Albumin Content Assay Kit (Bromocresol Green Colorimetry)

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

Operation Equipment: Microplate Reader/ Spectrophotometer

Catalog Number: BC5835

Size:100T/96S

Components:

Extract Solution:110 mL×1, stored at 2-8℃.

Reagent I: 0.28 mL×1, stored at 2-8℃.

Reagent II: 25mL×1, stored at 2-8℃.

Reagent III: 0.3mL×1, stored at 2-8℃.

Standard: 1 mL×1, 10 mg/mL albumin standard solution, stored at -20℃. Before use, 200 μL 10 mg/mL albumin standard solution is taken and 200μL Extract Solution is added to prepare 5 mg/mL albumin standard solution.

Preparation of Color developing solution: According to the number of samples before use, the color developing solution is prepared according to the ratio of reagent I : reagent II : reagent III = 10μL : 990μL : 10μL (1010μL, 5T).

Product Description

Albumin is the most important protein in human plasma, synthesized by the liver. It is an important nutrient in the body that maintains plasma osmolality and can be combined with a variety of nutrients, hormones and drugs. Albumin content can reflect the nutritional status of the organism, and can also detect diseases that affect the metabolic function of the liver, such as cirrhosis, liver injury, malnutrition, malignant tumors and so on.

Serum albumin is positively charged in pH 4.2 buffer, and in the presence of non-ionic surfactants, it can combine with the negatively charged dye bromocresol green to form a blue-green complex, with an absorption peak at a wavelength of 630 nm, and its color depth is positively proportional to the concentration of albumin.

Reagents and Equipment Required but Not Provided.

Centrifuge, microplate reader/ spectrophotometer, bowl/homogenizer/cell ultrasonic breaker, micro glass cuvette/ 96-well plate, ice and distilled water.

Procedure

I. Extraction of soluble protein in the sample:

1.Bacteria or cells: collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. According to bacteria or cells (10^6): Extract solution (mL) is 5~10:1 to extract. It is suggested to add 1 mL of Extract solution to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 200W, working time 3 seconds, interval 7 seconds,

repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

2.Tissue: according to tissue weight (g): Extract solution (mL) is 1:5~10 to extract. Add 1 mL of Extract solution to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 8000×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

3. Liquid samples: direct determination. If the liquid is turbid, centrifuge and take the supernatant for determination.

II. Measurement steps

a. Preheat the microplate reader/ spectrophotometer for 30 minutes, adjust the wavelength to 630 nm and spectrophotometer adjust zero with distilled water.

b. Operation table：（Add the following reagents to micro glass cuvette/ 96-well plate）

Reagent Name（μL）	Test Tube (A_T)	Standard Tube (A_S)	Blank Tube (A_B)
Sample	20	-	-
Standard Solution	-	20	-
Extract Solution	-	-	20
Color developing solution	200	200	200

Mix well, react at room temperature for 20s, and measure the absorbance of each tube at 630 nm, which are recorded as A_T, A_S and A_B, respectively. 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.

Note: The length of reacting time will affect the test results. It is recommended to react directly in micro glass cuvette/ 96-well plate for 20s to determine the absorbance value.

III. Calculation of Albumin Content:

1. Calculated according to Protein concentration:

Albumin Content（mg/mg prot）$= \Delta A_T \times (C_S \div \Delta A_S) \times V_S \div (V_S \times Cpr) = 5 \times \Delta A_T \div \Delta A_S \div Cpr$

2. Calculated according to the weight of the sample:

Albumin Content（mg/mg prot）$= \Delta A_T \times (C_S \div \Delta A_S) \times V_S \div (W \times V_S \div V_E) = 5 \times \Delta A_T \div \Delta A_S \div W$

3. Calculated according to liquid volume:

Albumin Content（mg/mg prot）$= \Delta A_T \times (C_S \div \Delta A_S) \times V_S \div V_S = 5 \times \Delta A_T \div \Delta A_S$

4. Calculated according to cell count:

Albumin Content（mg/mg prot）$= \Delta A_T \times (C_S \div \Delta A_S) \times V_S \div (N \times V_S \div V_E) = 5 \times \Delta A_T \div \Delta A_S \div N$

C_S：Concentration of standard solution，5mg/mL；

V_S：Sample volume，0.02mL；

V_E：Extract solution volume，1mL；

Cpr：Sample protein concentration，mg/mL；

W：Sample weight，g；

N：Total number of bacteria / cells，as 10^6 a unit。

Notes：

1. If the $\Delta A_T < 0.010$ or A_T is close to the blank tube, the sample size can be increased before the determination ; if the determination of $\Delta A_T > 0.5$, it is recommended that the sample supernatant be diluted with the extract before determination. Note the simultaneous modification of the calculation formula.
2. If the sample is turbid after adding the chromogenic agent, it is recommended that the sample supernatant be diluted with the extract and then determined. Note the simultaneous modification of the calculation formula.

Experimental example:

1. Take 20 μL of human serum sample, dilute 10 times with the extraction solution, follow the assay procedure, and measure with a 96-well plate. Calculation: $\Delta A_T = A_T - A_B = 0.426-0.124=0.302$, $\Delta A_S = A_S - A_B = 0.406-0.124=0.282$, by volume of liquid:
   Albumin content (mg/mL) $=5\times\Delta A_T \div \Delta A_S \times 10$（dilution factor） $=53.546$mg/mL。

References：

[1] Tada S, Yasukawa K, Yatomi Y, et al. A simple colorimetric assay to determine the concentration and proportion of human mercaptalbumin[J]. Practical Laboratory Medicine, 2022, 31: e00281.
[2] Muramoto Y , Matsushita M , Irino T .Reduction of reaction differences between human mercaptalbumin and human nonmercaptalbumin measured by the bromcresol purple method[J].Clinica Chimica Acta, 1999, 289(1-2):69-78.

Related Products:

BC1270/BC1275	Protein Carbonyl Content Assay Kit
BC3180/BC3185	Protein Content Assay Kit (Biuret Method)