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Total Iron Binding Capacity (TIBC) Colorimetric Assay Kit

Catalog No: E-BC-K071-M

Method: Colorimetric method

Specification: 96T (Can detect 79 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.14 mg/L

Detection range: 0.31-50 mg/L

Average intra-assay CV (%): 1.5

Average inter-assay CV (%): 2.3

Average recovery rate (%): 100

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ 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 Stock Solution	2 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Chromogenic Agent A	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 3	Chromogenic Agent B	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 4	Chromogenic Agent C	15 mL × 2 vials	2-8℃ , 12 months
Reagent 5	Iron Absorbent	Powder × 79 vials	2-8℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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

Microplate reader (510-530 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Consumptive material

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

Reagents

Double distilled water

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

Pre-assay preparation

▲Reagent preparation

1. Bring all the reagents to room temperature before use.
2. The preparation of chromogenic agent:
   Dissolve a vial of reagent 2 and a vial of regent 3 with 15 mL of reagent 4 fully. The prepared solution can be store at 2-8℃ for a month with shading light.

▲Sample preparation

Serum sample

Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection.

▲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 (0.31-50 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
Machin serum	1

Note: The diluent is double distilled water.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S0	S8	S16	S24	S32	S40	S48	S56	S64	S72
B	B	B	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
C	C	C	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
D	D	D	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
E	E	E	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
F	F	F	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
G	G	G	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
H	H	H	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79

Note: A-H, standard wells; S0, control well; S1-S79, sample wells.

▲Operating steps

1. The measurement of standard curve
   1) 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.
   2) Take 30 μL of standard solution with different concentration to the wells.
   3) Add 150 μL of chromogenic agent to the wells.
   4) Mix fully for 5 s with microplate reader, stand at room temperature for 5 min and measure the OD value at 520 nm.

2. The measurement of sample

1) The pretreatment of sample

Take 50 μL of serum, add 50 μL of 10 mg/L iron standard application solution, mix fully with a vortex mixer and stand at room temperature for 5 min. Then add a vial of reagent 5, mix fully with a vortex mixer for 3 s and stand at room temperature for 5 min. Centrifuge at 3000 g for 10 min and take the supernatant for detection.

2) Sample tube: Add 50 μL of pretreated sample into the 1.5 mL EP tube. Control tube: Add 50 μL of double distilled water into the 1.5 mL EP tube.

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

4) 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).

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

▲Operation table

The measurement of standard curve

	Standard tube
Standard solution with different concentration (μL)	30
Chromogenic agent (μL)	150
Mix fully for 5 s with microplate reader, stand at room temperature for 5 min and measure the OD value at 520 nm.	

The measurement of sample

	Control tube	Sample tube
Double distilled water (μL)	50	
Pretreated sample (μL)		50
Chromogenic agent (μL)	250	250
Oscillate fully with a vortex mixer for 3 s and incubate in 100℃ water bath for 5 min. 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). Take 180 μL of the supernatant to the corresponding wells of microplate and measure the OD value at 520 nm of each well.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is: y= ax + b.

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

$$\text{or } TIBC(\mu mol/L) = (\Delta A_{520} - b) \div a \times f \times c_1$$

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

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

Note:

y: $OD_{Standard} - OD_{Blank}$.

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before test.

ΔA_{520}: $OD_{Sample} - OD_{Blank}$.

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 Data

▲Example analysis

Take 50 μL of human serum and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.014 x – 0.0009, the average OD value of the sample is 0.080, the average OD value of the control is 0.038, and the calculation result is:

TIBC(mg/L) = (0.080 - 0.038 + 0.0009) ÷ 0.014 = 3.06 mg/L

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.