Protocol

Bradford Assay for Determining Protein Concentration

Clara L. Kielkopf, William Bauer, and Ina L. Urbatsch

The Bradford assay is a quick and fairly sensitive method for measuring the concentrations of proteins. It is based on the shift in absorbance maximum of Coomassie Brilliant Blue G-250 dye from 465 to 595 nm following binding to denatured proteins in solution.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Bradford stock solution <R>

Bradford working reagent <R>

Concentrated reagent with minimal batch-to-batch variations is commercially available (Bio-Rad, Sigma-Aldrich, Thermo Scientific Pierce, etc.) and may be more sensitive. Consult the manufacturer's guidelines for appropriate working dilutions.

Deionized H_2O

Immunoglobulin G (IgG; 1 mg/mL in deionized water)

Although commonly used, bovine serum albumin (BSA) gives an approximately twofold greater response to the Bradford reagent than an average protein. Therefore, IgG is recommended as a protein standard.

A UV absorbance assay is recommended for calibrating pure protein solutions for use as a standard. The A_{280} values for a 1 mg/mL solution of BSA is 0.63; bovine, human, or rabbit IgG is 1.38.

Aliquots of the protein standard can be stored at −20°C.

NaOH (1 M) (optional)

Protein sample of unknown concentration

Sample buffer

This buffer should match the composition of the protein storage buffer.

Equipment

Disposable cuvettes (polystyrene)

Disposable cuvettes are recommended because dye and denatured protein may be difficult to remove completely.

Plate reader (96-well) (see Step 3)

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Spectrophotometer with visible light source (e.g., Spectronic 20 Genesys, Spectronic Instruments; Beckman DU series, Shimadzu BioSpec-mini, or similar model)
Vortex mixer

METHOD

This protocol for the Bradford assay (Bradford 1976) is modified from Bollag and Edelstein (1991) for the 10-mL test tube format. The Lowry and BCA assays can be accomplished in a similar manner, following modifications of the reagents, incubation times, and temperatures. Guidelines for Lowry and BCA assays are offered by the suppliers of the assay reagents (e.g., Thermo Scientific Pierce or Bio-Rad). See also Introduction: ***Methods for Measuring the Concentrations of Proteins*** *(Kielkopf et al. 2020).*

1. To generate the standard curve, prepare several dilutions of the protein standard from 0 to 20 µg (0–20 µL of 1 mg/mL IgG) in a final volume of 20 µL of deionized water. Add 10 µL of sample buffer to each dilution. Test enough standard concentrations to assess the reproducibility of the assay. Eight concentrations (e.g., 0, 2.5, 5, 7.5, 10, 12.5, 15, and 20 µg) measured in duplicate or triplicate are usually sufficient.
2. In parallel with the standard curve, dilute three different volumes of the sample protein solution (e.g., 2, 5, and 10 µL) to a final volume of 10 µL in sample buffer. Add 20 µL of deionized water to each dilution.

   Protein dilution volumes may need to be adjusted based on the concentration. All readings should be done in triplicate.

   Protein sample volumes up to 100 µL may be assayed; however, the effect of interfering substances will increase. The volume of sample buffer added to the protein standards (in Step 1) should be adjusted accordingly.

   To improve solubility of membrane proteins and/or reduce batch-to-batch variation in signal, an equal volume of 1 M NaOH may be added to the diluted protein solution at this step (Stoscheck 1990).
3. Add 1 mL of Bradford working reagent to standards and samples, and vortex to mix thoroughly. Let the color develop for at least 5 min at room temperature.

   This assay may be adapted to the 96-well format by scaling down amounts and volumes by a factor of 5–10 (100–200 µL of Bradford working reagent). This format is advantageous in that it consumes a smaller volume of protein sample, the reagent can be rapidly dispensed using a multichannel pipette, and all standards and samples can be read simultaneously in a plate reader. If the plate reader does not have a monochromator, choose the nearest available wavelength filter. For the 96-well plate format, protein sample volumes up to 25 µL may be assayed. However, the effect of interfering substances will increase; adjust the volume of sample buffer added to the protein standards accordingly. A range of different sample concentrations that fall within the linear range of the standard curve should be assayed in triplicate.
4. Use the "blank" (0 µg of sample) to zero the spectrometer at 595 nm, and then record the absorbances of the standards and samples at 595 nm.

   Color may continue to develop during the reading process. Record each absorbance sequentially in the same order as the samples were prepared.
5. Generate a standard curve by plotting the average absorbance at 595 nm as a function of concentration of protein standard.
6. Determine the amount of protein for a given volume of sample using the standard curve above. Protein amounts should fall within the linear range of the standard curve. Calculate the concentration for each volume (in micrograms per microliter), and average the concentrations. Sample protein amounts can be directly read off the standard curve or calculated in Excel using linear regression analysis to calculate the slope of the standard curve.

   See also Box 2 in Introduction: ***Methods for Measuring the Concentrations of Proteins*** *(Kielkopf et al. 2020).*

RECIPES

Bradford Stock Solution

Reagent	Quantity (for 300 mL)	Final concentration
Ethanol (95%)	100 mL	31.67%
Phosphoric acid (88%)	200 mL	58.67%
Coomassie Brilliant Blue G-250	350 mg	1.41 M

The stock solution is stable indefinitely when stored in a brown glass bottle at room temperature.

Bradford Working Reagent

Reagent	Quantity (for 500 mL)	Final concentration
Distilled water	425 mL	
Ethanol (95%)	15 mL	2.85%
Phosphoric acid (88%)	30 mL	5.28%
Bradford stock solution <R>	30 mL	16.67 × dilution of the stock

The reagent should be a light brown color. Filter through Whatman No. 1 paper. This working reagent is stable for several weeks (with refiltering as needed) when stored at room temperature in a brown glass bottle.

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