Protein Extraction and Calibration Curve

If storing samples for more than 30 days, add a protease inhibitor to hemolymph extract.

- With the larva constrained and folded in half, exposing the dorsum, make an incision through the cuticle at the proleg to extract hemolymph
- Pipet the hemolymph into 100µL of PBS
- Place the test tube into ice to slow oxidation and add inhibitor(s).
- **Store supernatant overnight at -80°C**

Protein Quantification using Bradford Assay kit *See: Pierce Bradford Protein Assay Kit.pdf*

Prepare the Diluted Albumin (BSA) Standards

Dilution Scheme for Standard Test Tube and Microplate Protocols (Working Range = 100-1500µg/mL)				
Vial	**Volume of diluent (µl) dH_2O**	**Volume (µl) and source of BSA**		**Final BSA concentration (mg/ml)**
A	0	300	Stock	2
B	125	375	Stock	1.5
C	325	325	Stock	1
D	175	175	Vial B dilution	0.75
E	325	325	Vial C dilution	0.5
F	325	325	Vial E dilution	0.25
G	325	325	Vial F dilution	0.125
H	400	100	Vial G dilution	0.025
I	400	0		0 = Blank

- Standard Microplate Protocol (Working Range = 100-1500µg/mL)
  - 3 Reps of each dilution
  - Upto 7 treatments can fit on a plate
- Prepare three ~~100µl~~ dilutions of each of your protein supernatant samples: 1X, 10X, 20X.
  - [diluting volume/#'x' volume] = diluted volume
  - Ex: 100µl/1x = 30µl of unknown need to be added → undiluted 100% unk stock
  - Ex: 100µl/10x = 10µl of unknown need to be added → half 50% unk stock 50% water
  - Ex: 100µl/20x = 5µl of unknown need to be added → 10% 10% unk stock 90% water
- Mix each sample for 5 secs and Pipette 5µL of each standard or unknown sample into the appropriate microplate wells.
- Prepare Commassie reagent, allow it to equilibrate to room temperature (WR). 200µl needed per sample plus 30%
  - (#stds + #unk)*(3rep)*(250) +(0.3*WR needed) = WR needed
- Add 200µL of the Coomassie Reagent to each well.
- Cover plate with *Press'n Seal,* mix with plate shaker for 30 seconds, then incubate at room temperature for 10 minutes.
- Measure the absorbance at or near **595** nm on a plate reader.