Serum Total Iron Binding Capacity (TIBC) Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Cat No: BC2860

Size: 50T/48S

Components:

Reagent I: Liquid 50 mL×1, store at 2-8℃.

Reagent II: Liquid 5 mL×1, store at 2-8℃.

Reagent III: Liquid 1 mL×1, store at 2-8℃.

Reagent IVA: Liquid 2.5 mL×1, store at 2-8℃.

Reagent IVB: Liquid 2.5 mL×1, store at 2-8℃. Mix reagents accordance the ratio A:B=1:1 before use. Reagents are only stored on the same day.

Reagent V: Liquid 15 mL×1, store at 2-8℃.

Standard: Powder×1, store at 2-8℃. Add 0.9 mL of distilled water before use to prepare as 40 μmol/mL $FeSO_4$ standard solution, the unused reagent can be stored at 2-8℃ for 8 weeks.

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

Required but not provided:

Spectrophotometer, water bath/constant temperature foster box, centrifuge, 1mL glass cuvette, distilled water.

Procedure:

1. Dilution of standard solution: take 10μL40μmol/ml $FeSO_4$ standard solution, add 1590μL distilled water, fully mixed, this is the concentration of 0.25μmol/ml standard solution. (In the experiment, each tube needs 100 μL. In order to reduce the experimental error, a large volume is prepared.)
2. Preheat spectrophotometer for 30min, adjust wavelength to 562 nm, set zero with distilled water.
3. Preheat reagent I at 37 ℃ for 10min.
4. Add reagents in centrifuge tube according to the following table.

Reagent (μL)	Test tube	Blank tube	Standard tube
Serum	100	-	-

Standard solution	-	-	100
Distilled water	-	100	-
Reagent I	700	700	700
Reagent II	100	-	-
Reagent III	-	100	100
Mix thoroughly, incubate at 37℃ for 10min.			
Reagent IV	100	100	100
Mix thoroughly, incubate at 37℃ for 5min, detect the absorbance of A_{1T}、A_{1B}、A_{1S} at 562nm and calculate $\Delta A1_T = A1_T - A1_B$, $\Delta A1_S = A1_S - A1_B$. After the measurement, pour the reaction solution back to the corresponding tube and add reagent V.			
Reagent V	300	300	300
Mix thoroughly, incubate at 37℃ for 5min, detect the absorbance of A_{2T}、A_{2B}、A_{2S} at 562nm and calculate $\Delta A2_T = A2_T - A2_B$, $\Delta A2_S = A2_S - A2_B$. Standard tube and blank tube only need to be measured 1-2 times.			

Calculation

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

$TIBC(\mu mol/L) = C_S \times \Delta A2_T \div \Delta A2_S - C_S \times \Delta A1_T \div \Delta A1_S$

$= 250 \times (\Delta A2_T \div \Delta A2_S - \Delta A1_T \div \Delta A1_S)$

C_S: The concentration of standard, 0.25μmol/mL=250μmol/L.

Note:

1. If $A1_T < 0.1$, 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 times with distilled water and operate according to the determination steps. Calculate $\Delta A1_T = A1_T - A1_B = 0.356$, $\Delta A1_S = A1_S - A1_B = 0.669$, $\Delta A2_T = A2_T - A2_B = 0.819$, $\Delta A2_S = A2_S - A2_B = 0.519$.

   $TIBC\ (\mu mol/L) = 250 \times (\Delta A2_T \div \Delta A2_S - \Delta A1_T \div \triangle A1_S) \times 4 = 1045.897\ \mu mol/L$.
2. Take 100 μL of goose serum diluted 8 times with distilled water and operate according to the determination steps. Calculate $\Delta A1_T = A1_T - A1_B = 0.588$, $\Delta A1_S = A1_S - A1_B = 0.669$, $\Delta A2_T = A2_T - A2_B = 0.797$, $\Delta A2_S = A2_S - A2_B = 0.519$.

   $TIBC\ (\mu mol/L) = 250 \times (\Delta A2_T \div \triangle A2_S - \Delta A1_T \div \triangle A1_S) \times 8 = 1313.443\ \mu mol/L$.

Related Products:

BC2790/BC2795 Blood Magnesium Content Assay Kit

BC1650/BC1655 Blood Phosphate Content Assay Kit

BC2800/BC2805 Blood Sodium Content Assay Kit
BC1730/BC1735 Serum Ferri Ion Content Assay Kit

Technical Specifications:

Minimum detection limit: the detection limit of the first measurement is 0.0002 μmol/mL; the detection limit of the second measurement is 0.0017 μmol/mL.

Linear range: the linear range of the first measurement is 0.00195-0.5 μmol/mL; the linear range of the second measurement is 0.00195-0.5 μmol/mL.