Lazzara Lab
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Micro BCA Protein Concentration Assay

Use the Thermo Scientific Micro BCA Protein Assay kit.
Do triplicates of all samples (including the standards).

For standards:

1. Make diluted lysis buffer (1:10 in water). 400 µL is enough if doing a 1:100 dilution for unknown samples. Otherwise, make an additional 50 µL per sample.
2. Make diluted BSA standard: 10 µL stock BSA + 390 µL water = 50 µg/ml BSA concentration.
3. Label a 96-well plate so that you know where all your samples and standards are. Prepare the following standard dilutions in the plate:

µg/mL	µ**L water**	µ**L diluted BSA**	µ**L diluted LB**
2	86	4	10
4	82	8	10
8	74	16	10
12	66	24	10
16	58	32	10
20	50	40	10
blank	90	0	10

For unknowns:

4. Put 90 µL water in every well that you will add sample to. (You will add 10 µL of diluted sample). Use the multichannel pipette for this step and any other steps you can to speed things up.
5. For 1:100 dilutions: The lysates are already at 1:10, so just add 10 µL of each to the 90 µL water already in the wells.
   For 1:250 dilutions: In separate tubes or unneeded wells on the plate, add 30 µL of diluted LB and 20 µL of sample. Now add 10 µL of each of these dilutions to the appropriate wells.
   For 1:500 dilutions: Mix 40 µL of dilute LB and 10 µL of sample. Now add 10 µL of each of these dilutions to the appropriate wells.

Reagent:

6. Prepare BCA substrate cocktail in a vial by adding 50% A + 48% B + 2% C : (50 + 48 + 2 µL) x number of wells + extra. [Example: For 60 wells: 3250 A + 3120 B + 130 C]. Add 100 µL BCA cocktail to each well and incubate at 37ºC for 1 hr. Read absorbances on plate reader at 562 nm. Remember to correct the unknown protein concentrations by the dilution factor.