-04].DOI:10.1038/s41419-023-06185-1.
6. Wang X , Wang K , Mao W ,et al.Emerging perfluoroalkyl substances retard skeletal growth by accelerating osteoblasts senescence via ferroptosis[J].Environmental Research, 2024, 258.DOI:10.1016/j.envres.2024.119483.

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.