

Downstream Process – Measuring Purified Protein Concentration

Materials:

1. **Bradford Reagent (Coomassie Blue):** For determining protein concentration
1. **Micropipette tips:** For measuring small volumes of reagents or bacterial cultures
2. **Microfuge tubes of various sizes:** For containing small volumes of reagents or bacterial cultures
3. **Microfuge tube rack:** To hold microfuge tubes.
4. **Coomassie waste container:** This is the container you will put all Bradford Assay waste into.
5. **Sharpie markers:** For correct labeling of samples.
6. **Albumin protein standard:** A solution of albumin at 2,000 ug/mL that will be used to create a protein concentration standard curve.
7. **Elution Buffer (TE):** 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.
8. **Small Falcon Tubes:** For preparing mixing the Bradford reagent and the Albumin protein in
9. **Cuvettes:** For measuring the OD595 of your purified RFP or GFP in the spectrophotometer
10. **Protein Concentration Standard Curve Report:** A document on which the Process Engineers previously reported the standard curve data, the graph of this data and the equation of the best fit line of the data.

Equipment:

1. Spectrophotometer
2. Micropipettes
3. Heat Block

Protocol:

Done by the Quality Control Technician. The Process Engineers will tare (blank) the spectrophotometer.

The QC Technician and Process Engineer should work with their team members to carry out this procedure.

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. (Process Engineers do this.)
3. Turn on the heat block and set it to 85°C. Use a functional thermometer to verify that the block is actually at 85°C . (Process Engineers do this.)

4. The wavelength of red or green fluorescence interferes with the OD595 readings. For this reason, you will heat denature your purified RFP (or GFP) prior to measuring the protein concentration of the purified protein with the Bradford assay.
5. Using a p200 micropipette, remove 100uL of the purified RFP (or GFP) and put it into a fresh screw cap microfuge tube. Label this tube HD (for heat denatured). A screw cap tube is used so that if pressure builds up in the tube during heating, the lid will not pop off.
6. Place the tube in the 85°C heat block for 5 minutes. When you pull it out the purified protein should be clear rather than pinkish (RFP) or greenish (GFP). Why do you think the color has changed?
7. Obtain a small Falcon tubes. Label it HD RFP (or GFP).
8. Remove the caps and set it in a rack. Using a p200 micropipette, put 30uL of the heat denatured protein in the Falcon tube.
9. Using a p1000 micropipette, add 1.5mL of Bradford reagent to the tube. Cap the tube tightly and invert twice to mix well. Allow the tube to sit at room temperature for 10 minutes before reading the sample in the spectrophotometer.
10. Set the spectrophotometer wavelength to 595 nm.
11. Use the blank cuvette from the preparation of the albumin standard curve to 'blank' the spectrophotometer. If you do not still have the blank from the previous collection of standard curve data, make a new one.
 - a. Using a p200 micropipette, put 30uL of Elution buffer (TE) into a Falcon tube. Using a p1000 micropipette, add 1.5mL of Bradford reagent to the tube. Cap the tube tightly and invert twice to mix well. Allow the tube to sit at room temp. for 10 minutes before transferring the sample to a cuvette and using it to blank the spectrophotometer.
12. Using a p1000 micropipette, transfer the contents of the HD tube to a clean cuvette. Read the absorbance of this solution and record it on the Downstream Process Batch Record.
13. Remove the cuvette from the spectrophotometer. Dump the Coomassie solution into the Coomassie waste container. Dispose of the cuvette in biohazard waste.
14. Use the OD595 reading from your sample and the equation of the line from the albumin standard curve assignment to calculate the concentration of your purified RFP (or GFP). Record this value on the Downstream Process Batch Record.

15. Using the total volume of your purified RFP sample (eluate) recorded previously and the protein concentration of your sample, calculate your total protein yield. Record this value on the Downstream Process Batch Record.
16. Complete the Downstream Process Batch Record and file it in your Team file.