Bradford Assay

Project: iGEM2026
Author: Ella Bloch
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This protocol is a Bradford Assay to determine the concentration of a protein sample. Result are analysed using plate reader. The assay starts with obtaining the standard curve on a known dilution series of of Bovine Serum Albumin (BSA).

Materials

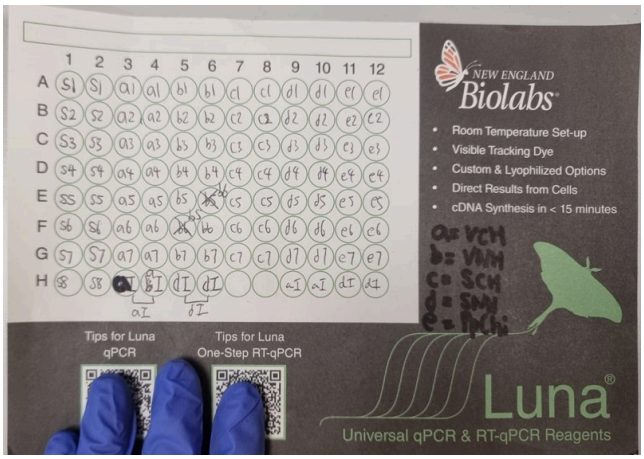
- Bovine Serum Albumin
- Unknown concentration of protein sample
- 96-well plate
- plate reader

Procedure

1. Plan the arrangement of samples and dilution series of BSA on a 96-well plate.

e.g. row 1 serial dilution and blank, following rows protein elution and repeats.

image.png



1. Prepare the following dilution series of BSA by firstly make the 2 mg/mL BSA standard stock solution

- Prepare BSA (Bovine Serum Albumin) Standard curve: Make 2mg/ml BSA stock: 1. In a 50ml falcon tube weigh out 20mg BSA. 2. Add 10ml Binding Buffer.
- Perform a serial dilution (8wells total): Pipette 100ul BSA into the first well. Pipette 50ul ddh into the next 6wells. Take 50ul from the first well and add it to the second well, mix thoroughly by pippeting up and down. Repeat for the rest of the standard curve wells. 50ul final volume in each well. In the 8th well add 50ul BB only as a blank.

1. Pipette 4ul of sample into the desired wells
2. Add 200ul Bradford assay reagent to all wells containing sample. Mix well without creating bubbles.
3. Put the 96-well plate into a plate reader and set the wavelength to 595 nm. The absorbance value is then plotted on a graph against the BSA concentration to obtain a standard BSA concentration curve. Any sample with unknown concentration can then be determined using the curve.