TECHNICAL MANUAL

Total Iron Binding Capacity (TIBC) Colorimetric Assay Kit

- **SKU CODE:** MAES0073
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Sample pretreatment
- Add standards and samples
- Measure the OD value

2. Intended use

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

3. Detection principle

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

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	100 mg/L Iron Standard Stock Solution	2 mL x 1 vial	2 mL x 1 vial	2 mL x 5 vials	2-8 °C, 12 months
Reagent 2	Chromogenic Agent A	Powder x 1 vial	Powder x 2 vials	Powder x 10 vials	2-8 °C, 12 months, shading light

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 3	Chromogenic Agent B	Powder x 1 vial	Powder x 2 vials	Powder x 10 vials	2-8 °C, 12 months, shading light
Reagent 4	Chromogenic Agent C	15 mL x 1 vial	15 mL x 2 vials	30 mL x 5 vials	2-8 °C, 12 months
Reagent 5	Iron Absorbent	Powder x 31 vials	Powder x 79 vials	Powder x 483 vials	2-8 °C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			

5. 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

6. 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 15 mL of chromogenic agent C, mix well to dissolve. Store at 2-8°C for 1 month protected from light.
3. **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 serial concentrations. The recommended dilution gradient is as follows: 0, 5, 10, 20, 25, 30, 40, 50 mg/L.

7. Sample preparation

Sample preparation:

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Dilution of sample:

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

- Human serum: 1
- Rat serum: 1
- Porcine serum: 1
- Rabbit serum: 1
- Chicken serum: 1
- Machine serum: 1

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

8. The key points of the assay

1. After 100°C 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 iron contamination.

9. Operating steps

The measurement of standard curve:

1. Dilute 100 mg/L iron standard stock solution with double distilled water to serial concentrations. 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 concentrations 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.

The measurement of sample:

1. 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 iron absorbent, mix fully with a vortex mixer for 3 s and stand at room temperature for 5 min. Centrifuge at 3000 xg 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°C water bath for 5 min.

4. Cool the tubes with running water, then centrifuge at 10000 xg 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.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute 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. Serum (plasma) sample:

TIBC (mg/L) = (ΔA_520 - b) ÷ a × f

or

TIBC (µmol/L) = (ΔA_520 - b) ÷ a × f × c_1

UIBC(µmol/L) = $c_3 - c_2$

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

[Note]

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 (%).

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Recovery

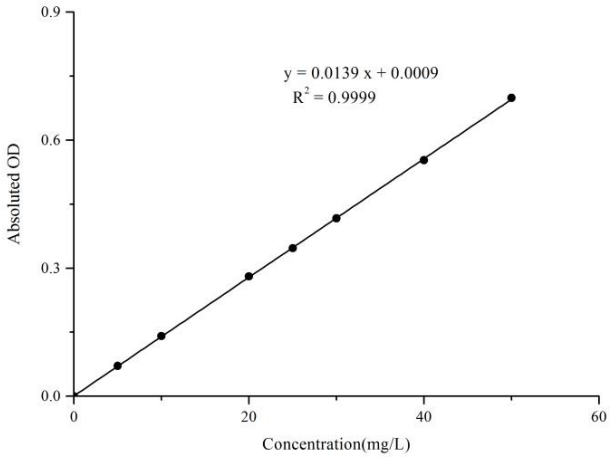
Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times in parallel to get the average recovery rate of 100%.

Sensitivity

The analytical sensitivity of the assay is 0.14 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.

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), the standard curve and data are provided as below for reference only:



12. Appendix II Example Analysis

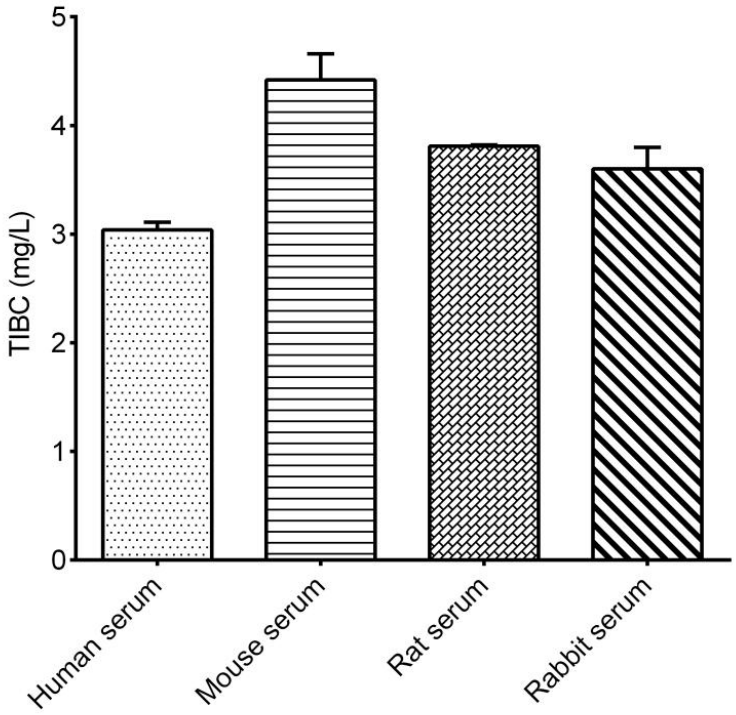
Example analysis:

Take 50 µL of human serum and carry out the assay according to the operating steps. 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

Detect human serum, mouse serum, rat serum and rabbit serum according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.