**His-tag purification**

1. Grow up 500 mL of bacteria expressing his-tagged protein
2. Resuspend pellet in 25 ml lysis buffer + 1:300 PIC’s (or alternate protease inhibitors) and 300 mM NaCl.
3. Lyse cells on cell disrupter.
4. Centrifuge at top speed for 1 hr to pellet debris.
5. Decant supernatant and add 0.25 ml of Ni-NTA equilibrated in lysis buffer-300 mM NaCl, stir at 4°C for 3 hr.
6. Centrifuge in 250ml conical tubes at 1000RPM for 3min (Wash with 25ml lysis buffer-300 and repeat).
7. Transfer resin to column and wash with 50ml lysis buffer-300.
8. Elute with 0.5 ml lysis buffer-100+250mM imidazole
9. Repeat elution to verify all protein is off the column

Lysis buffer: 10X Lysis Buffer 0.1 M KCl, 0.2 M immidazole, 0.5 M Hepes pH 7.8