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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: AK0373

Size: 100T/96S

Components:

Reagent I: 30 mL×1, store at 4℃.

Reagent II: 5 mL×1, store at 4℃.

Reagent Ⅲ: 1 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: 12 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 $FeSO_4 \cdot 7H_2O$, 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^{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, 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℃ for 10 min			
Reagent IV	40	40	40
Mix thoroughly, incubate at 37℃ 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℃ for 5mins, 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.

$$TIBC(\mu mol/L) = [C_S \times (A_{2T}-A_{2B})/(A_{2S}-A_{2B}) \times V_{SA} - C_S \times (A_{1T}-A_{1B})/(A_{1S}-A_{1B}) \times V_{SA}] \div V_{SA}$$
$$= [500 \times (A_{2T}-A_{2B})/(A_{2S}-A_{2B}) - 500 \times (A_{1T}-A_{1B})/(A_{1S}-A_{1B})]$$

C_S： The concentration of standard, 0.5 μmol/mL；

V_{SA}： The volume of added serum, 0.04 mL=40×10^{-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 μl of camel serum diluted twice with distilled water, and operate according to the determination steps. Calculate $\Delta A1_T = A1_T - A1_B = 0.342$, $\Delta A1_S = A1_S - A1_B = 0.746$, $\Delta A2_T = A2_T - A2_B = 0.735$, $\Delta A2_S = A2_S - A2_B = 0.550$.

Total iron binding capacity TIBC (μmol/L) = $500\times(\Delta A2_T \div \Delta A2_S - \Delta A1_T \div \Delta A1_S)\times2$ = 877.919 μmol/L.

2. Take 40 μL of goose serum diluted 2 times with distilled water, operate according to the determination steps, and calculate $\Delta A1_T = A1_T - A1_B = 0.191$, $\Delta A1_S = A1_S - A1_B = 0.746$, $\Delta A2_T = A2_T - A2_B = 0.732$, $\Delta A2_S = A2_S - A2_B = 0.550$

Total iron binding capacity TIBC (μmol/L) = $500 \times (\Delta A2_T \div \Delta A2_S - \Delta A1_T \div \Delta A1_S)\times2$ = 1074.877 μmol/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

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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.