Coomassie Plus Assay for Measuring Concentration of Purified Protein

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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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(blank) (BSA) (BSA)  (BSA) ->
(blank) (BSA) (BSA)  (BSA) ->
( 1:1 ) (1:5) (1:10) (1:20)
( 1:1 ) (1:5) (1:10) (1:20)
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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)