# Measuring Protein Concentration in Cell Lysates with BCA Kit

1. Make BCA Mix: 50% Component A ; 48% Component B ; 2% Component C

    * You will need 150 µL BCA mix per well

2. Dilute your cell lysate samples in 96-well plate: 148 µL $dH_2O$ + 0.5, 1, or 2 µL your sample

    * Prepare one well with 148 µL $dH_2O$ + 2 µL of cell lysis buffer to control for reactivity of your buffer with the BCA solution

3. Make standard curve in 96-well plate by diluting: 148 µL $dH_2O$ + 0, 0.5, 1, 2, 3, 4, 5, 6, or 10 µL of 2 mg/mL BSA

4. Add 150 µL of BCA mix prepared in step 1 to each well with samples

5. Mix gently for 30 sec at Room Temperature on orbital shaker

6. Incubate 1.5 – 2 hrs in a 37 ˚C incubator

7. Cool plate for ~ 5 mins at Room Temperature

8. Read plate (lid off) in microplate reader at 570 nm

9. Graph standard curve in Excel using XY Scatter Plot. Absorbance value on Y-axis and BSA protein amount (0, 1, 2, 4, 6, 8, 10, 12, 20 ug) on X-axis. **

10. Add Linear Trendline (under Chart Tools / Layout Menu) to the XY Scatter Plot

11. Add Equation and $R^2$ value to Chart by click on Trendline then Chart Tools / Layout / Trendline / More Trendline Options

    * $R^2$ should be ~ 0.99
    * Equation is Y=mX+b (b = x-intercept; m = slope; Y = Abs of sample; X = Protein amount (ug)

12. Determine your sample's concentration (ug/µL) using the standard curve equation. Plug in your Y-value (your unknown sample's Abs) and solve for X (ug). Then divide this X-value (ug) by the amount (µL) of sample used (0.5, 1, or 2 µL) to get final concentration (ug/uL).

** STEPS 9-12 CAN BE DONE USING THE LAB'S EXCEL WORKSHEET TEMPLATE