

BCA (PIERCE) PROTEIN ASSAY

1. Label 8 microfuge tubes used to prepare the standard curve. Keep lysate samples on ice during this time.
2. STANDARD CURVE:

µg/µl	2 µg/µl BSA Stock (µl)	Buffer Solution (RNase/DNase free H2O)
0	0	100
0.2	10	90
0.4	20	80
0.6	30	70
0.8	40	60
1.0	50	50
1.2	60	40
1.5	75	25

3. Vortex standards and add 10µl of each standard to 3 wells (triplicates) of a 96 well microplate.
4. For the lysate samples, make a dilution using 2µl of sample in 8µl of RNase/DNase free water (1:5 dilution). Add this to 3 wells (triplicates).

NB: Make sure this dilution factor is included in the concentration calculation.

5. Prepare a working BCA reagent. Add Reagent A to a 15ml falcon tube and then add Reagent B.

Reagent A	Reagent B
5ml	0.1ml
10ml	0.2ml
15ml	0.3ml
20ml	0.4ml
25ml	0.5ml

6. Add 200µl of the working reagent to each well of the microplate that contains standards and samples. The color should slowly start to turn purple.
7. Incubate the microplate in the 37°C oven for 30 minutes. Make sure the microplate is covered with the lid.
8. Read on the plate reader in Room 5S-20 at 562nm.