

Serum Total Iron Binding Capacity (TIBC) Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0374

Size: 50T/48S

Components:

Reagent I: 50 mL×1, store at 4°C .

Reagent II: 5 mL×1, store at 4°C .

Reagent III: 1.5 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: 15 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 FeSO₄•7H₂O, then dilute with distilled water to 0.25 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.

Fe²⁺ reacts with ferrozine to form a fuchsia compound which has an absorption peak at 562 nm. In alkaline condition, serum transferrin can bind with Fe³⁺, and the remaining unbound Fe³⁺ can be reduced to Fe²⁺. So the absorbance A1 is positively correlated with Fe³⁺. After acidification, the transferrin-bound Fe³⁺ is released and further reduced to Fe²⁺. The absorbance A2 has a positive correlation with Fe³⁺, A2 minus A1 was proportional to TIBC.

Required but not provided:

Spectrophotometer, water bath, centrifuge, 1mL glass cuvette, distilled water.

Procedure:

1. Preheat spectrophotometer for 30min, 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	100	-	-
0.25 μmol/mL standard	-	-	100
Distilled water	-	100	-
Reagent I	700	700	700

Reagent II	100	-	-
Reagent III	-	100	100
Mix thoroughly, incubate at 37°C for 10min.			
Reagent IV	100	100	100
Mix thoroughly, incubate at 37°C for 5min, detect the absorbance of A1 at 562nm, then add Reagent IV immediately after detecting.			
Reagent V	300	300	300
Mix thoroughly, incubate at 37°C for 5min, set zero with distilled water, detect the absorbance of A2 at 562nm.			

Calculation

Definition: Per liter of serum combining the μmol amount of Fe^{3+} at 37 °C.

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

$$= [250 \times (A_{2T} - A_{2B}) / (A_{2S} - A_{2B}) - 250 \times (A_{1T} - A_{1B}) / (A_{1S} - A_{1B})]$$

C_S : The concentration of standard, $0.25 \mu\text{mol/mL} = 250 \mu\text{mol/L}$;

V_{SA} : The volume of added serum, $0.1 \text{ mL} = 100 \times 10^{-6} \text{ L}$.

Note:

1. If $\text{OD} > 0.1$, 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 100 μl of camel serum diluted four with distilled water, and operate according to the determination steps. Calculate $\Delta A_{1T} = A_{1T} - A_{1B} = 0.356$, $\Delta A_{1S} = A_{1S} - A_{1B} = 0.669$, $\Delta A_{2T} = A_{2T} - A_{2B} = 0.819$, $\Delta A_{2S} = A_{2S} - A_{2B} = 0.519$.

Total iron binding capacity TIBC ($\mu\text{mol/L}$) = $250 \times (\Delta A_{2T} \div \Delta A_{2S} - \Delta A_{1T} \div \Delta A_{1S}) \times 4 = 1045.897 \mu\text{mol/L}$.

2. Take 100 μL of goose serum diluted 8 times with distilled water, operate according to the determination steps, and calculate $\Delta A_{1T} = A_{1T} - A_{1B} = 0.588$, $\Delta A_{1S} = A_{1S} - A_{1B} = 0.669$, $\Delta A_{2T} = A_{2T} - A_{2B} = 0.797$, $\Delta A_{2S} = A_{2S} - A_{2B} = 0.519$.

Total iron binding capacity TIBC ($\mu\text{mol/L}$) = $250 \times (\Delta A_{2T} \div \Delta A_{2S} - \Delta A_{1T} \div \Delta A_{1S}) \times 8 = 1313.443 \mu\text{mol/L}$.

Related Products:

AK0380/AK0379	Blood Magnesium Content Assay Kit
AK0272/AK0265	Blood Phosphate Content Assay Kit
AK0181/AK0180	Blood Sodium Content Assay Kit
AK0415/AK0414	Serum Ferri Ion Content Assay Kit

Technical Specifications:

Minimum detection limit: the detection limit of the first measurement is 0.0002 $\mu\text{mol/mL}$; the detection limit of the second measurement is 0.0017 $\mu\text{mol/mL}$.

Linear range: the linear range of the first measurement is 0.00195-0.5 $\mu\text{mol/mL}$; the linear range of the second measurement is 0.00195-0.5 $\mu\text{mol/mL}$.