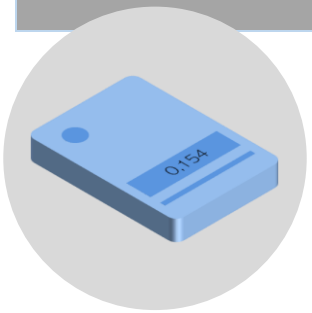


Bradford Protein Assay

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Bradford protein assay is a valuable method for quantifying the total protein content in a sample. This assay utilizes the protein-binding dye known as Coomassie Brilliant Blue G250. When proteins bind to this dye, it induces a noticeable shift in absorbance from 465 to 595 nm. By combining the Coomassie Reagent with the protein sample and measuring the absorbance peak at 595 nm, the assay provides a reasonably accurate, straightforward and rapid means of protein quantification.

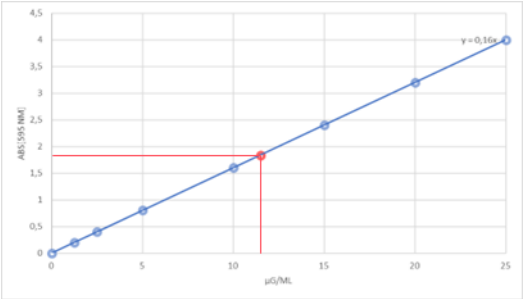
Material	
Bradford Reagent	0.01% w/v Coomassie Blue G250 4.7% w/v Methanol 8.5% w/v phosphoric acid filtered through blotting paper
Spectrophotometer	
Reagent tubes or 96-well plates	
BSA Stock Solution 1 mg/ml (Bovine serum albumin)	

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.

- 2 - Method

Standard Curve

Protein Concentration [µg/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



- Prepare a series of BSA dilutions (Please refer to the table) for your standard
- Transfer 20 µl of each BSA dilution into separate 1.5-ml vials
- Add 1 ml of the Bradford reagent to each vial containing the BSA dilution
- Mix thoroughly by gently inverting the vials
- Incubate the vials at room temperature for 5 minutes to allow the dye to bind with the proteins
- Set up a blank control by adding 1 ml of distilled water to a separate vial
  This will serve as a reference for background absorbance
- Using a spectrophotometer, measure the absorbance of each BSA dilution at a wavelength of 595 nm
  Remember to subtract the absorbance value obtained from the blank control (blue values in the graph)
- Utilize the absorbance values obtained from the BSA dilutions to construct a standard curve, which will allow for the evaluation of protein concentrations

Evaluation

- Transfer 20 µl of your sample into a 1.5-ml vial
- Add 1 ml of the Bradford reagent to the vial containing the sample
  If your sample is highly concentrated, you may need to dilute it before measurement. Please keep the appropriate dilution factor in mind when calculating the protein concentration
- Invert the vial gently to ensure thorough mixing
- Incubate the vial at room temperature for 5 minutes to allow the dye to bind with the proteins
- Use a spectrophotometer to measure the absorbance of the sample at a wavelength of 595 nm. To obtain the accurate protein concentration, subtract the absorbance value obtained from the blank control (red value in the 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.

- 3 - Trouble Shooting

Reason	Solution
Low Measurement Values	Allow the reagents to reach room temperature before measuring. This ensures optimal conditions for accurate readings. For lightweight proteins or small peptides, consider using the bicinchoninic acid (BCA) assay as an alternative technique. It may provide more reliable results in such cases.
Sample Precipitation	Dilute or dialyze the protein sample before use. This step helps to remove any impurities or precipitates that could interfere with the assay.
Spectrophotometer	Instead of measuring at 595 nm, try using a different wavelength between 580 and 610 nm as an alternative. Please keep in mind that this may result in a decrease in the specificity of the standard curve.
High Measurement Values	Dilute your sample to bring the measurement value within the range of your standard curve. This will ensure accurate quantification. Consider using a different protein, such as Gamma-Globulin, to generate your standard curve. This can potentially lead to more precise results.

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.