Serum Total Iron Binding Capacity (TIBC) Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: NA0661

Size: 50T/48S

Components:

Reagent I: 50 mL×1, store at 4℃.

Reagent II: 5 mL×1, store at 4℃.

Reagent III: 1.5 mL×1, store at 4℃.

Reagent IVA: 2.5 mL×1, store at 4℃.

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

Reagent V: 15 mL×1, store at 4℃.

Standard: Powder×1, store at 4℃. Add 0.9 mL of distilled water before use to prepare as 40 μmol/mL $FeSO_4$•$7H_2O$, 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.

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, 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℃ for 10min.			
Reagent IV	100	100	100
Mix thoroughly, incubate at 37℃ 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℃ 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.

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

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

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

V_{SA}：The volume of added serum,0.1 mL=100×10^{-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 100 μl of camel serum diluted four with distilled water, and operate according to the determination steps. Calculate $\Delta A1_T = A1_T - A1_B = 0.356$, $\Delta A1_S = A1_S - A1_B = 0.669$, $\Delta A2_T = A2_T - A2_B = 0.819$, $\Delta A2_S = A2_S - A2_B = 0.519$.

Total iron binding capacity TIBC (μmol/L) = $250\times(\Delta A2_T \div \Delta A2_S - \Delta A1_T \div \Delta A1_S)\times4 = 1045.897$ μmol/L.

2. Take 100 μL of goose serum diluted 8 times with distilled water, operate according to the determination steps, and calculate $\Delta A1_T = A1_T - A1_B = 0.588$, $\Delta A1_S = A1_S - A1_B = 0.669$, $\Delta A2_T = A2_T - A2_B = 0.797$, $\Delta A2_S = A2_S - A2_B = 0.519$.

Total iron binding capacity TIBC (μmol/L) = $250 \times (\Delta A2_T \div \Delta A2_S - \Delta A1_T \div \Delta A1_S)\times8 = 1313.443$ μmol/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 0.0002 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.