Notes specific to BTEC 2020’s first Bradford assay

* You will be performing a Bradford assay on the same protein you used for your first SDS-PAGE gel. The protein is diluted in water.
* To ensure you get an absorbance reading within the range of the assay, you will need to make dilutions of your protein sample. I suggest you run the assay on undiluted protein, as well as 1/2 and 1/5 dilutions. This means you will have 3 experimental samples.
* Each sample should be run by triplicate, which means three identical reactions per every sample examined.
* Make sure you report the original concentration of the protein in the tube I gave you. (For example, if your 1/2 diluted sample has a concentration of 200 µg/mL, then the original is 400 µg/mL.)
* After determining your protein concentration, calculate what volumes of protein solution contain 1, 2, 5, and 10 µg of protein. Tentatively, you will be loading those amounts of protein on your SDS-PAGE next time.

# Bradford Assay Protocol

The Bradford Assay allows estimation of the protein concentration in a sample on the basis of comparison to known concentrations of a standard protein, often BSA (bovine serum albumin). The best estimates of concentration are obtained when the sample concentration is within the linear range of the assay, and when the sample concentration is higher than the lowest standard concentration, but lower than the highest standard concentration. *It may be necessary to make dilutions of your sample, or change the range of your standards, to obtain these results.* Also, the protocol below explains how to make a single set of protein standards, but, as always, greater accuracy can be obtained from repeated trials. Depending on the accuracy required, you may wish to make your standards (and samples) in triplicate, as previously mentioned.

Caution: The dye reagent contains phosphoric acid and is slightly caustic. Promptly rinse any spills. Be sure to use only plastic, disposable cuvettes for this assay. The dye is very difficult to remove from quartz cuvettes.

## Preparation of BSA standards

Dilute standards with the same buffer in which your sample is diluted (H2O). Label 5 disposable plastic cuvettes: 300, 150, 75, 37.5, and 0. The previous numbers refer to the concentration (in µg/mL) of the standard protein in each cuvette prior to the addition of dye. Carry out the serial dilution of the BSA stock as indicated below.

1. Start with a stock of BSA that is 300 µg/mL. Put 20 µL of that stock into the disposable plastic cuvette labeled 300.
2. Put another 20 µL of the 300 µg/mL BSA stock into the cuvette labeled 150. Add 20 µL of diluent. (This will yield 40 µL of 150 µg/mL BSA.) Mix well by pipetting up and down.
3. Take 20 µL from cuvette 150, and move it to cuvette 75. Add 20 µL of diluent. (This will yield 40 µL of 75 µg/mL BSA.) Mix well by pipetting up and down.
4. Take 20 µL from cuvette 75, and move it to cuvette 37.5. Add 20 µL of diluent. (This will yield 40 µL of 37.5 µg/mL BSA.) Mix well by pipetting up and down.
5. Take 20 µL from cuvette 37.5, and dispose of it. (This will leave only 20 µL in cuvette 37.5, which is the same volume as in the other cuvettes.)
6. Put 20 µL of diluent (no protein) into cuvette 0.

## Performing the Bradford assay

1. Dilute Bio-Rad Protein Assay Dye Reagent Concentrate 5-fold with ddH2O. (1 part dye concentrate + 4 parts ddH2O). You will need 1 mL per cuvette (5 cuvettes for standards + however many experimental cuvettes you have. In our case we should have 3 dilutions X triplicate = 9).
2. Put 20 µL of each experimental sample into its own disposable cuvette. *(Remember that you should make several dilutions of your samples to ensure that one reading is in the usable range.)*

Undiluted X 3

1/2 dilution X 3

1/5 dilution X 3

1. Each cuvette should now contain 20 µL. Put your cuvettes in order: standards - lowest to highest concentration - then experimental samples, lowest to highest concentration. Add 1 mL of diluted dye to each cuvette, in order. Mix well. Wait at least 10 minutes, but not more than 1 hour, to allow the color change to develop before measuring absorbance at 595 nm. Measure absorbance in the same order as you added the dye to the cuvettes, to maintain *equal incubation time for each sample*.

## Using the Bio-Rad SmartSpec Plus

1. Press the Lambda (λ) button.
2. When it asks for the number of wavelengths to be read, enter 1 using the keypad, and press Enter.
3. Enter 595 as the wavelength to be read.
4. Select NO background correction.
5. When it is ready to read absorbance, insert your 0 BSA standard, and press the “Read Blank” button. Be sure that the cuvette is oriented properly. Our cuvettes have arrowheads on one side. Always point that arrowhead in the same direction as the arrowhead in front of the cuvette slot. If there’s a danger of fingerprints on the cuvette, wipe it off before reading.
6. After the blank is read, remove the cuvette, and press the right arrow button to continue. Don’t throw away this or any cuvette until your data are in hand.
7. Insert your next standard, and press “Read Sample”. You can write down the absorbance value, or wait until the end and print.
8. Continue on until all your standards, and all your experimental samples, have been read. Be sure to note the order in which you read them!
9. When you have read all your cuvettes, press enter, then the left arrow key to exit the assay. It will ask if you want to print; if so, press Select to toggle to yes, then Enter. If you do not write down or print the report before another assay key is pressed, the data will be lost. To protect yourself in case of this occurrence, do NOT throw away your cuvettes after each reading. Save them until you have the data in hand!
10. If you have printed out your results, use care when taping the printout into your notebook. Use clear (scotch-type) tape (it holds longer than lab tape), but tape only along the edges of the paper; DO NOT TAPE OVER THE PRINTING. Scotch tape on these printouts causes the ink to lighten dramatically, making your data unreadable!

## Graphing in Excel 2007

1. Open a new workbook. In column A, put the heading “µg/mL protein”, and then enter your standard concentrations (0, 37.5, 75, 150, 300) in the rows below.
2. In column B, enter the heading “A595”, and your absorbance values in the rows below, making sure that the values in columns A and B match up. Note that the absorbance for 0 protein is 0; though you didn’t get an explicit reading for 0 protein, you forced it to be 0 when you used it as a blank.
3. Highlight all the cells in which you have entered column headings and data.
4. Click on the Insert tab, then on Scatter under Charts. Click on the scatter plot without any lines.
5. You should now have a graph. Under Chart Layouts, choose Layout 1. Fill in the appropriate axis titles, with units as appropriate. Fill in the chart title with something meaningful, including the date. You will want to include this graph in your notebook, so label well.
6. Right-click on one of the data points on your graph, then select Add Trendline.
7. Select Linear as the trendline type (you could do others, but the subsequent math will be harder). At the very bottom, check the options to Display Equation and Display R-squared. Close this window.
8. You should now have a line on your graph, and an equation for that line. The R2 value tells you how good the fit is; the closer to 1, the better.
9. The equation is in the format y=mx+b, with m and b replaced with numbers. Here, y represents A595 values, and x protein concentrations. Plug in the A595 value of your sample for y, and then solve for x to determine its protein concentration.
10. Remember that a standard curve is useful only for the range in which you have data. For example, if the highest absorbance value you got from the standards was 0.9, and your experimental sample’s absorbance was 1.2, it is NOT appropriate to extrapolate the curve out to 1.2. You will need to start over, diluting your experimental sample so that its absorbance will be lower in the next trial. (You should also make new standards, and a new standard curve, because differences in incubation time affect results.)

Modified by Alejandro, July 26th, 2017