Lowry Protein Assay Protocol (from Scott Hsieh)

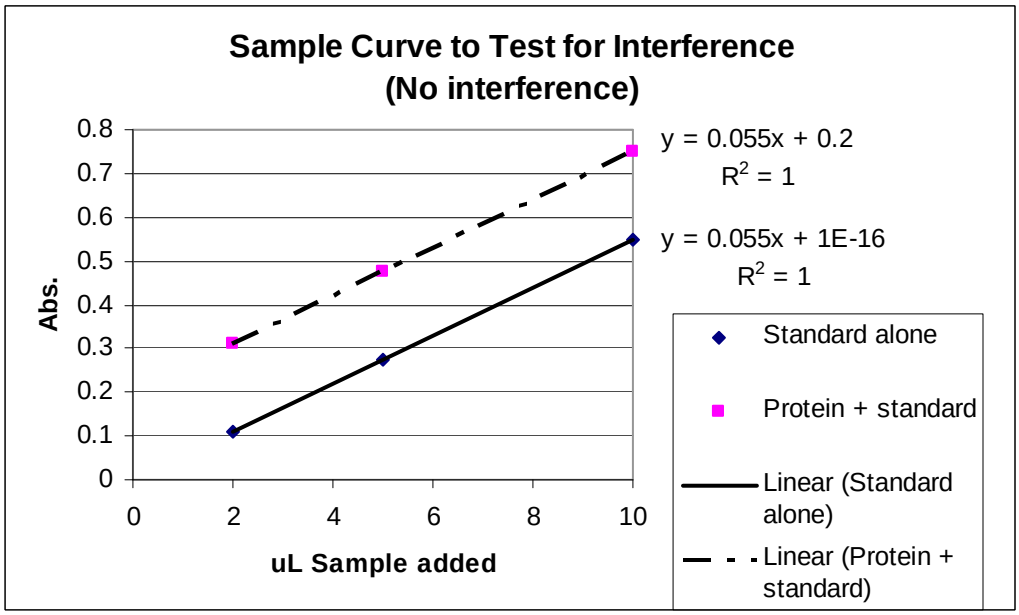
Solution A: 4 mg/mL NaOH and 20 mg/mL Na_2CO_3 in water
Add 2 g of NaOH and 10 g of Na_2CO_3 to 400 mL water while stirring until completely dissolved, then adjust volume to 500 mL.

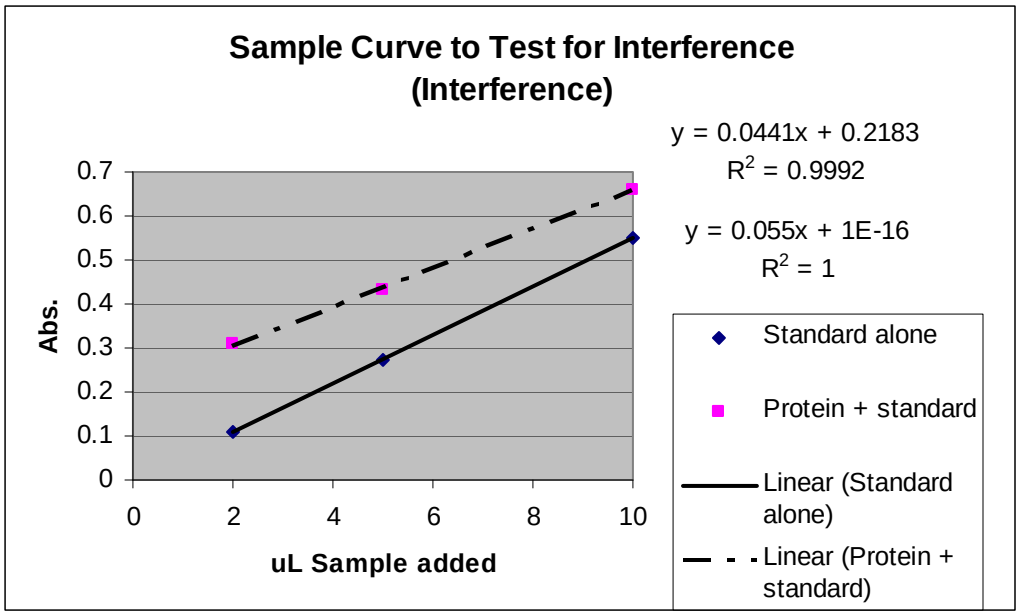
Solution B: 10 mg/mL Potassium Sodium Tartrate and 5 mg/ mL $CuSO_4$ in water
Add 100 mg Potassium Sodium Tartrate and 50 mg $CuSO_4$ (cupric sulfate) (**do not weigh on Merchant lab balances**) to 8 mL of water in a plastic falcon tube (**do NOT use Merchant lab glassware for solutions containing copper**). Shake the mixture until solids are completely dissolved, then adjust volume to 10 mL.

Mix solutions A and B and store at 4˚C this is called Lowry's solution and should be usable for up to six months. (50:1 mix of solutions A and B)

General comments

Before doing the assay it is important to know whether or not your samples contain interfering species. The easiest way to do this is to prepare your samples as described in step #2 but alongside also prepare a set of tubes containing your sample plus a known amount of protein (BSA) to each sample. Should there be no interfering species your curves should run roughly parallel to each other. If there is an interfering species then the curves will not run parallel.





Assay

1) Prepare Standards as indicated below in glass tubes (16 x 100 mm). For greater accuracy run this step in duplicate.

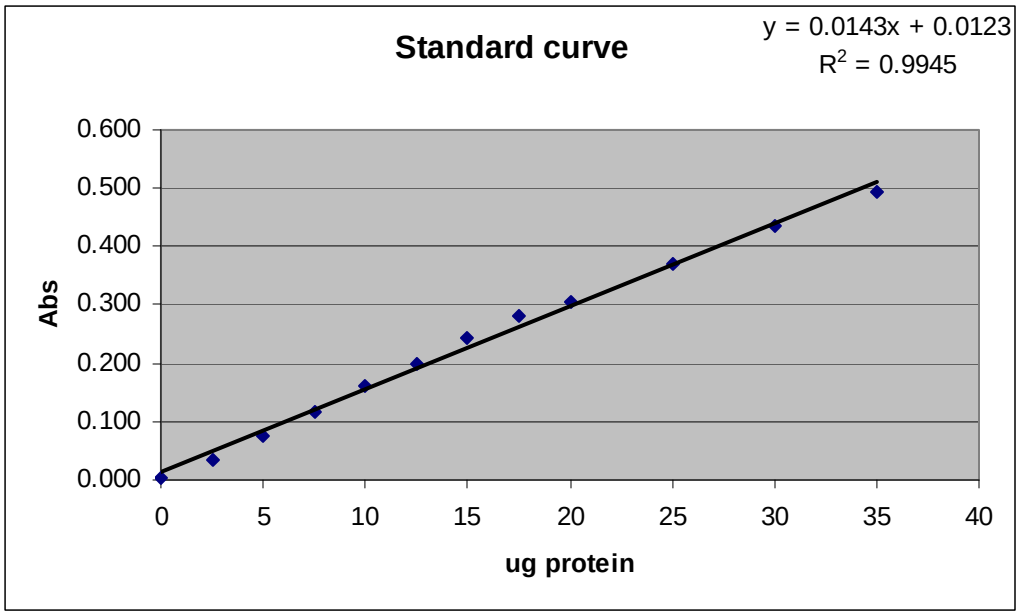
µg protein added	**0**	**2.5**	**5**	**7.5**	**10**	**12.5**	**15**	**17.5**	**20**	**25**	**30**	**35**
2mg/mL BSA (µL)	0	1.25	2.5	3.75	5	6.25	7.5	8.75	10	12.5	15	17.5
H_20 (µL)	200	198.75	197.5	196.25	195	193.75	192.5	191.25	190	187.5	185	182.5

2) Prepare samples by adding 2, 5, and 10 µL of sample into a glass tube and adjust total volume to 200µL. (The volumes given above are recommended for Chlamydomonas cell extracts (per our usual freeze-thaw protocol) but can be altered depending on expected protein concentration). It is suggested to prepare two sets of sample to determine precision.

3) To each tube add 1mL Lowry's Solution, vortex, wait 15 min.

4) To each tube add 100µL 1.0N Folin's Phenol reagent from Sigma (F-5292)
while vortexing, wait 30 min.

5) Measure A_{750nm}. Ideally, your absorbances should be between 0.1 and 0.5.



NOTE: Standard curve should appear similar to above with linearity between approximately 0.1 and 0.5 absorbance units. R^2 should between 0.9800 and 0.9999.