BRADFORD ASSAY

Materials
Spectrophotometer
Glass test tubes, 13 X 100mm
Bradford Reagent
Standard BSA protein solutions

Procedure
In order to determine the unknown concentrations of proteins in solution, you will need to construct a calibration curve using the standard protein solutions that have been previously prepared. The standard solution range is in concentration from 0 micrograms/100 microliters to 100 micrograms/100 microliters.

1. Transfer 100ul of each of the standard calibration protein solution to separate 13 X 100 mm test tubes. Be sure to label the test tubes with the appropriate concentration. (Make sure to label near the top of the tube, so label does not interfere with absorbance). These standards will be used to construct your calibration curve.

2. To each of these test tubes, add 9 mL of Bradford Reagent. Cover the tube with parafilm and gently invert to mix the solutions. Note the color of each of the standard solutions.

3. Follow the instructions for calibrating the spec. (See attached sheet) This is very important since it will affect the absorbance of each solution. Make sure the spec is set to 595 nm before making the reading. The 0 micrograms/100 microliters will be used as the blank. Be sure to wait at least 2 minutes before measuring the absorbance of the calibration solutions.

4. Measure the absorbance from the remaining calibration solutions at a wavelength of 595 nm.

5. Plot the absorbance for each solution on the Y-axis against the concentration (micrograms) of protein on the X-axis. This is the calibration curve.

6. Determine the slope and y-intercept of the curve.

Data Table 1—Calibration Curve Data

<table>
<thead>
<tr>
<th>Protein Concentration (ug)</th>
<th>Absorbance at 595 nm</th>
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