3p Mon Dopman

- Add wavelength to datasheet before saving to drive.
- Calculate the protein concentration in your samples using a standard curve and determine 'x' µL needed provide 40µg per well

Dilution factor =	Vol_f =	Vol of stock + dilution =	100µL stock + H_2O
	Vol_i	Vol of stock	X µL stock

  - (Dilution factor) * (Concentration) = µg of stock
  - Things to remember:
    - µg/µl = mg/ml
    - plot OD on y-axis, concentration on x-axis
    - make scatter plot>marked>select date>add>select x, select y
- Dilute 'x' µL sample in an equal amount of 2X Laemmli buffer (with β-mercaptoethanol)
  Must be less than 15µL as the well can hold a maximum of 30µL.
- Heat at 95°C for 5 min
- **Store at -80°C if needed**
  - *if storing for more than 30 days inhibitors need to have been added to PBS*

SDS-PAGE (Sodium Dodecyl Sulfate – PolyAcrylamide Gel Electrophoresis)

- Turn on heating block to 95°C
- Defrost samples
- Re-boil samples at 95°C for 2 min
- Setup precast gel in system
- Fill tank with 1X Tris/Glycine/SDS running buffer
  - 100ml 10X buffer + 900ml dH_2O
  - $C_1V_1=C_2V_2$
- Load appropriate amount of sample and 10µL of protein weight marker into gel
  - Place the lid on the tank and connect the leads to the power pack. Run the gel at 75V for 5 min
  - Increase the voltage to 150V and run the gel until the front has run off the bottom (~1h)
- Add water, seal in plastic, and store in a refrigerator.

$\frac{3.308mg}{1mL}$ $\frac{3308\mu g}{1000\mu L} = \frac{40\mu g}{x}$ 12.09µL

$\frac{135\mu L}{3.09} = \frac{x\,\mu L}{40\mu g}$

$\frac{40\mu g}{0.29\mu L} = \frac{40\mu g}{}$

0.29 (40µg)(0.29) = 40µg(5µL)