# Protocol

TD-P Revision 2.0

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# Bradford Protein Assay

## For Protein Quantitation

## Introduction

The Bradford Protein Assay is a quick, inexpensive and simple way to determine protein concentration. A protein is reacted with the Bradford reagent and absorbance is measured. The Bradford reagent contains Coomassie Brilliant Blue G-250 (CBBG) dye. The CBBG dye contains two sulfonic acid groups and six phenyl groups, which interact with proteins via positively charges residues and hydrophobic residues, respectively. The absorbance of CBBG increases from 465 nm to 595 nm once it forms a complex with the protein. The absorbance of various amounts of a known protein standard can be measured and a linear regression equation can be obtained by plotting absorbance versus protein amount. An unknown amount of protein can then be estimated by measuring the absorbance with CBBG and using the linear regression equation that was obtained from the standard.

The most commonly used protein standard is bovine serum albumin (BSA). BSA tends to be more sensitive to the assay than other proteins. Lysozyme is also sometimes used, as it tends to be a more typical protein in terms of hydrophobic content. No matter the standard protein, there is often considerable variation in the types of amino acid residues between proteins being assayed so the calculated protein concentration is an estimation. The protein being assayed would be the most accurate standard to use if there is a known concentration is available.

## Materials

- 95% Ethanol
- 85% Phosphoric Acid
- Coomassie Brilliant Blue G-250, GoldBio Catalog # C-460 [CAS 6104-58-1, mw. = 854.02 g/mol]
- Albumin Bovine Serum (BSA), Fraction V, Protease Free, GoldBio Catalog # A-420 [CAS 9048-46-8, mw. = 66 kDa]

Alternative to BSA:

  - Lysozyme, Egg White, GoldBio Catalog # L-040, [CAS 12650-88-3, mw. = 14.3 kDa]

## Storage and Handling

- Store BSA at 4°C.

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- These products may be shipped on ice and should be stored at -4°C immediately upon arrival. When stored under the recommended conditions and handled correctly, these products should be stable for at least 1 year from the date of receipt.

## Method

Bradford Reagent Preparation

1. Weigh 100 mg Coomassie Brilliant Blue G-250 dye.
2. Add 50 ml 95% ethanol.
3. **Slowly and carefully**, add 100 ml 85% phosphoric acid.
4. Mix until the blue dye is completely dissolved.
5. Add the dye/ethanol/phosphoric acid solution to 850 ml pure sterile $H_2O$.
6. Filter any precipitates.
7. Store Bradford reagent at 4°C for months.

Measuring Protein Standard

1. Warm up the spectrophotometer.
2. Pipet six different volumes [0 µl (0 µg), 10 µl (5 µg), 20 µl (10 µg), 30 µl (15 µg), 40 µl (20 µg), 50 µl (25 µg)] of 0.5 mg/ml BSA into separate cuvettes.
    a. Other protein standards can be substituted for BSA. The cuvette with no protein standard in it serves as a blank.
3. Add 1.5 ml of Bradford Reagent to each cuvette.
4. Cover the cuvettes with plastic paraffin film and mix by gently inverting.
5. Let the cuvettes incubate at room temperature for 10 minutes.
6. Measure the absorbance of each cuvette at 595 nm.

Measuring Unknown Quantity

1. Pipet between 10 to 50 µl of the protein that is to be quantified into a cuvette; a dilution may be necessary.

a. The goal is to get an absorbance reading that is in the middle of the standard curve.
b. An equal volume of 1M NaOH can be added to help solubilize membrane proteins. When choosing this option, be sure to include NaOH in the standards.

2. Add 1.5 ml of Bradford Reagent to the cuvette.

3. Cover the cuvette with plastic paraffin film and mix by gently inverting.

4. Let the cuvettes incubate at room temperature for 10 minutes.

5. Measure the absorbance of each cuvette at 595 nm.
   a. If the absorbance does not fall within the range of the standard cure, change the dilution of the sample and measure absorbance again.

Standard Curve Generation

1. Make a scatter plot for the standard curve values.

2. The X-axis will by micrograms (µg) of standard protein that were assayed; the Y-axis will be $Abs_{595}$ that was measured.

3. Fit a linear trendline to the graph.

4. Show the linear regression equation.

5. Calculate the unknown amount of protein by using the linear regression equation, as shown in the calculations section of this document.

6. The unknown concentration of the original protein solution can be calculated by dividing the amount of protein by the volume of protein assayed and multiplying the quotient by the dilution factor, as shown in the calculations section.

7. Alternatively, a curvilinear (polynomial) trendline can be fitted to the graph. This polynomial regression equation may sometimes give a better estimation of protein concentration.

## Calculations

$$\mu g\ of\ protein = \frac{Absorbance - y\ intercept}{slope}$$

$$\frac{\mu g\ of\ protein}{\mu l\ assayed} = concentration\ assayed$$

$$concentration\ assayed\ \times dilution\ factor = concentration\ of\ original\ solution$$

## Tips

- Phosphoric acid is corrosive! **Always wear protective gloves and goggles. To dilute, always add acid to water; do not add water to acid.**
- Ethanol is flammable! **Avoid open flames.**
- Always use a blank!
- Most detergents interfere with the Bradford Assay.
- Most reducing agents do not significantly interfere with the assay.
- Avoid quartz cuvettes because CBBG can bind to the quartz.

## References

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1), 248-254.

Stoscheck, C. M. (1990). Quantitation of protein. *Methods in enzymology*, *182*, 50.