Technical Manual

Total Iron Binding Capacity (TIBC) Colorimetric Assay Kit

- **Catalogue Code: MAES0073**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.31-50 mg/L

Sensitivity:

0.14 mg/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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 bound to 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 a 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).

5. Kit Components & Storage

Item	Specification	Storage
Iron Standard Stock Solution (100 mg/L)	2 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent A	lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent B	lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent C	15 mL × 2 vials	2-8°C 6 months
Iron Absorbent	lyophilized × 79 vials	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipette
- Vortex mixer
- Water bath
- Microplate Reader (510-530 nm, optimum wavelength: 520 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. After the 100°C incubation in the 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 prevent the contamination of iron.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. The preparation of **chromogenic agent:** Dissolve a vial of chromogenic agent A and a vial of chromogenic agent B with 15 mL of chromogenic agent C. The prepared solution can be stored at 2-8°C for a month by avoiding direct sunlight.

8. Sample Preparation

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

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 520 nm

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.

10. Operation Steps

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 5s with microplate reader, stand at room temperature for 5 min and measure the OD value at 520 nm.

The measurement of samples

1. **The pre-treatment 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 iron absorbent, mix fully with a vortex mixer for 3s 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 **pre-treated 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 3s and incubate in 100°C 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 (For standard curve)

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

Operation Table (For sample)

	Control well	Sample well
Double distilled water (μL)	50	
Pre-treated sample (μL)		50
Chromogenic agent (μL)	250	250
Oscillate fully with a vortex mixer for 3s and incubate in 100°C 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.		

11. Calculations

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.

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

Or

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

$$\text{UIBC (μmol/L)} = c_3 - c_2$$

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

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0)
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 (μmol/L)
c_3: Total iron binding capacity (TIBC) (μmol/L)
i: Iron saturation (%)

12. Performance Characteristics

Detection Range	0.31-50 mg/L
Sensitivity	0.14 mg/L
Average inter-assay CV (%)	2.3
Average intra-assay CV (%)	1.5
Average recovery rate (%)	100

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:

$$\text{TIBC (mg/L)} = (0.080 - 0.038 + 0.0009) \div 0.014$$
$$= 3.06\ \text{mg/L}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assaygenie.com