Appendix II

BCA Method to measure protein concentrations

1. Prepare working BCA reagent (see below).
2. Prepare standard dilutions in 96-well for standard curve as below:

	Standard Protein (BSA) µL	MQ µL
Std 1	0	20
Std 2	1	19
Std 3	5	15
Std 4	10	10

3. Add 4 µL sample lysate into a 96 well plate
4. Add 8 µL sample lysate into a 96 well plate
5. Add 200 µL of BCA working reagent into each sample and standard wells.
6. Incubate at 37 ºC for 30 min.
7. Let sample cool down to room temperature
8. Read OD at 562nm using Synergy HT.

(A) BCA Reagent Stock

20 g sodium carbonate
1.6 g sodium potassium tartrate
9.5 g sodium bicarbonate
3.0 g sodium hydroxide
1.25 g bicinchoninic acid (BCA)

Add Milli-Q water 950 mL, adjust pH to 11.25 using NaOH or $NaHCO_3$, then dilute to 1000 mL with Milli-Q water. Store at room temperature.

(B) CuSO4

4 g CuSO4 in 100 MQ, store at room temperature.

Working BCA Reagent (A+B)
A: 50 mL
B: 1 mL