

BRADFORD ASSAY

Generating Standard Curve - Preparing standard samples

- ⇒ Range for BSA is 0.2 mg/ml - 0.9 mg/ml (in -20°C (labeled box))
- ⇒ Re-suspend lyophilized powder w/ 20ml dH₂O to give 1.4 mg/ml concentration
- ⇒ Make 3-5 dilutions in linear range, in duplicate or triplicate (in 15 ml conical tubes):

	Concentration	Dilu factor	Sample Volume	dH ₂ O to 100 μ l*
#1	0.2 mg/ml	1:7	14.3 μ l	85.7 μ l
#2	0.4 mg/ml	1:3.5	28.6 μ l	71.4 μ l
#3	0.7 mg/ml	1:2	50 μ l	50 μ l
#4	0.9 mg/ml	1:1.6	62.5 μ l	37.5 μ l

- Do dilution of BSA in the buffer that your protein sample for co-IP is in
- Add 5ml of a 1:5 dilution of protein assay reagent (in 4°C) - should see color gradations (blues) change in a regular way across concentrations
- ⇒ Vortex briefly and add 1 ml each sample to each disposable 3ml cuvette.
- ⇒ Prepare standard curve on Smart Spec:
 - Protein buttons
 - Select Bradford
 - Subt
 - New standard curve: yes
 - # replicates = 2 or 3 depending on whether duplicate or triplicate samples generated to support curve
 - cuvette w/ just 1:5 Bradford reagent and no protein
 - Insert blank and press read blank
 - Enter # standards = 4 , then *enter*
 - Conc. = mg/ml
 - Put each standard sample
 - If $R^2 > 0.9$, the standard generated is good. Otherwise, repeat the standard (pipetting error).
- ⇒ Get Protein sample concentration:
 - Hit *enter*. (continued from above) Put in the first protein sample:
 - 20 to 50 μ l lysate
 - 5 μ l 1:5 diluted assay reagent, then transfer 1ml to cuvette
 - Get units in mg/ml