ISO 13485:2016 ISO 9001:2015

Ver.240816

Protein Estimation Kit

PC0010

Spectrophotometer (50 Tests)

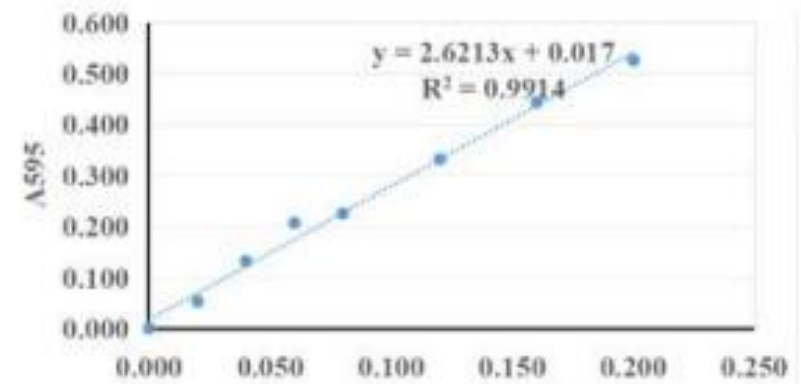
Microplate reading method (500 Tests)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description:

Coomassie brilliant blue G-250 dye combined with protein in acidic solution, so that the maximum absorption peak position (Imax) of the dye changed from 465 nm to 595 nm. In a certain concentration range, the measured absorbance value A_{595} was proportional to the protein concentration. The determination of protein concentration by Bradford method is not affected by the chemical substances in most samples. The concentration of mercaptoethanol in the sample can be as high as 1M, and the concentration of dithiothreitol can be as high as 5mM. However, due to the influence of slightly high concentration of detergent, it is necessary to ensure that the concentration of SDS is less than 0.1 %, Triton X-100 is less than 0.1 %, Tween 20,60,80 is less than 0.06 %.

Standard curve of the microplate Method



The horizontal axis is the standard of different concentration gradients, and the vertical axis is the absorption value at the corresponding 595 nm.

Product composition:

Reagent	Volume	Storage
5×G250 Staining Solution	50 mL	2-8°C
PBS Diluent	15 mL	2-8°C
BSA Protein Standard(5 mg/mL BSA)	0.5 mL	-20°C

Protocols

I. Microplate reader method

1. Completely dissolved protein standard, take 10 μL, diluted to 250 μL, so that the final concentration is 0.2 mg / mL. The protein sample and standard should be diluted using the same solution. However, for the sake of simplicity, the standard can be diluted with 0.9 % NaCl or PBS.
2. Before using 5 × G250 dyeing solution, please mix it upside down 3-5 times, take 1mL 5 × G250 dyeing solution, add 4 mL double distilled water, and mix it into 1 × G250 dyeing solution. The 1 × G250 dyeing solution can be stored at 4 °C for one week.
3. The standard was added to 96-well plates according to 0, 2, 4, 6, 8, 12, 16 and 20 μL, respectively, and the PBS diluent was added to 20 μL, which was equivalent to the standard concentration of 0, 0.02, 0.04, 0.06, 0.08, 0.12, 0.16 and 0.2 mg /μL respectively.
4. Dilute the sample appropriately (preferably several more gradients, such as 2 times, 4 times, 8 times dilution), and add 20 microliters to the sample hole of the 96-well plate. Due to the error of the pipette when taking a small amount, the point in front of the standard line may not be very accurate, so as far as possible to let the sample point fall after 1 / 2 of the standard line.
5. Each well was added with 200 microliters of diluted 1 × G250 staining solution and placed at room temperature for 3-5 minutes.
6. Determination of absorbance of A_{595}, or other wavelengths between 560-610 nm by microplate reader.
7. According to the standard curve, the protein concentration in the sample was calculated

II. Spectrophotometer method

If there is no microplate reader, the staining reaction can be carried out in a centrifuge tube. The reaction solution is mixed and added to a colorimetric dish, and the absorbance value is measured by a spectrophotometer.

The steps are as follows :

1. Take eight (or more) clean 10mL centrifuge tubes and mark the number.
2. A total of 100 μL BSA was added to 2.4 mL PBS and diluted to a final concentration of 0.2 mg / mL.

3. Please mix 3-5 times before using the 5 × G250 staining solution, take 10 mL of 5 × G250 staining solution, add 40 mL of double distilled water, and mix to form 1 × G250 staining solution. The 1 × G250 staining solution can be stored at 4 °C for one week.
4. According to the following table to join the reagent (5mL per hole, the excess is used to clean the cuvette), the corresponding standard concentration of the first six tubes were 0, 0.04, 0.08, 0.12, 0.16,0.2 mg/ mL

Centrifuge Tube number	1	2	3	4	5	6	7(Sample tube 1)	8(Sample tube 2)	9(Sample tube 3)
Standard protein BSA (μL)	0	100	200	300	400	500	500 μL of appropriately diluted sample 1	500 μL appropriately diluted sample 2	-
PBS(μL)	500	400	300	200	100	0	0	0	0
1X G250 staining solution(mL)	5	5	5	5	5	5	5	5	5

5. After 3 minutes of reaction, the OD values of A_{595}, or other wavelengths between 560-610 nm were measured. For the accuracy of the experiment, a tube of staining solution can be added every 2 minutes, and a tube of OD value can be measured every 2 minutes. The following table

Centrifuge tube number	1	2	3	4	5	6	7	8	-
Add Staining Solution(minutes)	0	2	4	6	8	10	12	14	-
OD value	3	5	7	9	11	13	15	17	-