Total Iron Binding Capacity (TIBC) Colorimetric Assay Kit

Catalog No: E-BC-K071-S

Method: Colorimetric method

Specification: 50 Assays (Can detect 48 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.03 mg/L

Detection range: 0.03-50 mg/L

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

General information

▲Intended use

This kit can be used to measure the total iron binding capacity (TIBC) content in serum samples. If you want to measure the unsaturated iron binding capacity (UIBC), the iron content of serum sample needs to be detected in addition (E-BC-K139-S/ E-BC-K139-M are recommended).

▲Background

Total iron binding capacity (TIBC) was used as a parameter to evaluate the maximum capacity of serum iron transport. Iron is an essential biological element in organisms because it is involved in many metabolic processes such as oxygen transport, DNA synthesis and electronic transport. TIBC is also indirectly used to assess the level of serum transferrin.

▲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. 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 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	Specification	Storage
Reagent 1	100 mg/L Iron Standard	7 mL × 1 vial	2-8℃ , 6 months
Reagent 2	Chromogenic Agent A	Powder × 2 vials	2-8℃ , 6 months, shading light
Reagent 3	Chromogenic Agent B	Powder × 2 vials	2-8℃ , 6 months, shading light
Reagent 4	Chromogenic Agent C	60 mL × 2 vials	2-8℃ , 6 months
Reagent 5	Iron Absorbent	Powder × 50 vials	2-8℃ , 6 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.

▲Materials prepared by users

Instruments

Spectrophotometer (520 nm), Micropipettor, Water bath, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲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 larger.
2. The experimental container must be clean to avoid the contamination of iron.

Pre-assay preparation

▲ Reagent preparation

1. The preparation of 1 mg/L iron standard application solution
   Dilute the reagent 1 with double distilled water for 100 times. The prepared solution can be store at 2-8°C for 3 days.
2. The preparation of chromogenic agent
   Dissolve a vial of reagent 2 and a vial of regent 3 with a vial of reagent 4 fully. The prepared solution can be store at 2-8°C for a month with shading light.

▲ Sample preparation

Serum

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.03-50 mg/L).

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).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	520 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.
(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. The preparation of sample supernatant
   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 reagent 5, 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 a 5 mL EP tube.
   Standard tube: Add 1.0 mL of 1 mg/L iron standard application solution into a 5 mL EP tube.
   Sample tube: Add 1.0 mL of sample supernatant in step 1 into a 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°C 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.

▲Operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	1.0		
1 mg/L Iron standard application solution (mL)		1.0	
Sample supernatant (mL)			1.0
Chromogenic agent (mL)	2.0	2.0	2.0
Oscillate fully with a vortex mixer and incubate in 100°C water bath for 5 min. 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. 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

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

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

$$\text{UIBC (μmol/L)} = c_4 - c_3$$

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

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_1: $OD_{Standard} - OD_{Blank}$

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, %

Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

Appendix I Performance characteristics			
Detection range	0.03-50 mg/L	Average intra-assay CV (%)	3.4
Sensitivity	0.03 mg/L	Average inter-assay CV (%)	4.7
Average recovery rate (%)	100		

▲Inter-assay CV

Take three kits of different batches to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 3 times (n=3) parallelly with each kit. Then calculate the corresponding values according to the following formula. The average Inter-assay CV is 4.7%.

$$\overline{x}_T = \frac{\overline{x}_1 + \overline{x}_2 + \overline{x}_3}{3}$$

$$R = \frac{\overline{x}_{max} - \overline{x}_{min}}{\overline{x}_T} \times 100\%$$

$$\overline{R} = \frac{R_1 + R_2 + R_3}{3} \quad (n=3)$$

$\overline{x}_{max}$--- The max values of $\overline{x}_i$

$\overline{x}_{min}$--- The min values of $\overline{x}_i$

$\overline{x}_T$---The average values of $\overline{x}_i$

R_i---The value of each batch number kit

▲Intra-assay CV

Take one kit to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 6 times (n=6) parallelly, and the average Intra-assay CV is 3.4%, which was calculated according to the following formula.

$$\mathrm{CV} = \frac{S}{\bar{x}} \times 100\%$$

S--- Standard deviation

▲Sensitivity

OD values of standard curve and 20 blank samples were measured according to the operation table. Plot the standard curve and calculate the standard deviation of blank, three standard deviations divided by the slope is the sensitivity (0.03 mg/L) according to the formula of IUPAC.

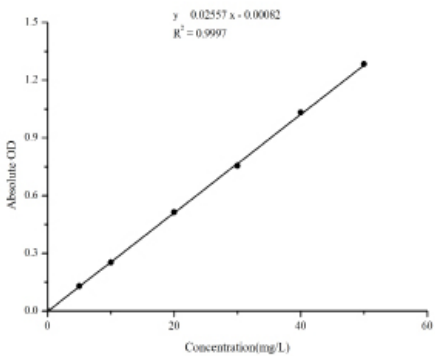
▲Recovery rate

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 3 times parallelly to get the average recovery rate of 100%.

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

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	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



▲Example analysis

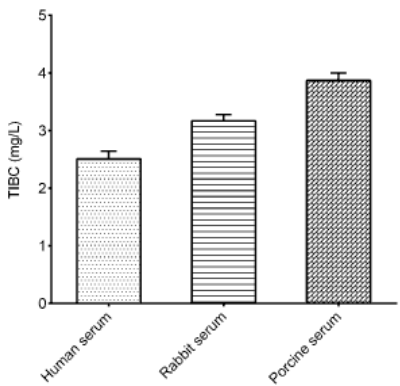
Take 1 mL of human serum, carry the assay according to the operation table.

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:

UIBC(μmol/L)=(0.069-0.002)÷(0.029-0.002)×17.91×1-21.963=22.48 (μmol/L)

Detect human serum, rabbit serum, porcine serum according to the protocol, the result is as follows:



Appendix II References

1. Kasvosve I, Delanghe J. Total iron binding capacity and transferrin concentration in the assessment of iron status. Clinical Chemistry & Laboratory Medicine Cclm:, 2002, 40(10): 1014-1018.
2. Emerit J, Beaumont C, Trivin F. Iron metabolism, free radicals, and oxidative injury. Biomedicine & Pharmacotherapy, 2001, 55(6): 333-339.
3. Conrad M E, Umbreit J N. Iron absorption and transport-An update. American Journal of Hematology, 2010, 64(4): 287-298.
4. Yamanishi H, Iyama S, Yamaguchi Y, et al. Total iron-binding capacity calculated from serum transferrin concentration or serum iron concentration and unsaturated iron-binding capacity. Clinical Chemistry, 2003, 49(1): 175-178.