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WB-RR-96 **Bradford Protein Assay Kit**

Size: 750 tubes Spectrophotometer

3000 assays Microplate Reader

Components	Size	Storage
Bradford Protein Assay Reagent*	6 x 100 ml	Store at 2-8°C for 12 months
Albumin Standard BSA 5 mg/ml (lyophilized)	3 x 5 mg	

*Each vial of Bradford Assay Reagent 100ml develops 125 tests /spectrophotometer and 500 microplate assays

Introduction

Bradford Protein Assay Kit is a ready to use kit, based on Bradford -binding colorimetric method for the total protein quantification.

Preparation of Standards and Assay Reagents

Albumin Standard Ampule (BSA standard):

- Reconstitute the lyophilized Albumin Standard vial with 1 ml of distilled water to get the 1 vial of liquid Albumin Standard with concentration 5 mg/ml.

Preparation of Albumin Standard (**BSA standard**): Once reconstituted the lyophilized Albumin Standard Ampules with 1 ml of distilled water see working range below in table 1.

Table 1. Preparation of Diluted BSA standards

Dilution scheme for Standard Test Tube and Microplate protocols- **Working range =125-1,500 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 Micro- Test Tube and Microplate protocols - **Working 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)

PROTOCOLS

Test Tube Procedures (Spectrophotometer)

A. Standard Test Tube Protocol（working range =125-1,500 μg/ml）

1. Pipette 0.05 ml (50μl) of BSA standard and unknown sample into an appropriately labeled test tube.
2. Add 1.5 ml of Bradford –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. Micro Test Tube Protocol（working range = 1-25 μg/ml）

1. Pipette 1 ml of BSA standard and unknown sample into an appropriately labeled test tube.
2. Add 1 ml of Bradford Regent to each tube and mix thoroughly.
3. Incubate at room temperature for 10 min (For optional reference).
4. With a 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
6. absorbance measurement of all other individual standard and unknown sample replicates.
7. 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

Microplate Procedure

Standard Microplate Protocol (working range: 125-1500 μg/ml)

1. Pipette 10 μl of 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.

Micro-Microplate Protocol (working range: 1-25 μg/ml)

1. Pipette 150 μl of 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.

IMPORTANT NOTES

1. Equilibrate Bradford Reagent "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.

Storage : 2-8°C for 12 months.

For Research use only

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