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

Catalog No：E-BC-K071-S

Specification：50 Assays(48 samples) / 100 Assays(98 samples)

Measuring instrument：Spectrophotometer（520 nm）

Detection range：0.03-50 mg/L

Elabscience®Total Iron Binding Capacity (TIBC) 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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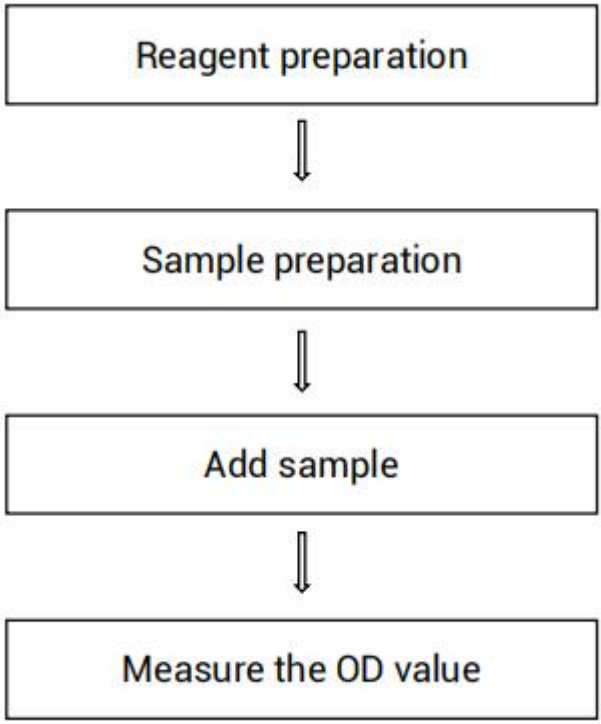
Assay summary



Intended use

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

Detection principle

The excess iron is added to the serum to bind all the ferritin in the serum, and the excess iron is adsorbed by adding the iron adsorbent. The iron bind with the ferritin is separated from the protein by the action of acid solution and reductant. Fe3+ in serum is reduced to Fe2+, Fe2+ binds with bipyridine to form pink complex. In a certain range, the amount of TIBC is positively correlated with the depth of color. The iron content measured is, minus serum iron value, which is called unsaturated iron binding force. Total iron binding capacity minus serum iron value is unsaturated iron binding capacity (UIBC).

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	100 mg/L Iron Standard Stock Solution	7 mL × 1 vial	14 mL × 1 vial	2-8℃, 12 months
Reagent 2	Chromogenic Agent A	Powder × 2 vials	Powder × 4 vials	2-8℃, 12 months shading light
Reagent 3	Chromogenic Agent B	Powder × 2 vials	Powder × 4 vials	2-8℃, 12 months shading light
Reagent 4	Chromogenic Agent C	60 mL × 2 vials	60 mL × 4 vials	2-8℃, 12 months
Reagent 5	Iron Absorbent	50 mg × 50 vials	50 mg × 100 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), Micropipettor, Water bath, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate reagents to room temperature before use.

② Preparation of chromogenic agent：
Dissolve one vial of chromogenic agent A and one vial of chromogenic agent B with 60 mL of chromogenic agent C, mix well to dissolve. Store at 2-8°C for 1 month protected from light.

③ Preparation of 10 mg/L iron standard application solution：
For each sample，prepare 1000 μL of 10 mg/L iron standard application solution (mix well 100 μL of 100 mg/L Iron Standard Stock Solution and 900 μL of double distilled water). Store at 2-8°C for 3 days.

④ Preparation of 1 mg/L iron standard application solution：
For each tube，prepare 1000 μL of 1 mg/L iron standard application solution (mix well 100 μL of 10 mg/L iron standard application solution and 900 μL of double distilled water). Store at 2-8°C for 3 days.

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.

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
Cynomolgus monkey serum	1

Note: The diluent is double distilled water or 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 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 to avoid the contamination of iron.

Operating steps

① The pretreatment of sample

Take 1 mL of serum, add 1 mL of 10 mg/L iron standard application solution, mix fully and stand at room temperature for 10 min. Then add a vial of iron absorbent, mix fully and stand at room temperature for 5 min, repeat the mix and stand steps for 4 times. Centrifuge at 2300×g for 10 min and take the supernatant for detection.

② Blank tube: Add 1.0 mL of double distilled water into 5 mL EP tube

Standard tube: Add 1.0 mL of 1 mg/L iron standard application solution into 5 mL EP tube.

Sample tube: Add 1.0 mL of sample supernatant in step 1 into 5 mL EP tube.

③ Add 2.0 mL of chromogenic agent into each tube. Oscillate fully with a vortex mixer and incubate in 100℃ water bath for 5 min.

④ Cool the tubes with running water, then centrifuge at 2300×g for 10 min (If the supernatant is turbid, collect the turbid supernatant into another new EP tube and centrifuge again). Take 1.0 mL of the supernatant.

⑤ Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 520 nm wavelength with 0.5 cm optical path cuvette.

Calculation

The sample:

Serum (plasma) sample:

$$\text{TIBC (mg/L)} = \frac{\Delta A_1}{\Delta A_2} \times C_1 \times f$$

or

$$\text{TIBC (μmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times C_2 \times f$$

$$\text{UIBC (μmol/L)} = c_4 - c_3$$

$$i = c_3 \div c_4 \times 100\ \%$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$.

ΔA_2: $OD_{Standard} - OD_{Blank}$.

c_1: Concentration of standard (1 mg/L).

c_2: Concentration of standard (17.91 μmol/L).

1 mg/L Iron = 1000 μg/L ÷ molecular weight of Iron (55.847) = 17.91 μmol/L.

f: Dilution factor of sample before test.

c_3: Serum iron concentration, μmol/L.

c_4: 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)	3.50	24.60	44.50
%CV	3.6	3.2	3.4

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)	3.50	24.60	44.50
%CV	4.5	4.7	4.9

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%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （mg/L）	13.8	34.7	45
Observed Conc. （mg/L）	13.7	34.0	46.4
recovery rate(%)	99	98	103

Sensitivity

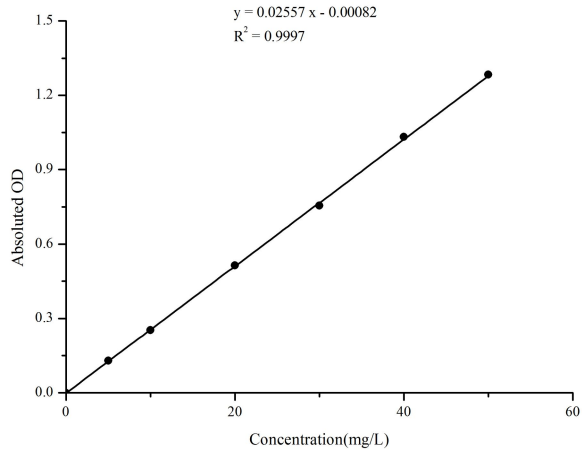
The analytical sensitivity of the assay is 0.03 mg/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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**	**30**	**40**	**50**
Average OD	0.001	0.130	0.253	0.515	0.755	1.034	1.283
Absoluted OD	0	0.129	0.252	0.514	0.754	1.033	1.282



Appendix Π Example Analysis

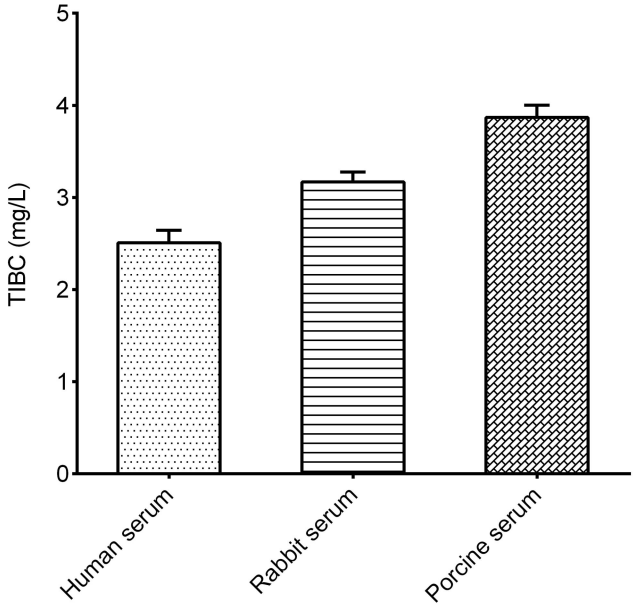
Example analysis：

Take 1 mL of human serum, carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.069, the average OD value of the blank is 0.002, the average OD value of the standard is 0.029, the concentration of standard is 17.91 μmol/L, serum iron concentration is 21.963 μmol/L, and the calculation result is:

$$\underset{(\mu mol/L)}{UIBC} = \frac{0.069-0.002}{0.029-0.002} \times 17.91 \times 1 - 21.963 = 22.48\ \mu mol/L$$

Detect human serum, rabbit serum, porcine 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.