

Bradford Protein Assay

Spectroscopic Analytical Method

www.davids-bio.com



GENERAL INFORMATION

The Bradford protein assay may be used to quantify the total protein content in a sample. Coomassie Brilliant Blue G-250 is a protein-binding dye. Binding of proteins leads in a change in absorbance from 465 to 595 nm. After mixing the Coomassie Reagent with the protein sample, measure the absorbance peak at 595 nm. Bradford is reasonably accurate, simple to conduct, and quick.



MATERIALS

Bradford Reagent

0.01% (w/v) Coomassie Blue G250
4.7% (w/v) Methanol
8.5% (w/v) phosphoric acid
Filtered through blotting paper

Additional Materials

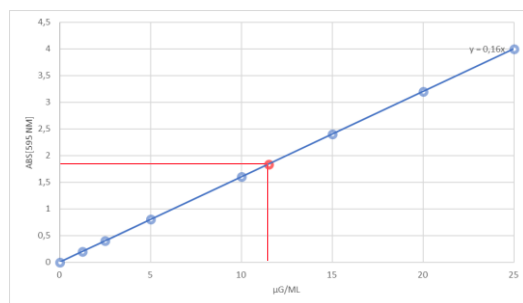
Spectrophotometer
Reagent tubes or 96-well plates
Bovine serum albumin (BSA Stock Solution: 1 mg/ml)



STANDARD CURVE

- Y Prepare a series of BSA dilutions for your standard (See table)
- Y Transfer 20 μl of each dilution in a 1.5-ml vial and add 1 ml Bradford reagent
- Y Invert and incubate the tubes for 5 min at room temperature
- Y Set Blank with 1 ml distilled water
- Y Measure the absorbance of each dilution with a spectrophotometer at 595 nm and subtract the blank value (blue values in graph).
- Y Make a standard curve by evaluating the values from the BSA dilutions.

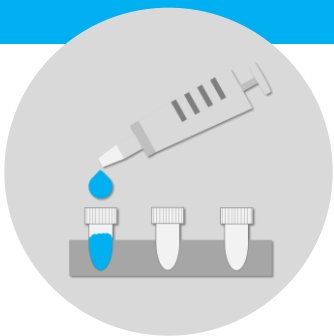
Protein Concentration [$\mu\text{g/ml}$]	BSA -Solution (1 mg/ml) [μl]	A. dest [μl]
25	25	975
20	20	980
15	15	985
10	10	990
5	5	995
2,5	2,5	997,5
1,25	1,25	998,75
0	0	1000



EVALUATION

- Y Transfer 20 μl of the sample in a 1.5-ml vial and add 1 ml Bradford reagent.
- Y You may need to dilute your sample prior to measurement. Please use the dilution factor when you want to calculate the protein concentration.
- Y Invert the tube and incubate for 5 min at room temperature
- Y Measure the absorbance with a spectrophotometer at 595 nm and subtract the blank value (red value in graph).

Not for diagnostic or therapeutic use. Not for human use. For research purposes only. This product is not a medical device. Not for use in in vitro diagnostic procedure for clinical diagnosis. Custom tests must be done to determine the ability for a specific use.



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TROUBLESHOOTING

Reason	Solution
Low Measurement Values	Allow the reagents to reach room temperature before measuring.
Measurement of Small Peptides	Bicinchoninic acid (BCA-Assay) may be used as an alternate technique for lightweight proteins.
Sample Precipitate	Dilute or dialyze the protein sample before use.
Spectrophotometer	A different wavelength between 580 and 610 nm can be utilized as an alternative to 595 nm measurement, at the cost of diminished standard curve specificity.
Values too high	You may want to dilute your sample. The measurement value must be within your standard curve range.
Standard	You may want to use a different protein like Gamma-Globulin for the generation of your standard curve to obtain more precise results.