Protein Content Assay Kit (Biuret Method)

Note: Take two or three different samples for prediction before test.
Operation Equipment: Spectrophotometer/microplate reader
Catalog Number: NA0398
Size:100T/96S

Components:
Extract Solution: Self provided. The enzyme extraction buffer or distilled water or normal saline is selected according to the needs.
Reagent 1: 20 mL×1, stored at 4℃.
Standard: 1 mL×1, 5mg/mL, stored at -20℃.

Product Description
The soluble protein content of the sample is often used to calculate the enzyme activity. In addition, soluble protein content is also used for quality analysis of food.
In strong alkaline solution, biuret forms purple complex with $CuSO_4$, the color of purple complex is directly proportional to protein concentration, but not related to protein molecular weight and amino acid composition, so it can be used to determine protein content. This method can be applied to the samples with high protein concentration, especially animal materials.

Reagents and Equipment Required but Not Provided.
Spectrophotometer/microplate reader, transferpettor, micro glass cuvette/96 well flat-bottom plate and distilled water.

Procedure
I. Extraction of soluble protein in the sample:
a. Liquid sample:
Clear and colorless liquid sample can be determined directly.
b. Tissue sample:
The proportion of tissue mass (g): volume of extract solution (mL): 1:5~10 (it is recommended to weigh about 0.1 g of tissue, add 1 mL of extract solution (self-prepared, select enzyme extraction buffer or distilled water or physiological salt water as required)) ice bath homogenate. Centrifugate at 10000 rpm for 10 minutes at 4℃, take the supernatant and place it on ice for testing (animal samples often need to be diluted).
c. Bacteria and fungi:
The number of cells (10^4): the volume of the extract solution(mL) is 500~1000:1 (1 mL of the extract solution is recommended to be added to 5 million cells), and the cells are broken by ultrasonic wave in ice bath (Power: 300W, ultrasonic: 3s, interval: 7s, total time: 3 minutes). Centrifugate at 8000 rpm for 10

minutes at 4℃, take the supernatant and place it on the ice for testing.

II. Measurement steps

a. Preheat the spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 540 nm and adjust zero with distilled water.

b. Blank tube: Take a 0.5 mL EP tube, add 40 μL of distilled water, 200 μL of Reagent I, mix well and leave it at room temperature for 15 minutes, take 200 μL into micro glass cuvette/96 well plate, colorimetry at 540 nm, record as A1 blank tube.

c. Standard tube: Take a 0.5 mL EP tube, add 40 μL of standard solution, 200 μL of Reagent I, mix well and leave it at room temperature for 15 minutes, take 200 μL into micro glass cuvette/96 well plate, colorimetry at 540 nm, record as A2 standard tube.

d. Measuring tube: Take a 0.5 mL EP tube, add 40 μL of solution to be measured, 200 μL of Reagent I, mix well and leave it at room temperature for 15 minutes, take 200 μL into micro glass cuvette/96 well plate, colorimetry at 540 nm, record as A3 measuring tube.

III. Calculation of Betaine Content:

1. Calculated according to liquid volume:

Protein (mg/mL $=C_S \div (A_S-A_B) \times (A_D-A_B) = 5 \div (A_S-A_B) \times (A_T-A_B)$

2. Calculated according to the fresh weight of the sample:

Protein (mg/g fresh weight): $C_S \div (A_S-A_B) \times (A_T-A_B) \times V_{ST} \div W = 5 \div (A_S-A_B) \times (A_T-A_B) \div W$

3. Calculated according to cell count:

Protein (mg/10^4 cell) $=C_S \div (A_S-A_B) \times (A_T-A_B) \times V_{ST} \div 500 = 0.01 \div (A_S-A_B) \times (A_T-A_B)$

C_S: 5 mg/mL;

V_{ST}: Total volume of sample, 1 mL;

W: Fresh weight of sample, g;

500: Total number of cells, 5 million;

Note:

1. The protein concentration of the sample must be in the range of 1-10 mg/mL. if it is lower than 1 mg/mL, this method cannot be used. If it is higher than 10 mg/mL, corresponding dilution must be done. Therefore, 1-2 samples are used for pre-test before determination to ensure that the protein concentration is in the range of 1-10 mg/mL.

2. The protein of the sample to be tested can be extracted with normal saline, distilled water or PBS without protein. This method is interfered by ammonium sulphate and Tris buffer, and these substances should not be contained in the extract; otherwise, BCA protein content determination kit is used instead