Bradford Protein Assay

Set up of Standards

1. Set up standards in duplicate in 13mm disposable test tubes.
2. The total volume in each tube should always equal 100µL.
3. BSA is used as the protein standard.

Example

The following is an example of the set-up of standards. For this example the standard had a concentration of 0.212µg/L. The tube you are using should have the concentration of the BSA written on it. BSA is stored @-20C, on the freezer door.

<table>
<thead>
<tr>
<th>BSA (µL)</th>
<th>ddH2O (µL)</th>
<th>Concentration (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>2.12</td>
</tr>
<tr>
<td>20</td>
<td>80</td>
<td>4.14</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
<td>6.36</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>8.48</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>10.6</td>
</tr>
<tr>
<td>60</td>
<td>40</td>
<td>12.72</td>
</tr>
<tr>
<td>70</td>
<td>30</td>
<td>14.84</td>
</tr>
</tbody>
</table>

1. Add 1.0mL diluted (1:5) Bradford Reagent per tube. Vortex.
2. Incubate at room temperature for 5 minutes.
3. Spec. each tube at multi-wavelength A595. (Sample extracts should be spec'd. right after standards.)

Set-up of Sample Extracts

1. Sample extracts should be done in duplicate in 13mm disposable test tubes.
2. 100µL of each sample extract is placed in test tube. If a dilution of the extract needs to be made, then ddH2O should be added so that the
final volume is still 100µL in each test tube.
3. Add 1.0mL diluted Bradford Reagent per tube. Vortex.
4. Incubate at room temperature for 5 minutes.
5. Spec. each tube at multi-wavelength A595. The standards and the sample extracts should be set-up at the same time so that the sample extracts can be specd. right after the standard.

Analysis of Samples

1. Set spectrophotometer on Multi-wavelength A595.
2. Calibrate spec. on ddH2O.
3. Read all standards then all sample extracts.

Calculations

\[
mg/mL = \text{mean in g} \times 1.0mg \times 1000L
\]

sample amt. in L 1000g 1.0mL

Solutions

1. Dilute Bradford Reagent (1:5 dilution)

Make a 1:5 dilution of Bradford protein dye to ddH2O.

EXAMPLE: 10mL Bradford Protein dye to 40mL ddH2O

Concentrated Bradford Reagent is stored at 4°C.

Diluted Bradford Reagent should be made fresh, it should not be stored for longer than a week at 4°C.

2. BSA. A stock of BSA of about 1mg/mL should be made.

Aliquot 1mL per microcentrifugr tubes tube.
3. BSA Standard.

   Take 2mL stock BSA 1mg/ml

   8mL ddH2O.

   Spec. 1mL at A260/280.

   \[(A280)\times(1\text{mg/mL}) = \text{prot mg/mL}\]

   \[(0.66)\]

   Aliquot 700µL per tube.

   Store at -20°C.