Coomassie Plus Assay for Measuring Concentration of Purified Protein
Jenny Chien and Daniel Haeusser, September 2006

Prepare Samples:
1. Make 1mL 10 mg/mL BSA in buffer appropriate for the purified protein, and divide into 500uL aliquots
2. Make 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, and 1.0 mg/mL BSA solutions from the stock solution (at least 25uL of each). All dilutions should be in appropriate buffer.
3. Dilute purified protein sample with appropriate buffer to 1:1, 1:5, 1:10, 1:20 (or other), at least 25uL each dilution.

Load into 96-well Plate:
1. Load 10uL of blank (buffer), BSA standard, and samples into a 96-well plate. Load duplicate rows of everything.
2. Add 300uL Coomassie Plus into each well containing 10uL of solution
   * Using the multichannel pipettor (max vol 150uL) can speed up this process
     - Aliquot a slight excess of Coomassie solution into the plastic trough
     - Load all 300uL of Coomassie first, then mix thoroughly in the wells
     - Avoid bubbles! Do not eject liquid all the way to the second stop on the pipettor

96-well plate schematic

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Read:
1. Read the plate using: SoftMax Pro 4.8 □ Assays □ Basic protocols □ Basic endpoint protocols □ Plate □ Setup □ Change Lm1 to 595nm □ Read
2. Within SoftMax Pro 4.8, set the Template: Experiment □ Protocols □ Basic endpoint protocols □ Template
   Highlight the wells and select an ID from the “Group” pulldown menu
   If defining a Blank, select Blank
   If defining a Standard, set the concentration in the upper right
   If defining unknowns, select New
   The group will automatically be given a “Group #” name
   Select “Unknown” from the pulldown menu
   Do not select “Unknown [Dilution]” unless you know what you’re doing
3. If the two duplicate wells are defined as a single Unknown Group, the program will output individual absorbance readings for each well, in addition to an average reading for each Group. Copy these values from the standards and blank into Excel to plot a best-fit curve. Look for an r-value > 0.95.

OD 280 for Measuring Concentration of Purified Protein
If the protein contains tryptophan, concentration may also be measured using OD280 through a quartz cuvette
   ~1:100 dilution generally works
   Cuvette holds 110uL; make a 150uL dilution and load all 150uL into the cuvette
To convert between absorbance and mg/mL, use the protein extinction coefficient (find on the web or with Gene Inspector)