Serum copper ion Content Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: BC5640

Size: 50T/48S

Components:

Reagent I: Liquid 45 mL×1, store at 2-8℃. If any reagent precipitates, it can be dissolved in a water bath at 37℃.

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

Standard: Liquid 1 mL×1, 10mmol/L (10000 μmol/L) copper sulfate standard solution.

Description:

Copper is one of the essential trace elements of human body, is an important component of many enzymes, it can combine with proteins to form cupric protein, has the function of protecting cells; Most of the copper in plasma binds to globulins to form ceruloplasmin, which plays an important role in the production of red blood cells. Therefore, the determination of serum copper can tell whether the body is deficient in copper.

Under acidic conditions, Cu^{2+} is dissociated from ceruloplasmin and albumin and reacts with complexing agent 3, 5-dibromo-PAESA to produce a purple complex, which has a characteristic absorption peak at 580nm, and the absorbance is proportional to the concentration within a certain range, thus calculating the Cu^{2+} concentration.

Required but not provided:

Spectrophotometer, cryogenic centrifuge, water bath/constant temperature incubator, adjustable pipette, 1mL glass cuvettes, mortar/homogenizer, ice and distilled water

Operation procedure:

I. Extraction of citric acid from samples

1. Plasma/serum: Direct measurement. If there is turbidity, centrifuge, take the supernatant and put it on the ice to be measured. (Note: EDTA cannot be used as an anticoagulant in plasma samples, heparin is recommended as an anticoagulant).

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 580 nm and set zero with distilled water.
2. Preparation of 80μmol/L standard solution: Take 100μL of 10mmol/L standard solution and add 400 μL of distilled water to mix, that is, 2000μmol/L standard product; Then take 40μL 2000μmol/L standard

product and 960μL distilled water to mix, that is, to prepare 80μmol/L standard solution.

3. Preheat the Reagent I in water bath at 37℃ for more than 10 minutes.
4. Add the corresponding reagent into the 1.5 mL EP tube according to the following table.

Reagent name (μL)	Black tube (B)	Test tube (T)	Standard tube (S)
Distilled water	50	-	-
Sample	-	50	-
Standard	-	-	50
Reagent I	750	750	750
Reagent II	250	250	250
After fully mixing, leave it for 5 minutes at 37℃, measure the absorbance at 580 nm, and record it as A_B, A_T, A_S. Calculate $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$, Blank tube and standard tube only need to measure 1-2 times.			

III. Calculation:

The content of Serum copper ion (μmol/L) = $\Delta A_T \div (\Delta A_S \div C_S) = 80 \times \Delta A_T \div \Delta A_S$

C_S: Standard concentration, 80 μmol/L;

Note:

1. Test the absorbance immediately after incubation at 37℃ for 5min. If the number of samples is too large, test them in batches and try to ensure that the determination is completed within 20min.
2. If the measured light absorption value of the sample is greater than 0.5, it is recommended to dilute the sample with distilled water for determination, and pay attention to the simultaneous modification of the calculation formula.
3. If the measured absorption value of the sample is less than 0.005 or close to the absorption value of the blank tube, the sample size can be appropriately increased, and the blank tube and standard tube also need to be adjusted accordingly.

Experimental example:

1. Take 50μL Horse serum and then operate according to the determination steps. Use 1mL glass cuvettes to measure and calculate $\Delta A_T = A_T - A_B = 0.125-0.071=0.054$, $\Delta A_S = A_S - A_B = 0.384-0.071=0.313$. Calculated:

The content of Serum copper ion (μmol/L) = $80 \times \Delta A_T \div \Delta A_S = 13.802$ μmol/L.

2. Take 50μL Human serum and then operate according to the determination steps. Use 1mL glass cuvettes to measure and calculate $\Delta A_T = A_T - A_B = 0.169-0.071=0.098$, $\Delta A_S = A_S - A_B = 0.384-0.071=0.313$. Calculated:

The content of Serum copper ion (μmol/L) = $80 \times \Delta A_T \div \Delta A_S = 25.048$ μmol/L.

Related Products:

BC1730/BC1735 Serum Ferri Ion Content Assay Kit
BC5410/BC5415 Ferrous ion Content Assay Kit