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Coomassie (Bradford) Protein Assay Kit

Cat No.: PC0010

Size: 2500T (Microplate Reader) /100T(Spectrophotometer)

Storage: BSA protein standard is stored at 4℃ for 3 months or at -20℃ for 1 year, other reagents are stored at 4℃ for 9 months. Please keep it sealed after opening and use it as soon as possible

Kit contents

5×G250	100ml
PBS Dilution Buffer	30ml
BSA (5mg/ml)	1ml

Description

Coomassie G-250 dye is a colorimetric reagent used for the detection and quantitation of total protein. In an acidic medium Coomassie dye binds protein causing an immediate shift in absorption maximum from 465nm to 595nm with a concomitant color change from green to blue. In a certain concentration range, the absorbance value A595 measured is in direct proportion to the protein concentration. Protein concentration determined by Bradford method is not affected by most of the chemical substances in the sample. 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, This method is is affected by slightly high concentration of detergent. It is necessary to ensure that the concentration of SDS, Triton X-100 are lower than 0.1% and Tween 20, 60, 80 less than 0.06%. BCA Protein Assay Kit (PC0020) is recommended for the samples containing detergent.

Protocol

- **Microplate Reader**

1. Completely dissolve BSA standard, dilute 10μl to 250μl, the final concentration of 0.2mg/ml. Dilution buffer was depend on the measured protein sample. For the sake of simplicity, suggested to use 0.9%NaCl or PBS.
2. 5×G250 mix well before use. 1ml 5×G250 diluted with 4ml ddH2O. 1×G250 solution can be stored for one week at 4℃.
3. The standard according to 0, 2, 4, 6, 8, 12, 16, 20μl respectively added to 96well plates, add PBS Dilution Buffer to 20μl.
4. Dilute the sample(prepare a few gradients, such as 2 times, 4 times, 8 times dilution), add 20μl sample to 96 well plate.
   To avoid errors, sample points need set after the standard line of 1/2.
5. Add 200μl diluted 1×G250 to each well, incubate at room temperature for 3-5 minutes.

6. Measure the absorbance of A595 or other wavelengths between 560-610nm.
7. According to the standard curve calculate the sample protein concentration.

- **UV-Vis Spectrophotometer**

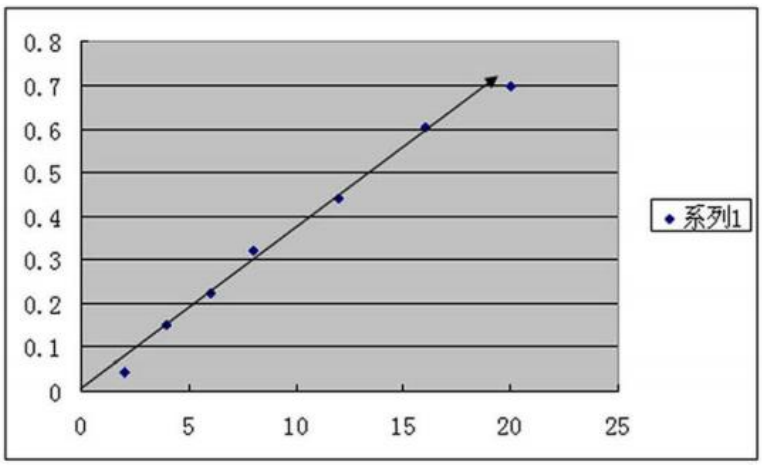
1. 8*10ml centrifuge tube(or more) and labeled with 1~8.
2. Dilute 100μl BSA with 2.4ml PBS to 0.2mg/ml.
3. 5×G250 mix well before use. 10ml 5×G250 diluted with 40ml ddH2O. 1×G250 solution can be stored for one week at 4℃.

4. Add reagents according to the following table (each well 5ml, the extra is used to clean the cuvette)

Tube No.	1	2	3	4	5	6	7(sample 1)	8(sample 2)	9(sample 3)
BSA	0μl	100μl	200μl	300μl	400μl	500μl	500μl diluted sample 1	500μl diluted sample 2	……
PBS	500μl	400μl	300μl	200μl	100μl	0μl	0μl	0μl	0μl
1×G250	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml

5. Measure OD value after 3 minutes reaction. In order to ensure the accuracy of the experiment, add one tube of stain solution every 2 minutes, measure OD value every 2 minutes

Tube No.	1	2	3	4	5	6	7	8	……
Time of adding Stain Solution(min)	0	2	4	6	8	10	12	14	……
Time of Measuring OD value(min)	3	5	7	9	11	13	15	17	……



Bio-rad 680，A570nm, room temperature for 3 minutes

Related products：

PC0001 Protein standard solution (5mg/ml BSA)
PC0015 5×G250 (Protein quantitative analysis)
PC0021 BCA reagents
PC0030 Lowry Protein Assay Kit
PC0020 BCA Protein Assay Kit
R0010 RIPA buffer(high)
PR1600 Prestained Protein Marker(14.4kD-97.4kD)
R0050 Nuclear Protein Extraction Kit

Reference:

[1] Zhongyuan Li,Xiumei Li,Tianhui Liu,et al. The critical roles of exposed surface residues for the thermostability and halotolerance of a novel GH11 xylanase from the metagenomic library of a saline-alkaline soil. International Journal of Biological MacromolecμLes. JμLy 2019;133:316-323. (IF 4.784)

[2] Qinlu Zhang,Qian Liu,Menghan Du,et al. Cetuximab and Doxorubicin loaded dextran-coated Fe3O4 magnetic nanoparticles as novel targeted nanocarriers for non-small cell lung cancer. Journal of Magnetism and Magnetic Materials. June 2018. (IF 3.046)