**PROTEIN CONCENTRATION**

**BCA Assay**

(adapted from Smith et al., Anal. Biochem. 150:76-85 1985)

Reagent A: 2% Na2CO3 for 100 mL: 2 g

 0.95% NaHCO3 0.95 g

 1% Bicinchoninic acid 1 g

 0.16% Sodium tartrate 0.16 g

 0.4% NaOH 0.4 g

 make to pH 11.25

Reagent B: 4% CuSO4 4 g

Combine a sufficient amount of Reagents A and B in a 50:1 ratio (e.g. 4.9 mL Reagent A and 100 µL Reagent B) immediately before use. Mix with protein solution as follows:

 190 µL Reagent A + B mixture

 10 µL protein solution (brought to 10 µL with appropriate buffer)

a) Incubate 37°C for 30 min.

b) Equilibrate at room temperature for 10 min.

c) Measure A562. Estimate unknown protein concentration from standard curve.

For standards, use 10 µL of 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL BSA stocks (or other protein standard such as ovalbumin). *It is important to use the identical buffer for the BSA stock solutions as the one containing your protein of interest.*

For the unknown protein solution, typical amounts used are 1, 2, 4 and 8 µL/assay although this can vary greatly. The total volume should be brought to 10 µL.

The unknown protein concentration should be calculated from the standard curve as follows. This approach uses slopes to eliminate bias and the effect of non-zero intercepts.

• Plot A562 vs. concentration of BSA stock (in mg/mL) and A562 vs. volume of the unknown protein solution. The slopes of these plots are A562/(mg/mL BSA) and A562/(µL unknown), respectively.

• Note that multiplication of A562/(mg/mL BSA) by 0.01 mL, the volume of BSA stock solutions added to the assays, yields A562/(mg BSA).

• The concentration of the unknown protein, in mg/mL, is then calculated as follows:



**Coomassie Gel**

Standard lanes: - dilute 3 µL 2 mg/mL BSA with 37 µL 2X SB (––> 150 ng/µL)

 - load 1, 2, 3, 4, 5, and 6 µL/lane

For overexpressed protein, typical dilution is 1:2 with 2X SB and using 1, 2, 4, and 8 µL/lane. After staining, estimate protein concentration via densitometry.