Bradford - Solution for Protein Determination

Solution for the rapid and accurate estimation of protein concentration
Product code A6932

Introduction

The Bradford assay is very fast and uses about the same amount of protein as the Lowry assay. It is fairly accurate and samples that are out of range can be retested within minutes. The Bradford assay is recommended for general use, especially for determining protein content of cell fractions and assesing protein concentrations for gel electrophoresis.

The Bradford protein assay is a simple procedure for determination of total protein concentrations in solutions that depends upon the change in absorbance based on the proportional binding of the dye Coomassie Blue G-250 to proteins. The Coomassie blue G250 dye appears to bind most readily to arginyl and lysyl residues of proteins (not to the free amino acids). This specificity can lead to variation in the response of the assay to different proteins, which is the main disadvantage of the method. Therefore, it is advisable to choose a protein standard that is likely to give absorbance values close to those for the protein samples of interest (e.g. if you determine the concentration of an immunoglobulin, use IgG as a standard). The Bradford assay is much more sensitive to immunoglobulin G (IgG) than to bovine serum albumin (BSA), so that with IgG the investigator is likely to overestimate the amount of protein in a sample. With BSA the investigator is likely to underestimate the amount. The assay is less accurate for basic or acidic proteins.

Unlike many other assays, including the Lowry procedure, the Bradford assay is not susceptible to interference by a wide variety of chemicals present in samples. The notable exception is high concentrations of detergents.

Principle

The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible color change. The assay is useful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range. Within the linear range of the assay (~5-25 µg/ml), the more protein present, the more Coomassie binds. The protein concentration of a test sample is determined by comparison to that of a series of protein standards known to reproducibly exhibit a linear absorbance profile in this assay.

Effects of Common Reagents on the Bradford Assay (acc. to Ref. 3; Absorbance at 600 nm)

Compound	**Blank**	**5 mg IgG**
Control	0.005	0.264
0.02% SDS	0.003	0.250
0.1% SDS	0.042	0.059
0.1% Triton	0.000	0.278
0.5% Triton	0.051	0.311
1M 2-Mercaptoethanol	0.006	0.273
1M Sucrose	0.008	0.261
4M Urea	0.008	0.261
4M NaCl	0.015	0.207
Glycerol	0.014	0.238
0.1M HEPES, pH 7.0	0.003	0.268
0.1M Tris, pH 7.5	0.008	0.261
0.1M Citrate, pH 5.0	0.015	0.249
10 mM EDTA	0.007	0.235
M $(NH_4)_2SO_4$	0.002	0.269

Strongly alkaline solutions can interfere with the assay as well.

References

(1) Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
(2) Stoscheck, C.M. (1990) Quantitation of Protein. *Methods in Enzymology* **182**, 50-69.
(3) Stoscheck, C. (1990) Increased uniformity in the response of the Coomassie blue protein assay to different proteins. *Anal. Biochem*. **184**, 111-116

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Protocols

Standard

A set of standards is created from a stock of protein whose concentration is known. The Bradford values obtained for the standard are then used to construct a standard curve to which the unknown values obtained can be compared to determine their concentration. Use a protein as your standard that most closely resembles the protein you are assaying. BSA and IgG are typical standards used to construct the curve. For BSA, use 0 - 1 mg/ml as your standard curve concentration; for IgG, use 0 - 1.6 mg/mL.

Prepare a standard curve of absorbance versus micrograms protein and determine amounts from the curve.

*__Note__: The ubiquitously used BSA is a very poor standard for the Bradford assay. To adjust for this, multiply the results you get for your protein concentration by 2.1 to get a closer approximation of your protein's concentration. Lysozyme, ovalbumin and catalase make much better standards, and no adjustment is necessary for these standards.

Glass or polystyrene (cheap) cuvettes may be used, however the color reagent stains both. Disposable cuvettes are recommended.

The Bradford reagent should be a light brown in color. Filter through Whatman #1 paper just before use. Filtration may have to be repeated to rid the reagent of blue components. Do not use directly from the bottle. Transfer the volume you are going to use to another recipient.

(1) Prepare a stock solution of 1 mg/ml BSA. **Dissolve** BSA in saline (150 mM NaCl) or water and store it frozen in 1 ml aliquots for quick use. The standard should be dissolved in a buffer similar to that the unknowns will be dissolved in.

(2) Protein has a tendency to absorb water during storage. Therefore obtain exact concentration of BSA by measuring ist absorbance at 280 nm (quartz cuvets). A 1 mg/ml solution of BSA should have an absorbance of 0.66.

(3) Using the result, prepare a solution of **100 µg/ml BSA**

(4) With this standard, pipet duplicates of known concentrations, i.e. 1 - 10 µg BSA in a total volume of 100 µl into 1.5 ml reaction tubes. This equivalent to 10, 20, 40, 60, 80 and 100 µl of the BSA standard.

µg BSA	µl BSA-Standard	H_2O or buffer	
0	0 µl	100 µl	reagent blank
1	10 µl	90 µl	
2	20 µl	80 µl	
4	40 µl	60 µl	
6	60 µl	40 µl	
8	80 µl	20 µl	
10	100 µl	0 µl	

(5) To each tube add 1 ml of Bradford Reagent, and mix gently by vortexing.

(6) Using reagent blank to zero the spectrophotometer. Transfer the samples to disposable cuvets and measure the absorbance at 595 nm.

Samples can be measured from 2 - 60 minutes after addition of Bradford Reagent.

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Unknown Samples

Assay I

(1) Pipet 2 x 5 µl of cell lysate from each sample into two separate labelled reaction tubes.
(2) Make up to 100 µl with distilled water.
(3) Add 1 ml of assay reagent to each tube.
(4) Mix thoroughly, but gently by vortexing
(5) Transfer each sample to a disposable cuvet.
(6) Make sure the spectrophotometer has been zeroed at 595 nm with the reagent blank.
(7) Measure the absorbance of each sample at 595 nm between 2 - 60 minutes after addition of reagent.

Using the standard curve find the equivalent protein concentration for the unknown samples. Remember that the sample was diluted 1 in 20 therefore multiply by twenty to get the protein concentration in a 100 µl sample. This is euqivalent to the sample which is used in ONPG assay. Therefore can be used to express the Units of β-galactosidase from the transfection assay in terms of total protein.

Alternative Assay II

(1) Warm up the spectrophotometer before use.
(2) Dilute unknowns if necessary to obtain between 5 and 100 µg protein in at least one assay tube containing 100 µl sample
Optional: If desired, add an equal volume of 1 M NaOH to each sample and vortex. Add NaOH to standards as well if this option is used.
Comment: The addition of 1 M NaOH was suggested by Stoscheck (1990) to allow the solubilization of membrane proteins and reduce the protein-to-protein variation in color yield.
(3) Prepare standards containing a range of 5 to 100 micrograms protein (albumin or gamma globulin are recommended) in 100 µl volume.
(4) Add 1 ml dye reagent and incubate 5 min.
(5) Measure the absorbance at 595 nm.

Alternative Assay III
Using a plate reader

Note: do all determinations in duplicate or triplicate

1.) Standard: Pipette 0, 2, 4, 6, 10, 15 and 20 µl of BSA standard solution (**100 µg/ml**) into assigned wells of a 96-well plate.
Make up to 20 µl with distilled water.
2.) Unknown samples: Pipette up to 20 µl of unknown samples into individual wells of a 96-well plate.
3.) Add 200 µl of Bradford Reagent into all wells containing standard or sample.
4.) Read absorbance at 595 nm without any prior incubation.

Comments

- Generate test samples for the reference cell, blank, BSA standards and the protein sample to be tested in disposable cuvettes.
- Note that the "reference cell" and "blank" are identical. A reference cell test sample is only required when using a double-beam UV-visible spectrophotometer for absorbance measurements.
- Note that a dilution of the protein sample may be required for the resulting absorbance to fall within the linear range of the assay.
- Allow each sample to incubate at room temperature for 10-30 minutes. (We recommend to record the actual incubation time in your lab book.)
- Plot the absorbance of each BSA standard as a function of its theoretical concentration. The plot should be linear.
- Note: If the absorbance of the test sample is outside of the absorbance range for the standards, then the assay must be repeated with a more appropriate dilution, if any. The linear range for the assay (and for most spectrophotometers is 0.2 - 0.8 O.D. units.