

## Bradford Protein Assay

### Quiz:

- 1) What is the application of Bradford assay?
  - a) Isolation of DNA
  - b) Determination of protein concentration
  - c) Protein purification
  - d) Separation of proteins
- 2) In Bradford protein assay, what is the dye used in the experiment?
  - a) Benedict's reagent
  - b) Coomassie brilliant blue
  - c) Methylene blue
  - d) Ethidium bromide
- 3) In Bradford protein assay, when Bradford dye reagent binds protein, the dye color will change from?
  - a) Blue to Red
  - b) Red to Blue
  - c) Yellow to Blue
  - d) Blue to Yellow
- 4) At which of the below wavelength that absorbance is maximum when Bradford dye reagent binds protein?
  - a) At 295nm
  - b) At 320nm
  - c) At 595nm
  - d) At 800nm
- 5) Which of the following statements is false about Bradford assay?
  - a) This method relies on the dye binding to protein.
  - b) At low pH, the free dye has absorption maxima at 470 and 650nm.

- c) The practical advantage of the method is that many proteins dissolve well in the acidic reaction medium.
  - d) To read the result, the more intense color means the more protein is present.
- 6) What is the purpose of the Bradford Assay in this practical test?
- a) To measure the protein concentration
  - b) To determine the enzyme activity
  - c) To create a standard curve
  - d) To dilute the unknown sample
- 7) How many standards should be made for the standard curve using Bovine Serum Albumin (BSA)?
- a) 1 standard
  - b) 2 standards
  - c) 3 standards
  - d) As many as necessary for an accurate and reliable standard curve
- 8) What is the purpose of preparing dilutions of an unknown solution in water for a Bradford assay?
- a) To maximize the likelihood of obtaining an absorbance reading within the accepted range for absorbance
  - b) To minimize the likelihood of obtaining an absorbance reading within the accepted range for absorbance
  - c) To calculate the concentration of the unknown solution
  - d) To determine the enzyme activity of the unknown solution
- 9) What does an  $R^2$  value indicate in the context of a standard curve?
- a) The reliability and validity of the data
  - b) The concentration of the unknown enzyme solution
  - c) The absorbance reading of the unknown solution
  - d) The dilution factor of the unknown solution

10) Which amino acids are primarily involved in the binding of Coomassie dye in the Bradford assay?


- a) Alanine and Glycine
- b) Arginine and Lysine
- c) Aspartic acid and Glutamic acid
- d) Leucine and Isoleucine

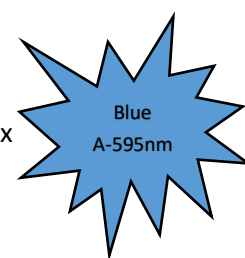
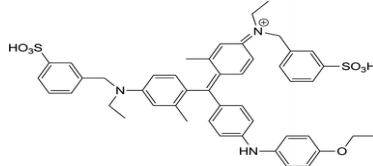
### **Objective:**

To find the concentration of protein using Bradford Assay.

### **Principle:**

The Bradford protein assay is based on the principle of the binding of a dye molecule i.e., Coomassie Brilliant Blue, to proteins results in a shift in the dye's absorption spectrum. In this assay, differential color change of a dye occurs in response to various concentrations of protein. The absorbance maxima of an acidic solution of Coomassie Brilliant Blue G-250 dye shifts from 465nm to 595nm when binds with protein, that results in a change in color from brown to blue. The Coomassie Blue dye binds to primarily basic and aromatic amino acid residues, especially arginine. Spector found that the extinction coefficient of a dye-albumin complex solution was constant over a 10-fold concentration range. Thus, Beer's law may be applied for accurate quantification of protein by selecting an appropriate ratio of dye volume to sample concentration. The amount of protein in a sample (milk protein) is determined by measuring the absorbance of the blue-colored solution at 595 nm and comparing it to a standard curve generated using known concentrations of protein (BSA).

Protein (Basic and aromatic side chains) + Coomassie Brilliant blue G-250 dye  Protein-dye Complex



### **Theory:**

Bradford protein assay is popular due to its ease of performance and relative sensitivity. It can detect as little as 1 microgram of protein in a sample that makes it suitable for use with small amount of protein, such as those obtained from cell culture or tissue samples. The assay is also compatible with a wide range of sample types, including serum, plasma, cell lysates, and

purified protein solutions.

Accurate protein concentration in unknown samples can be determined by using a standard curve plotted for known protein concentrations versus absorbance values of the analyte. The assay's sensitivity and dynamic range make it suitable for detecting protein concentrations between 1-200  $\mu\text{g/mL}$ .

- Mechanism of CBBG dye binding to proteins:

The Coomassie brilliant blue G-250 (CBBG) dye binds to proteins through ionic and hydrophobic interactions, primarily targeting basic amino acid residues such as arginine and lysine in the protein, which are abundantly present in most proteins. The dye-protein binding is also influenced by the hydrophobic interactions and Van der Waals forces. The binding process is rapid and stable.

- Color shift mechanism and its relation to protein concentration:

When CBBG binds with proteins, it undergoes a visible color shift from brown to blue. It occurs due to the transition of the dye from its acidic form (brown; maximum absorption at 465 nm) to its basic form as a protein-dye complex (blue; maximum absorption at 595 nm). This color shift occurs due to the light absorbed by the bound dye, maximally at 595 nm. The degree of color shift is directly proportional to the concentration of protein. As protein concentration increases, more CBBG binds, resulting in a deeper blue color hence more absorbance.

## **Materials Required:**

- Apparatus and equipments:

For standard assay:

- Spectrophotometer
- Cuvettes with 1cm path length matched to laboratory spectrophotometer. (Disposable polystyrene cuvettes are recommended)
- 13 x 100mm test tubes/ Eppendorf tubes
- Vortex Mixer
- Whatmann #1 filter (or equivalent) and funnel for dye reagent preparation
- Graduated cylinders,
- pipettes

- Chemicals and reagents:

- 1x Bradford reagent

- Protein sample
- 1x PBS
- Bovine serum albumin (BSA) standard solutions

### **Procedure:**

1. Prepare the Bradford reagent according to the manufacturer's instructions. Briefly, the Bradford reagent can be prepared by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 mL of 95% ethanol. Then, add 100 mL of 85% phosphoric acid and 850 mL of distilled water. Mix well and store the solution in a brown glass bottle.
2. Prepare a series of BSA standard solutions of known concentrations (e.g: 20, 40, 60, 80, 100  $\mu\text{g/mL}$ ) by diluting a stock solution of BSA in distilled water.
3. Prepare 100  $\mu\text{L}$  of 1:50 diluted protein sample in PBS. For this, pipette 98  $\mu\text{L}$  of 1x PBS and 2  $\mu\text{L}$  of sample into the eppendorf tube and mix it. This mixture has diluted the sample to one part in 50. The concentration of protein sample should be within the linear range of the standard curve.
4. Next, take two cuvettes and label them as 'blank' and the other 'sample.' Add 20  $\mu\text{L}$  of diluted (1:50) protein sample to the sample cuvette and 20  $\mu\text{L}$  of 1x PBS to the blank cuvette.
5. Serially dilute the protein standards i.e. BSA. Label one cuvette each for the standards and label it appropriately. For the protein standard, 20  $\mu\text{L}$  of each is added into the corresponding cuvette, followed by 1 mL of 1x Bradford reagent to all the cuvettes including blank and sample and mixed thoroughly.
6. After incubating for 5 minutes, measure the protein concentration using a spectrophotometer. Use a blank to set the spectrophotometer to zero absorbance or 100% transmittance. Then, measure the absorbance values of the protein standards and sample at 595nm.
7. Create a standard curve by plotting the absorbance values measured at 595 nm (y-axis) against the corresponding protein concentrations in  $\mu\text{g/mL}$  (x-axis). Visually compare the cuvette visually compare the cuvette Use the standard curve to determine the protein concentration in your sample.

- Data analysis and interpretation:

Accurate data analysis is vital for obtaining reliable results in the Bradford assay. The process involves generating a standard curve to establish the relationship between protein concentration and absorbance that serves as the reference for determining the concentration of unknown samples. Proper interpretation of the data requires careful consideration of factors that may influence accuracy and reproducibility.

- Generating and analyzing a standard curve:

A standard curve is generated by plotting the absorbance values (at 595 nm) against the known protein concentrations of protein standards (typically BSA). The data for a curve is generated using the following range of concentrations of the BSA standard: 0, 20, 40, 60, 80, 100 µg/ml. Linear regression analysis is used to fit the data and ensure a strong correlation coefficient ( $R^2 > 0.95$ ) for the best results. The standard curve should be linear within the chosen concentration range.

- To determine the protein concentration in an unknown sample, follow these steps:

- 1) Record the absorbance of the unknown sample at 595 nm.
- 2) Apply the linear regression equation derived from the standard curve,  $y=mx+c$ , where:
  - $y$  = absorbance
  - $x$  = protein concentration
  - $m$  = slope of the line
  - $c$  = y-intercept
- 3) Now calculate the slope ( $m$ ). For this, identify two points on the line (the dashed green line on the standard curve), noting their (BSA concentration, x-axis) and (Absorbance, y-axis) values.
- 4) The y-intercept ( $c$ ) is calculated with one data point on the standard curve. Next, the  $m$  and  $c$  values are used for further calculations using the  $mx+c$  equation.
- 5) Measure the absorbance of your unknown protein sample and place the absorbance value into the standard curve equation. Rearrange the equation to solve for  $x$  (protein concentration) =  $(y - c) / m$
- 6) If the absorbance of the unknown sample exceeds the linear range of the standard curve, dilute the sample and remeasure.

### **Differences Encountered in a Real Laboratory:**

In an actual laboratory setting, there are certain important steps that are not necessarily applicable in a virtual lab:

1. Always wear personal protective equipment when you are in the lab. When you enter the lab, switch on the exhaust fan and make sure that all the reagents required for the experiment are available. If it is not available, prepare the reagents using the components shown in the reagent preparation.
2. Ensure that the Bradford reagent is freshly prepared and stored in a brown glass bottle to prevent photodegradation.
3. The Coomassie dye reagent contains acidic components that may irritate the skin and eyes.
4. Dispose of waste solutions according to local regulations.
5. Avoid exposure to open flames.
6. Wash hands and equipment after completing the assay.

### **Factors Affecting Data Accuracy:**

Several factors, such as;

- the presence of detergents and ampholytes in the reagents
- CBBG dye purity
- pH

can impact the accuracy of Bradford assay results. To minimize errors and ensure reliable results:

- Freshly prepare or purchase CBBG dye reagents and protein standards of high quality.
- Use compatible buffers, avoid detergents, and centrifuge samples.
- Regularly calibrate the spectrophotometer and pipettes.
- In some instances of inaccurate results, the standard can be prepared in the same buffer with potential interfering substances. The sample may be desalted or dialyzed to remove small interfering substances ( $< 3$  kDa).
- Average multiple readings to reduce variability.
- Ensure  $R^2 > 0.95$  and check for deviations.

### **For Reading:**

[https://pubs.acs.org/doi/pdf/10.1021/acs.jchemed.1c00756?ref=article\\_openPDF](https://pubs.acs.org/doi/pdf/10.1021/acs.jchemed.1c00756?ref=article_openPDF)

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