Bradford Assay

Purpose

To determine the concentration of protein

Materials

- Unknown protein sample
- BSA protein sample (2 mg/mL stock, Bio-Rad)
- 10X PBS (Bio-Rad), contains 137 mM NaCl, 2.7 mM KCl, 10 nM Na2HPO4, 2mM KH2PO4
- Diluted Bradford reagent (Bio-Rad Protein Assay Dye reagent), contains Coomassie Brilliant Blue G-250, phosphoric acid and methanol

Equipment

- Multiskan Plate Reader with Accent Software
- 96- well plate

Procedure

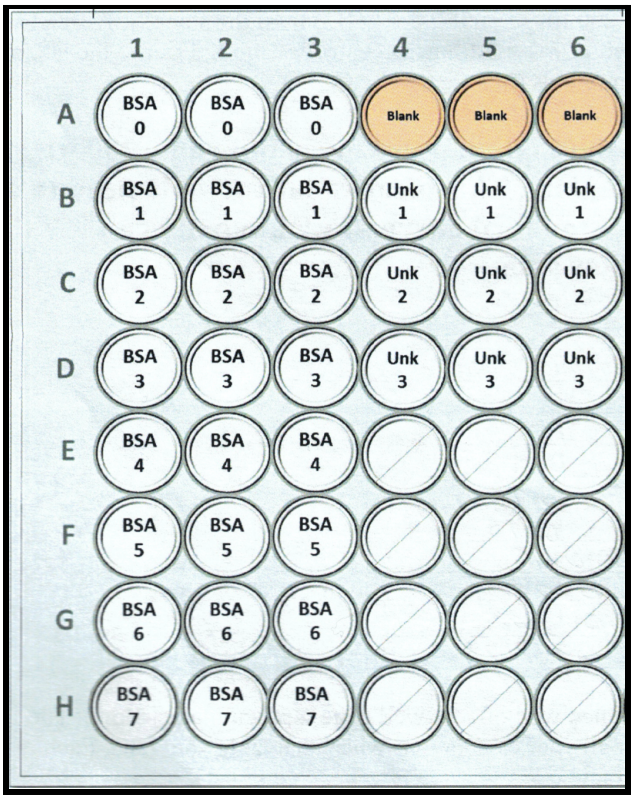
1. Make 7, 200 µL serial dilutions (½) from the stock BSA protein sample using PBS has dilution buffer. Below is a table of desired BSA concentrations.

Dilution	BSA concentration (mg/mL)
0	2
1	1
2	0.5
3	0.25
4	0.125
5	0.0625
6	0.03125
7	0.015625

1. Add 10 µL of each dilution into each designated well of a 96-well plate.
2. Perform 1/10 and 1/50 dilution of murA protein
3. Add 10 µL of unknown murA concentration from stock (Unk 1), 1/10 (Unk 2), and 1/50 dilution (Unk 3) into each designated well of the 96-well plate
4. Add 10 µL PBS dilution buffer into 3 wells as the “Blank”.

5. Add 200 µL of diluted Bradford reagent dye to all loaded wells. Leave the plate undisturbed for 5 minutes
6. Measure and record the absorbance of the sample in 96-well plate using MultiSkan Plate Reader
7. Create standard curve and calculate unknown protein concentration

Below is an image of our desired 96-well plate.



Safety Precautions

Remember personal protective equipment (including lab coat, gloves, goggles, close toed shoes, and long hair tied back) must be worn at all times during experimentation. May be harmful if ingested or inhaled. Do not bring into contact with skin or eyes.

Reference

Vulcu, F. (2019). *BiomedDC 3C09: Laboratory Manual.* Hamilton, ON: The Campus Store and Media Production Services.