WB-RR-96

Bradford Protein Assay Kit

Size: 5000 Tests with Microplate Method or 1000 Tests with Test Tube Method

Lot # Check on the product label

Introduction

1. Bradford Protein Assay Kit is based on the method of Bradford which is one of the two most popular protein assay methods, can determine the protein concentration fast, stably and high sensitively.
2. Can complete 10-80 samples assay within 10 min.
3. The minimum detection level is 25 μg/ml. The minimum testing volume of protein is 0.5 μg, and the size of the sample is 1-20 μl.
4. Good liner range at 50-1000 μg/ml.
5. Determination of the protein concentration with Bradford is not influenced by chemical substances in majority of the samples, but will be influenced by detergents with high concentration. Thus, concentration of the following detergents should be SDS < 0.01%, Triton X-100 < 0.05%, Tween 20, 60 , 80 < 0.015%. It is recommended to use BCA Protein Assay Kit to assay detergents contained samples.
6. Each kit can be sufficient to test 1000 samples.

Kit Components

Components	Size	Storage Instruction
Bradford Protein Assay Dye Reagent	100 ml × 10	Store at 4℃ for one year
Albumin Standard Ampules（5 mg/ml BSA）	5x 5 mg, lyophilized	

Protocol

Preparation of Albumin Standard Ampules (BSA standard): Reconstitute the lyophilized Albumin Standard Ampules with 1 ml of distilled water to get the liquid Albumin Standard Ampules with concentration 5 mg/ml.

For Standard Test Tube and Standard Microplate Method, follow this instruction to dilute BSA standard (Detection range =100-1,500 μg/ml)

Tube No.	Volume of Diluent	BSA Standard	Final concentration
A	600 μl	400 μl (From 5 mg/ml tube)	2,000 μg/ml
B	125 μl	375 μl (Pipette from Tube A)	1,500 μg/ml
C	250 μl	250 μl (Pipette from Tube A)	1,000 μg/ml
D	175 μl	175 μl (Pipette from Tube B)	750 μg/ml
E	325 μl	325 μl (Pipette from Tube C)	500 μg/ml

F	325 µl	325 µl (Pipette from Tube E)	250 µg/ml
G	325 µl	325 µl (Pipette from Tube F)	125 µg/ml
H	400 µl	100 µl (Pipette from Tube G)	25 µg/ml
I	400 µl	0	0 µg/ml (blank)

For Microassay Test Tube and Microassay Microplate Method, follow this instruction to dilute BSA standard (Detection range = 1-25 µg/ml）

Tube No.	Volume of Diluent	BSA Standard	Final concentration
A	3,950 µl	50µl (From 5mg/ml tube)	25 µg/ml
B	800 µl	3,200 µl (Pipette from Tube A)	20 µg/ml
C	1,000 µl	3,000 µl (Pipette from Tube B)	15 µg/ml
D	1,000 µl	2,000 µl (Pipette from Tube C)	10 µg/ml
E	1,500 µl	1,500 µl (Pipette from Tube D)	5 µg/ml
F	1,500 µl	1,500 µl (Pipette from Tube E)	2.5 µg/ml
G	4,000 µl	0	0 µg/ml (blank)

- **Test Tube Method**

A. Standard Procedure（Detection range =100-1,500 µg/ml）

1. Pipette 0.05 ml of each BSA standard and unknown sample into an appropriately labeled test tube.
2. Add 1.5 ml of Bradford Protein Assay Dye Regent to each tube and mix thoroughly.
3. Incubate at room temperature for 10 min (For optional reference).
4. With the spectrophotometer set to 595 nm, zero the instrument on a cuvette filled only with water. Then, measure the absorbance of all the samples.
5. Subtract the average 595 nm absorbance measurement of the Blank standard replicates from the 595 nm absorbance measurement of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595 nm measurement for each BSA standard & its concentration (µg/ml). Use the standard curve to determine the protein concentration of each unknown sample.

B. Microassay Procedure（Detection range = 1-25 µg/ml）

1. Pipette 1 ml of each BSA standard and unknown sample into an appropriately labeled test tube.
2. Add 1 ml of Bradford Protein Assay Dye Regent to each tube and mix thoroughly.
3. Incubate at room temperature for 10 min (For optional reference).
4. With the spectrophotometer set to 595 nm, zero the instrument on a cuvette filled only with water. Then, measure the absorbance of all the samples.
5. Subtract the average 595 nm absorbance measurement of the Blank standard replicates from the 595 nm absorbance measurement of all other individual standard and unknown sample replicates.

6. Prepare a standard curve by plotting the average Blank-corrected 595 nm measurement for each BSA standard & its concentration (μg/ml). Use the standard curve to determine the protein concentration of each unknown sample.

- **Microplate Method**

A. Standard Procedure（Detection range =100-1,500 μg/ml）

1. Pipette 10 μl of each BSA standard and unknown sample to corresponding marked microplate wells.
2. Add 300 μl of Bradford Protein Assay Dye Regent to each well and mix thoroughly on the plate shaker for 30 seconds.
3. Stop shaking. Generally, incubate at room temperature for 10 min.
4. Measure absorbance at 595 nm.
5. Subtract the average 595 nm absorbance measurement of the Blank standard replicates from the 595 nm absorbance measurement of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595 nm measurement for each BSA standard & its concentration (μg/ml). Use the standard curve to determine the protein concentration of each unknown sample.

B. Microassay Procedure（Detection range = 1-25 μg/ml）

1. Pipette 150 μl of each BSA standard and unknown sample to corresponding marked microplate wells.
2. Add 150 μl of Bradford Protein Assay Dye Regent to each well and mix thoroughly on the plate shaker for 30 seconds.
3. Stop shaking. Generally, incubate at room temperature for 10 min.
4. Measure absorbance at 595 nm.
5. Subtract the average 595 nm absorbance measurement of the Blank standard replicates from the 595 nm absorbance measurement of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595 nm measurement for each BSA standard & its concentration (μg/ml). Use the standard curve to determine the protein concentration of each unknown sample.

Notes

1. Equilibrate G250 Dye Reagent to room temperature, and reverse 3-5 times to mix thoroughly before use.
2. Mix BSA standard thoroughly after dissolved, then dilute to a series of concentrations.
3. For the assay accuracy, please measure the absorbance within 5-20 min after all reagents added, since the color development is the most stable during this period.
4. Some cation, like K^+ , Na^+, Mg^{2+}, $(NH_4)_2SO_4$, ethanol etc. do not impact the determination, but a lot of detergent like Triton X-100, SDS etc. will seriously interference the determination.
5. Please wear the lab coat and disposable gloves to operate.