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/ Microplate reader

Cat No: BC2865

Size: 100T/96S

Components:

Reagent I: Liquid 30 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 12 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/microplate reader, water bath/ constant temperature foster box, centrifuge, micro glass cuvette/ 96 well plate, distilled water.

Procedure:

1. Dilution of standard solution: take 20μL 40μmol/ml $FeSO_4$ standard solution, add 1580μL distilled water, fully mixed, this is the concentration of 0.5μmol/ml standard solution. (In the experiment, each tube needs 40 μL. In order to reduce the experimental error, a large volume is prepared.)
2. Preheat spectrophotometer/microplate reader for more than 30min, adjust the wavelength to 562nm and set spectrophotometer counter to zero with distilled water.
3. Preheat reagent I at 37℃ for 10min.
4. 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	-
Reagent I	280	280	280
Reagent II	40	-	-
Reagent III	-	40	40
Mix thoroughly, incubate at 37℃ for 10 min.			
Reagent IV	40	40	40
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	120	120	120
Mix thoroughly, incubate at 37℃ for 5min, detect the absorbance of A_{2T}、A_{2B}、A_{2S} at 562nm and calculate $\Delta A_{2T} = A_{2T} - A_{2B}$, $\Delta A_{2S} = A_{2S} - A_{2B}$. 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.

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

$= 500 \times (\Delta A2_T \div \Delta A2_S - \Delta A1_T \div \Delta A1_S)$

C_S：The concentration of standard, 0.5 μmol/mL= 500 μmol/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 40 μl of camel serum diluted twice with distilled water and operate according to the determination steps. Calculate $\Delta A1_T = A1_T - A1_B = 0.342$, $\Delta A1_S = A1_S - A1_B = 0.746$, $\Delta A2_T = A2_T - A2_B = 0.735$, $\Delta A2_S = A2_S - A2_B = 0.550$.

   $TIBC\ (\mu mol/L) = 500 \times (\Delta A2_T \div \Delta A2_S - \Delta A1_T \div \triangle A1_S) \times 2 = 877.919\ \mu mol/L$.
2. Take 40 μL of goose serum diluted twice with distilled water and operate according to the determination steps, and calculate $\Delta A1_T = A1_T - A1_B = 0.191$, $\Delta A1_S = A1_S - A1_B = 0.746$, $\Delta A2_T = A2_T - A2_B = 0.732$, $\Delta A2_S = A2_S - A2_B = 0.550$.

   $TIBC\ (\mu mol/L) = 500 \times (\Delta A2_T \div \triangle A2_S - \Delta A1_T \div \triangle A1_S) \times 2 = 1074.877\ \mu mol/L$.

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 0.00098 μmol/mL; the detection limit of the second measurement is 0.0012 μmol/mL.

Linear range: the linear range of the first measurement is 1.95×10^{-3}-0.5 μmol/mL; the linear range of the second measurement is 1.95×10^{-3}-0.5 μmol/mL.