Protein quantification – E. Vierling's method modified for 96-well plate use
Modified by Jessie 4-1-08

With one 96-well plate, you can quantify up to 88 protein samples with 6 standards and 2 negative controls.

Equipment and Supplies:

- BSA Stock Solution 8ug/uL in protein extraction buffer
- Sample buffer used in protein extractions
- Coomassie blue stain (see recipe below)
- Coomassie destain (see recipe below)
- 2% SDS Solution
- Filter Paper
- 96 well plates, flat bottomed
- 3 small containers (for destaining)
- Hole punch
- Spectrophotometer
- Shaking/rotating platform

1. **Make up BSA standards of 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 ug/uL:**
   a. Start with stock of 8.0 ug/uL diluted in sample buffer, NOT water
   To change concentrations or final volume, see spreadsheet "serial dilutions calc.xls"
   b. label 6 0.5mL microfuge tubes with the 6 concentrations and date
   c. make up the dilutions according to the chart below

Standard concentrations (ug/ul)	volume of stock solution to add (ul)	volume of solvent to add (ul)	final volume (ul)
0.5	1.875	28.125	30
1	3.75	26.25	30
2	7.5	22.5	30
4	15	15	30
6	22.5	7.5	30
8	30	0	30

   d. After using, store standards in the refrigerator. Since we use 2 ul per sample, this should be enough for 14-15 protein concentration quantifications.

2. **Plan 96-well plate and record a well # for each sample including the 6 standards and 2 negative controls.**
   a. for now, record the well # and sample ID or standard concentration next to the well in a spreadsheet *Once we have our own spec up and running, we should be able to enter this into a spreadsheet so that the absorbances will be matched up with each sample.*

3. Set up the Plate

a. Cut a piece of filter paper to just smaller than a 96-well plate.
b. Cut a small triangle off of the top right corner. This will allow you to orient your paper correctly later.
c. Place the filter paper on top of a regular 96 well plate and under the 96-well plate with the bottoms cut out. This plate will simply serve as a guide for pipetting into the correct place.

4. Pipette samples onto filter paper.

a. Spot 2ul of each standard and each sample onto the corresponding well of the 96-well plate onto the filter paper.
b. Change pipette tip between EVERY replicate and EVERY sample!
c. Allow filter paper to dry thoroughly. *(fans or hood?)*

5. Coomassie Blue Stain:

a. Place the filter paper in a plastic box.
b. Pipette enough Coomassie Blue to just cover the paper, 5-10 mL.
c. **DO NOT** pipette directly onto stain spots!!!
d. **DO NOT** shake or agitate during staining!!!
e. Stain for 15 minutes.

6. Destaining

a. Prepare 3 baths of destain. *I need to find small containers for this.*
b. Using forceps, remove filter paper from 96-well plate lid.
c. Place into bath.
d. Secure bath to shaking/rotating platform.
e. Shake/Rotate for 15-20 minutes in first bath.
f. Repeat for next 2 baths.
e. Remove papers from final bath and dry in test tube racks (time)?

7. Protein elution

a. Get a clean 96-well plate
b. Fill each well that you will use with 300 uL of 2% SDS.
c. Orient the filter paper with the triangle cut out at the top left corner. You should be able to see light blue protein "spots" in the 96-well pattern.
d. With a hole punch, cut out each spot from the filter paper and place into the appropriate well.
e. Negative Control: punch out a circle of filter paper from the edge of the paper (where there should be no protein).
f. cover plate tightly with a plate seal so that samples cannot spill into other wells.
g. Place tubes in rotator overnight.

8. Read absorbance of samples – if using the Tonsor Lab microplate reader, see the microplate reader protocol for this step. (microplate spec prtcl.doc)

a. set spectrophotometer to a wavelength of 595 nm
b. the negative control should be used as a "blank."
c. Read and record absorbance of all samples..

9. Protein sample quantification

a. Graph the BSA standards absorbance against the concentration:
the easiest way is to plot concentration on the y axis and absorbance on the x-axis.
b. perform a linear regression and calculate r^2 and the equation of the line. In excel:
i. graph absorbance vs concentration with a scatterplot
ii. label axes and create title
iii. click on the points on the scatterplot
iv. go to chart then choose "add trendline."
v. Under type, choose linear. Under options, choose "display equation on chart" and "display r^2 value on chart".
vi. click ok
c. calculate protein concentrations of samples *(if your r^2 value is less than 0.95, be aware that your concentrations are not as accurate as they should be)*
i. if you plotted concentration on the y-axis and absorbance on the x axis, the concentration of each sample can be calculated by substituting the absorbance of the sample in for x.

Recipes

Coomassie Stain Solution (1L)
450 mL Methanol
450 mL dd H_2O
100 mL glacial Acetic Acid
2.0 g of Commassie Brilliant Blue **R-250**

Destain (5 L)		**Concentration**
Methanol	1.5 L	30% by volume
Glacial Acetic Acid	500 mL	10% by volume
ddH_2O	3 L	60% by volume