

Bradford Assay for Protein quantification

To measure the protein concentration in an extract use the dye-binding assay of Bradford:

(A) The Assay:

(1) Dilute the Bradford reagent fivefold in dH₂O (1 part Bradford: 4 parts dH₂O). Filter the diluted reagent through Whatman 540 paper (or equivalent; Eric uses the Millipore filtration unit). Store the diluted Bradford reagent at 4°C.

(2) Add 10-20 µl of the protein extract to 1 ml of the diluted reagent and mix. Measure the blue color formed at the wavelength 595nm. If using cuvettes, use the disposable plastic cuvettes; if you use the Beckman DU 640 spec housed in Al Barta's lab, then you have the option of using the sipper, which means that the sample can be sucked right out of a microfuge tube directly into the spec eliminating the need to use cuvettes. The minimum volume for the sipper is 800µl, the set of microcell cuvettes can read as little 100µl.

(3) Prepare a standard curve using a serial dilution series (0.1-1.0 mg/ml) of a known protein sample concentration; e.g., BSA dissolved in whatever buffer your protein is in.

Note that the operation of the spec below is base on ROUTINE MEASURE at a FIXED WAVELENGTH. However the DU640 also has the capability to measure protein concentrations, DNA & RNA concentrations and create the standard curves for the user; I do not yet know how to work these functions. However there is a manual for the spec in the drawer and it should explain how to use these functions.

(B) Operating the Beckman DU 640 spec:

(1) Turn on the power. The switch is located in the back on the upper right hand corner. Allow the machine to warm up for about 5min. The machine should complete a self test.

(2) Click quit in the upper right hand corner of the screen.

(3) Under ROUTINE MEASURE, click on FIXED WAVELENGTH.

(4) In the top row click on SAMPLING DEVICE. Select choice; e.g., ONE CELL for a single measurement, AUTO SAMPLER for the 6-cuvette cell, SIPPER for the sipper, etc. Click OK.

(5) In the row SAMPLE ID, enter the first wavelength (λ) number by entering this value into the blue calculator keypad that pops up. Enter as many as 3 λ s; e.g., 260, 280 and 320 for DNA & RNA)

(6) In the bottom tool bar, in the top row turn on the lamp by clicking in the lamp you need; e.g., VIS ON (for wavelengths greater than 420) and/or UV ON (for wavelengths less than 320).

(a) Using the 6 cell rack:

(i) Place up to 6 cuvettes into the cell holder.

(ii) The blank goes in the back, cell # 1.

(iii) In the bottom tool bar, in the top row, click on BLANK. The machine should now auto zero.

(iv) It will continue reading all 6 cells. If not then right click anywhere on the screen and it will then take the readings.

(b) Using the Sipper:

(i) Be sure the cuvette attached to the sipper is in the last cell (#1), arrow down and on your left. Note that there are four buttons in front of the sipper that indicate each of their functions:

RETURN	FILL	FILL	FLUSH
	BLANK	READ	

(ii) Condition the flowcell. All solutions are provided next to the spec. If you finish one, replenish it. All of the following entail inserting the sipper tube into the indicated solution and pressing the FLUSH button.

- (a) Rinse with dH₂O.
- (b) Flush with air.
- (c) Rinse with the spectral-grade methanol.
- (d) Flush with air a second time.
- (e) Rinse with dH₂O.
- (f) The cell is now conditioned and ready to use.

(iii) Fill the blank by inserting the sipper tube into your blank solution and pressing the FILL BLANK button.

(iv) FLUSH the line with air.

(v) Insert the sipper tube into the solution to be read and press the FILL READ button.

(vi) FLUSH the line with air (or dH₂O or blank solution).

(vi) Proceed to the next sample.

(vii) PRINT the readings.

(viii) When finished rinse out the flow cell:

- (a) FLUSH with ~ 1ml dH₂O.
- (b) FLUSH with ~ 2ml 10% Trace-Klean solution.
- (c) FLUSH with > 5ml dH₂O.
- (d) FLUSH with 95% dH₂O – 5% methanol.
- (e) Be sure the line (and cell) is left containing the 95/5% dH₂O/methanol.
- (f) Before the next use flush with methanol and then dH₂O.

(ix) Click on QUIT when completed and turn off the computer/spec.

Note:

Using the sipper with Bradford reagent is always a little bit tricky. This is because the Bradford reagent will stick to the tubing. To minimize this problem I do the following:

- (1) Turn on the Spec as described under spec operations and let go through its initialization phase (about 2 minutes).
- (2) Turn on the vis lamp
- (3) Set all ODs (in this instance to 595)
- (4) Choose the sipper
- (5) Place the sipper cuvette into the slot at the **Back** of the cuvette holder (furthest from you)
- (6) Condition the flow cell as described under spec operations.
- (7) Run (**FLUSH**) several mls of ddH₂O through.
- (8) Run (**FLUSH**) several mls of the Bradford reagent through. You'll notice that the tubing picks up a blue color. This is good because it will help to minimize variability later.
- (9) Run (**FLUSH**) several mls of ddH₂O through.
- (10) Now fill the blank by inserting the sipper tube into one of your blank solutions (be sure there is a minimum of 1.0ml of blank, usually I add some of a blank to another to be sure when that sipper sucks, it gets

what it needs) and press the FILL BLANK button. It takes about 30 seconds for the machine to complete this process.

- (11) Run (**FLUSH**) several mls of ddH₂O through.
- (12) Insert the sipper tube into the solution to be read and press the FILL READ button.
- (13) After this is complete, **FLUSH** several mls of ddH₂O through.
- (14) Insert the sipper tube into the next solution to be read and press the FILL READ button.
- (15) Repeat this cycle, **FLUSH**ing several mls of ddH₂O through between each sample reading.

Read several of your blank samples as well. Usually I do this after reading all of my samples. Most protein samples come out at 0.2 to 0.6. A lot of times when you run a blank sample invariably as high as 0.02 (or higher). I cannot seem to get around this. Thus these numbers that we get from the blank will be averaged and then that average will be subtracted from each samples' OD₅₉₅ reading.

Be sure to print this and to annotate the readings with the sample. This then will get entered into an excel spreadsheet.

When you are completely done it usually takes a bit of effort to rid the tubing of the Bradford reagent sticking to it. Usually I can accomplish this by two cycles of first rinsing with several mls of ddH₂O, then flushing several mls of Methanol through, then rinsing with ddH₂O, then the Trace Clean solution, then rinsing with ddH₂O...

Reagents and Supplies needed:

- (1) Bradford reagent (BioRad Protein Assay Dye Reagent; cat# 500-0006; kept at +4°C in the Deli).
- (2) dH₂O
- (3) Filtration set up
- (4) Microfuge tubes, tips and (cuvettes)
- (5) Spec

Reference:

Bradford, M.M. (1976) A dye binding assay for protein. *Anal. Biochem.* **72**:248-254.