Cell copper Content Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: BC5750

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.

Preparation of 20nmol/mL standard product: Dilute 10000nmol/mL standard solution into 200nmol/mL standard solution with distilled water first, and then dilute 200nmol/mL standard solution into 20nmol/mL standard solution for use. The specific dilution can be referred to as follows: Take 20μL of 10000nmol/mL of standard liquid and add 980μL of distilled water to mix, that is, 200nmol/mL of standard product; Then draw 100μL 200nmol/mL standard liquid and add 900μL distilled water to mix, that is, 20nmol/mL standard product.

Description:

Copper (Cu) is one of the essential trace elements of human body, and is also an important part of protein and enzyme. It can exist inside and outside the red blood cells, and its main function is to assist hematopoietic, that is, to catalyze the synthesis of hemoglobin. Copper can properly promote the development of the human bone, promote the development of the human nervous system and the brain, and maintain the normal growth and development of infants and young children, therefore, the determination of copper ion content in the tissue can know 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 in 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. Cells: The cells were collected into the centrifuge tube, and the supernatant was discarded after centrifugation. According to the number of bacteria or cells (10^6): the volume of distilled water (mL) is

5-10:0.4 to prepare (it is recommended that add 0.4 mL of distilled water to 500 million of cells). Bacteria/cells is split by ultrasonication (power 200W, ultrasonic 3s, interval 7s, total time 3 min).

Centrifuge at 10000 g and 4℃ for 10 minutes. Take the supernatant on ice for test.

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 580 nm and set zero with distilled water.
2. Preheat the Reagent I in water bath at 37℃ for more than 10 minutes.
3. 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	250	-	-
Sample	-	250	-
Standard	-	-	250
Reagent I	550	550	550
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:

1. Calculated according to the number of cells:

   The content of copper (nmol/10^6 cell) = $\Delta A_T \div (\Delta A_S \div C_S) \times V_T \div N = 8 \times \Delta A_T \div \Delta A_S \div N$

2. Calculated according to the content of mitochondrial protein:

   The content of copper (nmol/g prot) = $\Delta A_T \div (\Delta A_S \div C_S) \times V_T \div (Cpr \times V_T) = 20 \times \Delta A_T \div \Delta A_S \div Cpr$

   C_S: Standard concentration, 20 μmol/L;

   V_T: Reagent I volume, 0.4mL;

   N: The total number of cells, millions;

   Cpr: Protein concentration of supernatant, mg/mL.

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. Collect about 5 million SHSY5Y cells and add 0.4mL of distilled water, Ultrasonic crushing (power 200w, ultrasonic 3s, interval 7s, total 3min), 10000g, centrifugation at 4℃ for 10min. Remove the superclear ice to be measured. Then operate according to the determination steps. Use 1mL glass cuvettes to measure and calculate $\Delta A_T = A_T - A_B = 0.187 - 0.071 = 0.116$, $\Delta A_S = A_S - A_B = 0.441 - 0.071 = 0.370$. Calculated according to the number of cells:

The content of copper (nmol/10^6 cell) $= 8 \times \Delta A_T \div \Delta A_S \div N = 0.5016$ nmol/10^6 cell.

2. Collect about 5 million U937 cells and add 0.4mL of distilled water, Ultrasonic crushing (power 200w, ultrasonic 3s, interval 7s, total 3min), 10000g, centrifugation at 4℃ for 10min. Remove the superclear ice to be measured. Then operate according to the determination steps. Use 1mL glass cuvettes to measure and calculate $\Delta A_T = A_T - A_B = 0.129 - 0.071 = 0.058$, $\Delta A_S = A_S - A_B = 0.441 - 0.071 = 0.370$. Calculated according to the number of cells:

The content of copper (nmol/10^6 cell) $= 8 \times \Delta A_T \div \Delta A_S \div N = 0.2508$ nmol/10^6 cell.

Related Products:

BC1730/BC1735	Serum Ferri Ion Content Assay Kit
BC5410/BC5415	Ferrous ion Content Assay Kit
BC5630/BC5635	Tissue copper Content Assay Kit
BC5640/BC5645	Serum copper ion Content Assay Kit