

## Serum Total Iron Binding Capacity (TIBC) Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/ Microplate reader

**Cat No:** NA0420

**Size:** 100T/96S

### Components:

Reagent I: 30 mL×1, store at 4°C.

Reagent II: 5 mL×1, store at 4°C.

Reagent III: 1 mL×1, store at 4°C.

Reagent IVA: 2.5 mL×1, store at 4°C.

Reagent IVB: 2.5 mL×1, store at 4°C. (Mix reagents accordance the ratio A:B=1:1 before use).

Reagent V: 12 mL×1, store at 4°C.

Standard: Powder×1, store at 4°C. Add 0.9 mL of distilled water before use to prepare as 40 μmol/mL FeSO<sub>4</sub>•7H<sub>2</sub>O, then dilute with distilled water to 0.5 μmol/mL.

### Description:

Total iron-binding capacity (TIBC) refers to the ability of serum transferrin to bind iron, and its content is closely related to the diseases such as iron deficiency anemia and acute hepatitis.

Fe<sup>2+</sup> reacts with ferrozine to form a fuchsia compound which has an absorption peak at 562nm. In alkaline condition, serum transferrin can bind with Fe<sup>3+</sup>, and the remaining unbound Fe<sup>3+</sup> can be reduced to Fe<sup>2+</sup>. So the absorbance A1 is positively correlated with Fe<sup>3+</sup>. After acidification, the transferrin-bound Fe<sup>3+</sup> is released and further reduced to Fe<sup>2+</sup>. The absorbance A2 has a positive correlation with Fe<sup>3+</sup>, A2 minus A1 was proportional to TIBC.

### Required but not provided:

Spectrophotometer/Microplate reader, water bath, centrifuge, micro glass cuvette/ 96 well plate, distilled water.

### Procedure:

1. Preheat spectrophotometer/microplate reader for 30 min, adjust wavelength to 562 nm, set zero with distilled water.
2. Add reagents in centrifuge tube according to the following table.

| Reagent name(μL)    | Test tube | Blank tube | Standard tube |
|---------------------|-----------|------------|---------------|
| Serum               | 40        | -          | -             |
| 0.5μmol/mL standard | -         | -          | 40            |
| Distilled water     | --        | 40         | 40            |

|  |     |     |     |
|--|-----|-----|-----|
| Reagent I  | 280 | 280 | 280 |
| Reagent II   | 40  | -   | -   |
| Reagent III  | -   | 40  | 40  |
| Mix thoroughly, incubate at 37°C for 10 min.   |     |     |     |
| Reagent IV   | 40  | 40  | 40  |
| Mix thoroughly, incubate at 37°C for 5mins, set zero with distilled water, detect the absorbance of A1 at 562 nm, then add Reagent IV immediately after detecting. |     |     |     |
| Reagent V  | 120 | 120 | 120 |
| Mix thoroughly, incubate at 37°C for 5mins, set zero with distilled water, detect the absorbance of A2 at 562nm.   |     |     |     |

### Calculation

Definition: Per liter of serum combining the  $\mu\text{mol}$  amount of  $\text{Fe}^{3+}$  at 37 °C.

$$\text{TIBC}(\mu\text{mol/L}) = [C_S \times (A_{2T} - A_{2B}) / (A_{2S} - A_{2B}) \times V_{SA} - C_S \times (A_{1T} - A_{1B}) / (A_{1S} - A_{1B}) \times V_{SA}] \div V_{SA}$$

$$= [500 \times (A_{2T} - A_{2B}) / (A_{2S} - A_{2B}) - 500 \times (A_{1T} - A_{1B}) / (A_{1S} - A_{1B})]$$

$C_S$ : The concentration of standard, 0.5  $\mu\text{mol/mL}$ ;

$V_{SA}$ : The volume of added serum, 0.04 mL =  $40 \times 10^{-6}$  L.

### Note:

1. If OD > 0.1, test after diluting, multiply the dilution multiple in equation.
2. Reagent II and Reagent IV is poisonous, please take precautions when operating.

### Experimental example:

1. Take 40  $\mu\text{L}$  of camel serum diluted twice with distilled water, and operate according to the determination steps. Calculate  $\Delta A_{1T} = A_{1T} - A_{1B} = 0.342$ ,  $\Delta A_{1S} = A_{1S} - A_{1B} = 0.746$ ,  $\Delta A_{2T} = A_{2T} - A_{2B} = 0.735$ ,  $\Delta A_{2S} = A_{2S} - A_{2B} = 0.550$ .

Total iron binding capacity TIBC ( $\mu\text{mol/L}$ ) =  $500 \times (\Delta A_{2T} \div \Delta A_{2S} - \Delta A_{1T} \div \Delta A_{1S}) \times 2 = 877.919 \mu\text{mol/L}$ .

2. Take 40  $\mu\text{L}$  of goose serum diluted 2 times with distilled water, operate according to the determination steps, and calculate  $\Delta A_{1T} = A_{1T} - A_{1B} = 0.191$ ,  $\Delta A_{1S} = A_{1S} - A_{1B} = 0.746$ ,  $\Delta A_{2T} = A_{2T} - A_{2B} = 0.732$ ,  $\Delta A_{2S} = A_{2S} - A_{2B} = 0.550$

Total iron binding capacity TIBC ( $\mu\text{mol/L}$ ) =  $500 \times (\Delta A_{2T} \div \Delta A_{2S} - \Delta A_{1T} \div \Delta A_{1S}) \times 2 = 1074.877 \mu\text{mol/L}$ .

### Related Products:

NA0668/NA0427 Blood Magnesium Content Assay Kit

NA0737/NA0426 Blood Phosphate Content Assay Kit

NA0667/NA0425 Blood Sodium Content Assay Kit

NA0736/NA0494 Serum Ferri Ion Content Assay Kit

### Technical Specifications:

Minimum detection limit: the detection limit of the first measurement is 0.00098  $\mu\text{mol/mL}$ ; the detection limit of the second measurement is 0.0012  $\mu\text{mol/mL}$ .

Linear range: the linear range of the first measurement is  $1.95 \times 10^{-3}$ - $0.5 \mu\text{mol/mL}$ ; the linear range of the second measurement is  $1.95 \times 10^{-3}$ - $0.5 \mu\text{mol/mL}$ .