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

Catalog No：E-BC-K758-M

Specification：48T(25 samples)/96T(50 samples)

Measuring instrument：Microplate reader (510-530 nm)

Detection range：0.31-50 mg/L TIBC

Elabscience®Total Iron Binding Capacity (TIBC) and Serum Iron 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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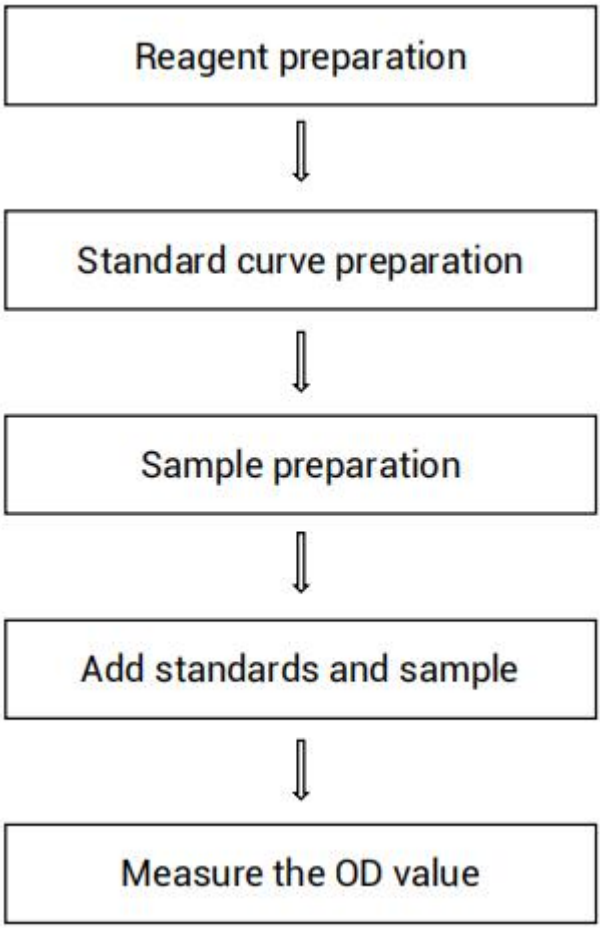
Assay summary



Intended use

This kit can be used to measure the total iron binding capacity (TIBC) and serum iron content in serum and plasma samples.

Detection principle

The excess iron is added to the serum to bind all the transferrin in the serum, and the excess iron is adsorbed by adding the iron adsorbent. The iron bind with the transferrin is separated from the protein by the action of acid solution and reductant. Fe^{3+} in serum is reduced to Fe^{2+}, Fe^{2+} binds with bipyridine to form pink complex. In a certain range, the amount of iron ions is positively correlated with the color, and the measured iron content is TIBC. Subtracting serum iron value from TIBC is called unsaturated iron binding capacity (UIBC).

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	100 mg/L Iron Standard Stock Solution	2 mL × 1 vial	2 mL × 1 vial	2-8℃, 12 month
Reagent 2	Chromogenic Agent A	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 month shading light
Reagent 3	Chromogenic Agent B	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 month shading light
Reagent 4	Chromogenic Agent C	15 mL × 1 vial	15 mL × 2 vials	2-8℃, 12 month
Reagent 5	Iron Absorbent	Powder × 50 vials	Powder × 100 vials	2-8℃, 12 month
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 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, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water

Reagent preparation

① Equilibrate reagents to 25℃ before use.

② The preparation of iron chromogenic agent:
Dissolve one vial of chromogenic agent A and one vial of chromogenic agent B with 15 mL of chromogenic agent C, mix well to dissolve. Store at 2-8°C for 1 month protected from light.

③ The preparation of 10 mg/L iron standard application solution:
Before testing, please prepare sufficient 10 mg/L iron standard application solution. For example, prepare 500 μL of 10 mg/L iron standard application solution (mix well 50 μL of 100 mg/L iron standard stock solution and 450 μL of double distilled water). Store at 2-8°C for 12 h protected from light.

④ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 100 mg/L iron standard stock solution with double distilled

water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 25, 30, 40, 50 mg/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/L)	**0**	**5**	**10**	**20**	**25**	**30**	**40**	**50**
100 mg/L standard (μL)	0	10	20	40	50	60	80	100
Double distilled water (μL)	200	190	180	160	150	140	120	100

Sample preparation

① Sample preparation

Serum and plasma: detect directly.

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Rat serum	1
Porcine serum	1
Rabbit serum	1
Chicken serum	1
Machin serum	1

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

The key points of the assay

① After 100℃ water bath, the supernatant obtained by centrifugation must be clarified, otherwise the experimental results will be affected.

② The experimental container must be clean with double distilled water over 10 times to avoid the contamination of iron.

③ The chromogenic agent A and chromogenic agent B are difficult to

dissolve during the preparation of iron chromogenic agent, so it is recommended to prepare before the experiment.

Operating steps

The measurement of standard curve:

① Standard well: Take 30 μL of standard solution with different concentration to the wells.

② Add 150 μL of iron chromogenic agent to the wells.

③ Mix fully for 5 s with microplate reader, stand at room temperature (25℃) for 5 min and measure the OD value at 520 nm.

The measurement of sample:

The preparation of TIBC sample supernatant: Take 50 μL of serum, add 50 μL of 10 mg/L iron standard application solution, mix fully with a vortex mixer for 10 s or more and stand at room temperature (25℃) for 5 min. Then add a vial of iron absorbent, mix fully with a vortex mixer for 10 s or more and stand at room temperature (25℃) for 5 min. Centrifuge at 3000×g for 10 min and take the supernatant for detection.

The preparation of serum iron sample supernatant: Take 50 μL of serum, add 50 μL of double distilled water and a vial of iron absorbent, mix fully with a vortex mixer for 10 s or more and stand at room temperature (25℃) for 5 min. Centrifuge at 3000×g for 10 min and take the supernatant for detection.

① The sample tube of TIBC: Add 50 μL of TIBC sample supernatant into the 1.5 mL EP tube.

The sample tube of serum iron: Add 50 μL of serum iron sample supernatant into the 1.5 mL EP tube.

Control tube: Add 50 μL of double distilled water into the 1.5 mL EP

tube.

② Add 250 μL of iron chromogenic agent into each tube. Mix fully with a vortex mixer for 3 s and incubate in 100℃ water bath for 5 min.

③ Cool the tubes with running water, then centrifuge at 10000×g for 10 min (If the supernatant is turbid, collect the turbid supernatant into another new EP tube and centrifuge again).

④ Take 180 μL of the supernatant to the corresponding wells of microplate and measure the OD value at 520 nm of each well.

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. The TIBC in serum (plasma) sample:

$$\underset{(mg/L)}{TIBC} = (\Delta A_1 - b) \div a \times f \times 2$$

or

$$\underset{(\mu mol/L)}{TIBC} = (\Delta A_1 - b) \div a \times f \times c_1 \times 2$$

2. The serum iron content in serum (plasma) sample:

$$\underset{(mg/L)}{Fe} = (\Delta A_2 - b) \div a \times f \times 2$$

or

$$\underset{(\mu mol/L)}{Fe} = (\Delta A_2 - b) \div a \times f \times c_1 \times 2$$

$$UIBC\ (\mu mol/L) = c_3 - c_2$$

$$i = c_2 \div c_3 \times 100\ \%$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Control}$. ($OD_{sample}$ is the OD value of the TIBC sample tube).

ΔA_2: $OD_{Sample} - OD_{Control}$. ($OD_{sample}$ is the OD value of the serum iron sample tube).

f: Dilution factor of sample before test.

2: The dilution ratio of sample at the preparation of sample supernatant.

c_1: 17.91 μmol/L (1 mg/L Iron = 17.91 μmol/L).

c_2: The concentration of serum iron.

c_3: Total iron binding capacity (TIBC) (μmol/L).

i: Iron saturation (%).

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)	3.80	24.60	43.80
%CV	1.7	1.6	1.2

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)	3.80	24.60	43.80
%CV	2.5	2.1	2.3

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)	8.5	22.6	32
Observed Conc. (mg/L)	8.6	22.4	32.0
recovery rate(%)	101	99	100

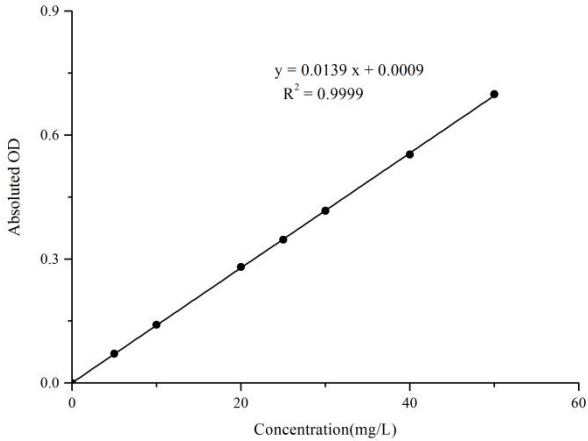
Sensitivity

The analytical sensitivity of the assay is 0.14 mg/L TIBC. 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**	**5**	**10**	**20**	**25**	**30**	**40**	**50**
Average OD	0.037	0.108	0.178	0.318	0.384	0.454	0.590	0.736
Absoluted OD	0	0.071	0.141	0.281	0.347	0.417	0.553	0.699



Appendix Π Example Analysis

Example analysis：

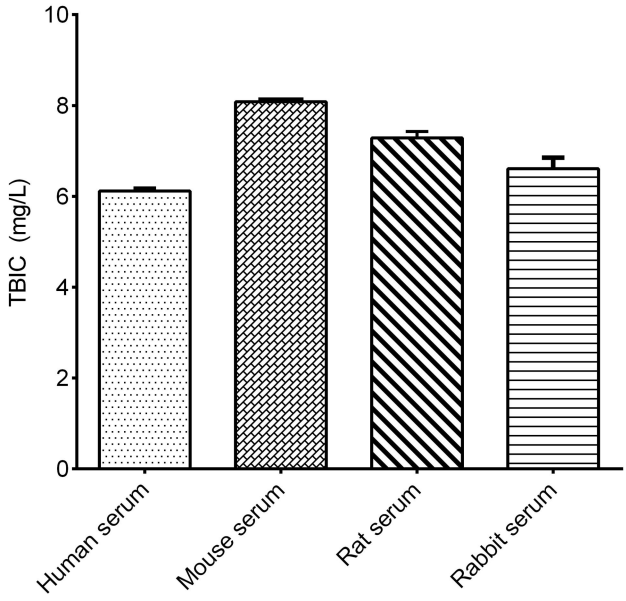
Take 100 μL of human serum and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.014 x - 0.0009, the average OD value of the TIBC sample well is 0.080, the average OD value of the serum iron sample well is 0.063, the average OD value of control well is 0.038, and the calculation result is:

$$\underset{(mg/L)}{TIBC} = (0.080 - 0.038 + 0.0009) \div 0.014 \times 2 = 6.18\ mg/L$$

$$\underset{(mg/L)}{Fe} = (0.063 - 0.038 + 0.0009) \div 0.014 \times 2 = 3.7\ mg/L$$

Detect human serum, mouse serum, rat serum and rabbit serum according to the protocol, the result of TIBC 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.