

## Measuring Protein Concentration (Bradford Assay)

1. In 0.6ml tubes, make the following dilutions:

BSA (10 mg/ml)	+	1X PBS	= [Final Concen]
a. 1 $\mu$ L	+	99 $\mu$ L	= 0.1 $\mu$ g/ $\mu$ L
b. 2 $\mu$ L	+	98 $\mu$ L	= 0.2 $\mu$ g/ $\mu$ L
c. 5 $\mu$ L	+	95 $\mu$ L	= 0.5 $\mu$ g/ $\mu$ L
d. 7.5 $\mu$ L	+	92.5 $\mu$ L	= 0.75 $\mu$ g/ $\mu$ L
e. 10 $\mu$ L	+	90 $\mu$ L	=1.0 $\mu$ g/ $\mu$ L

2. To each cuvette, add 20 $\mu$ L of this dilution.
3. For your protein samples, add (18 $\mu$ L PBS + 2  $\mu$ L of sample OR 19 $\mu$ L PBS + 1  $\mu$ L sample) together in the cuvette and mix well.
4. Prepare the Bradford assay solution. Dilute the solution 1:5 (1 part Bradford solution + 4 parts dH<sub>2</sub>O). Mix and add 980 $\mu$ L diluted Bradford reagent to each cuvette and make sure solution is mixed.
5. Measure OD at 595 nm (visible light). Be sure to blank and generate BSA curve before measuring your samples.

### Notes:

1. Using the Sansam Lab Denovix, the Standard Curve is saved and can be re-used. Be sure to check the dilution factor- either 10 (if you used 2 $\mu$ L of sample) or 20 (if you used 1 $\mu$ L sample).
2. You do not have to remake the standard curve each time. It is good practice to check your pipetting skills though!
3. The protein in the cuvettes will be "stable" on the benchtop, but do not add the Bradford solution until you are ready to measure the OD, for over time, the solutions will all turn the same color and will be useless.