Bradford Protein Assay Kit

User Manual

Catalog # CRG1002

Detection and Quantification of Protein Concentration.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Bradford coomassie-binding, colorimetric method for total protein quantitation is one of dye binding methods. Coomassie Briliant Blue G-250 in the free state shows brown, and its maximum light absorbance is 488nm. When Coomassie Briliant Blue G-250 combines with protein, it turns brown into blue, and the maximum absorption is 595 nm. The absorbance at 595nm is proportional to the protein concentrations. Protein binding Coomassie Briliant Blue G-250 reaches a balance in about 2 minutes. The complex remains stable in 1 hour at room temperature. The method has superiority such as simple and convenient operation, sensitive reaction (4 times higher than Lowry Method), etc. Bradford protein assay kit is developed based on Bradford coomassie-binding method. This method has traits of simple and convenient operation, high sensitivity, accurate quantification and stable effect.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Coomassie Bradford Assay Reagent	25 ml x 4	RT
Standard	2 mg x 5	4 °C
Technical Manual	1 Manual	

Note:

Standard: Add 1ml ddH2O into each tube and mix by vortex. The concentration of the standard is 2mg/ml. Store at 4°C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 595 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Timer
6. Plate shaker

IV. STANDARD PREPARATION

Label 9 test tubes with A-I and prepare the standards as indicated below. The diluent used should be the same as used for the protein samples. The following dilutions are suitable for duplicate Standard assays.

Tube	BSA	Diluent	Concentration
A	200 µl from Stock Solution	0 µl	2000 µg/ml
B	100 µl from Tube A	100 µl	1000 µg/ml
C	100 µl from Tube B	100 µl	500 µg/ml
D	100 µl from Tube C	100 µl	250 µg/ml
E	100 µl from Tube D	100 µl	125 µg/ml
F	100 µl from Tube E	100 µl	62.5 µg/ml
G	100 µl from Tube F	100 µl	31.25 µg/ml
H	100 µl from Tube G	100 µl	15.625 µg/ml
I	0 µl	100 µl	0 µg/ml (Blank)

V. ASSAY PROCEDURE

1. Pipette 10 µl of each standard or unknown sample replicate into a microplate well (working range = 15.625 - 2000 µg/ml).
2. Add 200 µl of the Coomassie Bradford Assay Reagent to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate and incubate for 5 minutes at RT.
4. Measure the absorbency at or near 595 nm on a plate reader.
5. Use the standard curve to calculate the protein concentration of each unknown sample.

VI. CONSIDERATIONS

1. Make Coomassie Bradford Assay Reagent return to RT before use, and mix well.
2. Please wear the lab coat and gloves to operate.
3. Make sure the absorbency at 595nm within the range of the standard curve.
4. Good linear range for samples is from 20-2000 μg/ml.
5. Period of validity is 1 year.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES