**Expression and purification of a N-His tagged protein**

**DAY 1**

1. transform BL21 cells with your plasmid of interest

**DAY 2**

1. Inoculate 50 ml of TB + ANTIBIOTIC in a flask with a single colony and grow overnight at 37 deg. C

**DAY 3**

1. Inoculate 1 L of TB WITHOUT antibiotic to an initial OD of 0.05
2. Grow at 37 deg. C to an OD600 of 0.4-0.6 (I normally aim for 0.5)
3. Cool flask on ice for 20 min. Swirl the flask regularly so that it cools quickly and the cells don’t overshoot the target OD.
4. Be sure to take a 1 ml sample of the culture an put it into a 1.5 ml epi labeled “pre-induction.” Spin down for 1 min at 13000 on the table top centrifuge, pour off supernatant, and freeze at -20 deg. C
5. Induce protein expression with 0.25 mM IPTG
6. Grow overnight (~17 hours) at 16 deg. C with shaking at 200 rpm

**DAY 4**

1. Take a 1 ml sample of the culture and label it “post-induction.” Spin down for 1 min at 13000 on the table top centrifuge, pour off supernatant, and freeze at -20 deg. C
2. Pellet cells in centrifuge bottle at 8,000 x g for 7 minutes at 4 deg. C. Be *very careful* not to mix anything up if you are expressing more than 1 protein at a time
3. Store pellets at -80 deg. C.
4. Check expression of cultures on a gel. I would check pre-induction, post-induction, insoluble, and soluble fractions at least.

**Purification**

1. When ready, thaw pellet on ice
2. Re-suspend in 5 mL of lysis buffer/g cell pellet. Add BME and protease inhibitor cocktail to lysis buffer before resuspension.
3. Homogenize cell pellet
4. French press (doing this twice can yield better lysis)
5. Centrifuge lysate in 50 ml falcon tube at 12, 000 x g for 20 min at 4 deg. C. *Take insoluble sample.*
6. Precipitate by slow addition of 0.15% w/v poly(ethyleneimine) on ice while stirring (in a little beaker)
7. Pour into 50 ml falcon tubes and centrifuge again at 12,000 x g for 20 min at 4 deg C
8. Pour supernatant into yet another clean falcon tube and centrifuge again at 12, 000 x g for 20 min at 4 deg. C
9. Equilibrate Ni-NTA resin in a column with loading buffer (w/10 mM imidazole). 10 column volumes should be good.
10. Add about 1 ml resin/g cell paste and rock in cold room to bind. You might want to bind with less resin if your protein did not express well.
11. Load resin onto column and collect flow-through. *Take flow-through sample*
12. Run a 3 column volumes of extra loading buffer through the column
13. Prepare Bradford reagent. It is in the 4 deg. delicase as a 5x stock. You can dilute it by a factor of 5 into a 50 ml conical. Pipette 100 µL of the 1x stock into wells of a 96-well plate. As you go through the purification process, you can estimate protein concentration by pipetting 5 µL of sample from what is dripping off of your column into each well to see if it turns blue
14. Wash resin with 5-10 CV wash buffer (loading buffer with 20 mM imidazole) or until the Bradford reagent is not too blue. *Take wash sample*
15. elute with 300 mM imidazole in buffer A. *Take elution sample*
16. Concentrate with Amicon Ultra spin concentrator at 4 deg. C and 5,000 x g. BE SURE TO PICK THE CORRECT MW CUTOFF SIZE
17. Buffer exchange into storage buffer, check with Bradford and/or nanodrop
18. Aliquot, flash-freeze, and store at -80 deg. C

**BUFFERS**

Lysis Buffer (pH 7.5)

50 mM sodium phosphate

300 mM sodium chloride

20% glycerol

10 mM imidazole

+ 20 mM BME (Don’t add to initial stock. Add fresh before use!)

Wash Buffer (pH 7.5)

50 mM sodium phosphate

300 mM sodium chloride

20% glycerol

20 mM imidazole

Elution Buffer (pH 7.5)

50 mM sodium phosphate

300 mM sodium chloride

20% glycerol

300 mM imidazole

Storage Buffer (pH 7.5)

50 mM HEPES

100 mM sodium chloride

2.5 mM EDTA

20% glycerol

1 mM DTT