Purification of His-tagged Proteins

Materials for expression:

- Transformed BL21(DE3)pLysS (pLysS is Cm-R). [Glycerol stocks are @-80oC (box A1a)].
- Sterile MMI medium (add antibiotics just before use). (25ml in 125ml flask for O/N culture and 0.5L in 2L flask for expression).
- 1M IPTG stock solution
- Cold 20mM Tris-HCl, pH8.0 (4oC)

Materials for purification:

- 1M sodium phosphate mono- and di-basic stocks (4oC)
- 5M NaCl stock (RT)
- 1M Imidazole, pH 8.0 stock (4oC)
- 0.2M PMSF stock (-20oC), 1M benzamidine (-20oC), protease inhibitors (-20oC, 4oC)
- Cold milliQ water (4oC)
- **Buffer A**: 50mM sodium phosphate buffer, pH 7.4 (for 1L: 42ml of 1M sodium dibasic + 8ml 1M sodium monobasic, pH to 7.4), 300mM NaCl (60ml per 1L), 5mM Imidazole (5ml per 1L), (add 0.1mM PMSF and just before use). (120ml of Buffer A is sufficient for one prep).
- Buffer B1: 10ml Buffer A + 0.15ml 1M Imidazole (to a final conc. 20mM) – make before use.
- Buffer B2: 10ml Buffer A + 0.45ml 1M Imidazole (to a final conc. 50mM) – make before use.
- Buffer E: 8ml Buffer A + 2ml 1M Imidazole (to a final conc. 205mM) – make before use.
- 1-2ml Cobalt column (4oC)
- Co-Regeneration buffer (to neutralize imidazole): 20mM MES buffer, pH 5.0, 0.1M NaCl.
- 20% ethanol – 5ml
- for complete Co-column regeneration: 100mM EDTA, pH 7.5
- 50mM $CoCl_2$

I. Expression:

1. Grow 25ml of O/N culture from glycerol stock or from a single colony in MMI medium containing appropriate antibiotic (for plasmid of interest) and Cm (for pLysS).
2. Next morning spin cells for 10 min @ 5000g. Decant supernatant, resuspend cell pellet in fresh 1-2ml of MMI medium containing appropriate antibiotics.
3. Inoculate the cell suspension into 2L flask containing 0.5L of MMI with antibiotics to OD_{600}=0.05-0.1.
4. Grow with shacking @ 37^{o}C until OD_{600}=1-1.5. Take a small aliquot of the uninduced cells to analyze on PAGE.
5. Add 1mM IPTG and grow for additional 5 HR O/N @ 15-37^{o}C (specific conditions depend on the construct and must be determined empirically).
6. Spin cells for 20 min @ 5000g @4^{o}C. Decant supernatant, resuspend cells in 50ml 20mM Tris-HCl, pH8.0 and spin again for 10 min @ 5000g @4^{o}C. Freeze the cell pellet @ -80^{o}C.

II. Purification:

Keep cells, all buffers, columns and collected samples on ice (or @4°C) at all times!!! For freshly prepared buffers use COLD (4°C) H_2O.

1. Take an aliquot of Buffer A (10ml per 1g of cell pellet) and add a protease inhibitor cocktail tablet, leupeptin (1:500), and trypsin inhibitor (1:500), 1mM PMSF, 2mM benzamidine. [~30ml of Buffer A per cell pellet from 0.5L culture].
2. Resuspend the cell pellet in Buffer A from step 1 (supplemented with the protease inhibitors). Use homogenizer on ice.
3. Freeze-thaw cell lysate if necessary to break the cells. Use sonicator (8th floor) to break DNA (10 sec pulse with 10 sec intervals @60% for 2min - keep cell lysate on ice). Take a small aliquot of the total cell lysate to analyze on PAGE.
4. Spin lysate for 30 min @ 30,000 rpm (70Ti rotor – ultra C/F on 7th floor) @4°C.
5. Filter the supernatant through a paper filter. Take a small aliquot of the soluble cell lysate to analyze on PAAG.
6. Equilibrate Cobalt-column with 10 bed Vol. of Buffer A (10 ml).
7. Load cleared lysate from step 5 to the equilibrated column. Take a small aliquot of the input to analyze on PAGE.
8. Collect flow-though, load back to column. Repeat this step at least two more times.
9. Collect flow-through (and keep it in case if Co-column did not work), take a small aliquot for PAGE.
10. Wash column with Buffer A applied in small fractions until Bradford is no longer blue.
11. Collect 10ml wash fraction with Buffer B1 (20mM Imidazole). Check by Bradford. Take an aliquot for PAGE.
12. Collect 10ml wash fraction with Buffer B2 (50mM Imidazole). Check by Bradford. Take an aliquot for PAGE.
13. Collect 10x1ml elution fractions with Buffer E (200mM Imidazole). Check each by Bradford. Take aliquots for PAGE.
14. **Regenerate column** with 10 V of regeneration buffer. Wash column with 20 V of H_2O. Store column in 20% ethanol (for long term storage) or in Buffer A (for short term storage).
15. **Keep all collected fractions @4°C!**
16. Run PAGE (include uninduced culture, total cell lysate, a small sample of pellet from step 4, input aliquot, flow-through aliquot, B1 and B2 washes, and elution fractions).