

Bradford Protein Assay Standard Curve Protocol

Materials:

1. **Bradford Reagent (Coomassie Blue):** For determining protein concentration
2. **Micropipette tips:** For measuring small volumes of reagents or bacterial cultures
3. **Microfuge tubes of various sizes:** For containing small volumes of reagents or bacterial cultures
4. **Microfuge tube rack:** To hold microfuge tubes.
5. **Bradford Reagent waste container:** This is the container you will put all Bradford Assay waste into.
6. **Sharpie markers:** For correct labeling of samples.
7. **Albumin protein standard:** A solution of albumin at 2,000 ug/mL that will be used to create a protein concentration standard curve.
8. **Elution Buffer:** The buffer your RFP or GFP was eluted in, which will be used as the blanking solution and also as the diluent for the serial dilution of albumin.
9. **5 mL Falcon Tubes:** For preparing mixing the Bradford reagent and the Albumin protein in
10. **Cuvettes:** For measuring the OD595 of your standard curve samples in the spectrophotometer
11. **Protein Concentration Standard Curve Report:** A document on which to report your standard curve data

Equipment:

1. Spectrophotometer
2. Micropipettes - p1000 and p200

Protocol:

Done by the Process Engineers. Dispose of all waste properly.

You are STRONGLY ENCOURAGED to check off each step below as you complete it.

1. Remove the tube containing the Bradford reagent from the refrigerator and allow it to come to room temperature.
2. Turn on the spectrophotometer to allow it to warm up.
3. You will need to create a 1:2 serial dilution of the albumin standard (2,000ug/mL).
4. Obtain six 500uL (small) microfuge tubes and label them as follows:
 - a. 1,000 ug/mL
 - b. 500 ug/mL
 - c. 250 ug/mL
 - d. 125 ug/mL
 - e. 62.5 ug/mL

5. Using a p200 micropipette, put 40uL of Elution Buffer in each microfuge tube. See Fig. 1.

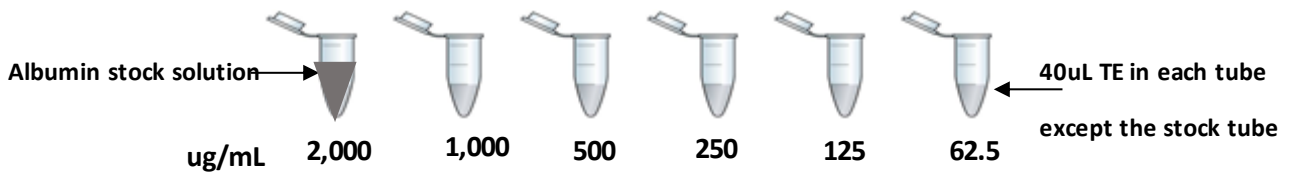


Fig. 1

6. Using a p200 micropipette, remove 40uL of the albumin protein standard (2,000 $\mu\text{g/mL}$) from the tube it is in. Mix this into the Elution Buffer that is in the tube labeled 1,000 $\mu\text{g/mL}$. You have now diluted the albumin 2-fold, so it is at 1,000 $\mu\text{g/mL}$.
7. Continue with the serial dilutions until you have completed the dilution labeled 62.5 $\mu\text{g/mL}$. See Fig. 2.

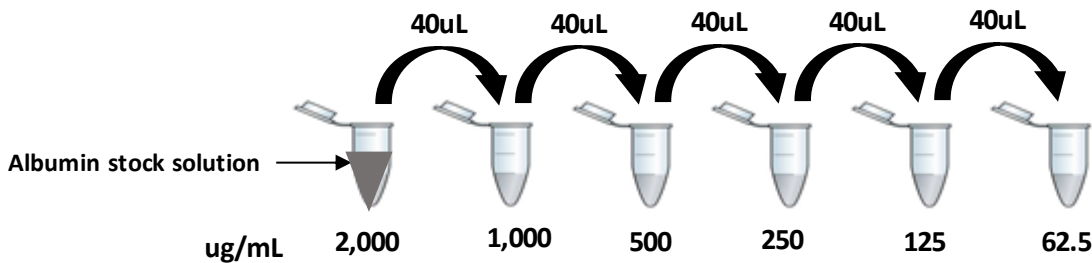


Fig. 2

8. Obtain seven 5mL Falcon tubes. Label them as follows:
- 2,000 $\mu\text{g/mL}$
 - 1,000 $\mu\text{g/mL}$
 - 500 $\mu\text{g/mL}$
 - 250 $\mu\text{g/mL}$
 - 125 $\mu\text{g/mL}$
 - 62.5 $\mu\text{g/mL}$
 - 0 $\mu\text{g/mL}$
9. Remove their caps and set them in a rack. Using a p200 micropipette, put 30uL of the albumin standard in the Falcon tube labeled '2,000'.
10. Using a p200 micropipette, put 30uL of the 1,000 $\mu\text{g/mL}$ solution into the Falcon tube labeled '1,000'. Continue this process until you have put 30uL of each serial dilution solution into the appropriate Falcon tubes.
- NOTE: Be sure to change tips between each sample.

11. Using a p200 micropipette, put 30uL of Elution buffer into the Falcon tube labeled '0'. This is the tube contains the 'blank' solution.
12. Using a p1000 micropipette, add 1.5mL of Bradford reagent to each tube. Cap each tube tightly and invert twice to mix well. Allow the tubes to sit at room temperature for 10 minutes before reading the samples in the spectrophotometer.
13. Using a p1000 micropipette, transfer the contents of the '0' tube to a clean cuvette.
14. Set the spectrophotometer wavelength to 595 nm.
15. Use this cuvette to 'blank' the spectrophotometer. Save this cuvette when you need to blank the spectrophotometer when teams are measuring the concentration of their purified protein.
16. Using a p1000 micropipette, transfer the contents of the '62.5' tube to another clean cuvette. Read the absorbance of this solution and record it on the Protein Concentration Standard Curve Report.
17. Remove the solution from the cuvette and put it back in the tube labeled '62.5'. Use this same cuvette to read the next sample.
18. Repeat steps 16 and 17 for each of the remaining standard curve tubes.
19. Remain ready to read samples for each team.
20. If teams will read their samples during another class period, give the blank tube to the teacher to cover with parafilm to keep for use as a blank.
21. Complete the Protein Concentration Standard Curve Report and make it available to all teams.
22. Each team will use the standard curve data and the Generation of a Standard Curve protocol to plot the standard curve and obtain the equation of the line.