Purification of His6-tagged proteins (1L cells)

1. Grow 20 ml overnight culture of LB from single colony in the presence of the appropriate antibiotic.

2. Inoculate fresh LB cultures with a 1:100 dilution of overnight culture

3. Grow until OD 600 is between 0.5-1.0 [generally 3 hrs. for BL21 (DE3) pLysS cells]

4. Induce culture with 0.4 mM IPTG (200 ul of a 1M stock/500 mls)

5. Grow at 37^{o}C for 3 hrs.

6. Spin down cells at 4000 rpm (2700 x g) in GS-3 rotor for 20 minutes

7. Resuspend pellet in 10 mls ice cold PBS

8. Combine washes and spin as in step 6

9. You can freeze pellet at this point or continue with purification

10. Thaw cell pellet on ice

11. Resuspend pellet in 40 ml lysis buffer (PBS/ 1% TX-100/ 10 mM Imidazole/ 1 mM PMSF). Note: Add PMSF immediately prior to use.

12. Add lysozyme to a final concentration of 1 mg/ml and incubate on ice for 30 minutes.

13. Split lysate into two tubes and sonicate. Using microtip set at 3- sonicate for 3 minutes at a 50% duty cycle while tube is in an ice bath.

14. Spin sonicated lysate for 30 minutes at 10,000 x g (9500 rpm in SS-34)

15. While spinning lysate prepare Ni-NTA superflow matrix (Qiagen). Wash 3 mls of a 50% slurry with 50 mM sodium phosphate/300 mM NaCl/ 10 mM imidazole using 10 mls of wash buffer. Resuspend washed matrix in 3 mls of wash buffer.

16. Add 1.5 mls of 50% slurry of matrix to clarified lysate. Incubate on rotator in cold room for 1 hour.

17. Combine lysate/matrix slurry and spin for 3 minutes at 1000 x g.

18. Resuspend matrix in 4-5 volumes wash buffer (50 mM sodium phosphate/300 mM NaCl/ 50 mM imidazole). Transfer into 15 ml conical tube. Wash by mixing 10 minutes in cold.

19. Spin and wash as above 2X

20. Elute with 4-5 volumes of elution buffer (50 mM sodium phosphate/300 mM NaCl/ 300 mM imidazole). Mix in cold for 20 minutes.

21. Spin as before. Collect eluate. Re-elute as above, spin, collect eluate with previous.

22. Concentrate eluate using Centricon concentrators with appropriate molecular weight cut-off.

For endotoxin removal use **Detoxi-Gel columns (Pierce).**

Wash columns with 5 volumes 1% sodium deoxycholate
Wash columns with 3-5 volumes with endotoxin-free PBS or water
Apply sample in approximately 1 ml volume. Allow to run into column, cap the bottom and incubate for at least 1 hour.
Collect eluate using endotoxin-free PBS as elution buffer. Concentrate as before.