Protein Purification Basic Protocol

1. Thaw pellet on ice (or in water if in a hurry). Resuspend frozen pellet with Lysis Buffer, using 5 ml buffer per gram of dry pellet.

2. Incubate with 1 mg/ml Lysozyme on ice for 30min-1hr.

(Meanwhile, equilibrate Ni-NTA resin (1 ml for each 10 ml lysate) with 10 CV of Lysis buffer.)

3. Sonicate cells on ice for 1.5 min total, 2 sec on 4 sec off.

4. Spin down at 9500 rpm, 4C for 20 min. *Get rid of cell debris*

(Keep ~20 ul of pellet as insoluble lysate.)

5. Take supernatant, add streptomycin sulfate to final concentration of 0.9% (w/v) slowly while stirring, let sit on ice for 5 min. *Get rid of DNA*

6. Spin down at 9500 rpm, 4C for 20 min.

(Keep ~20 ul of supernatant for soluble lysate.)

7. Take supernatant, incubate with pre-equilibrated Ni-NTA at 4C rotating for 1 hour.

8. Load supernatant onto column

(Keep 20 ul of flowthrough fraction.)

9. Wash with 10 CV wash buffer or until protein concentration is low by Bradford.

(Keep 20 ul of wash as wash fraction.)

10. Elute with 5-10 CV of elution buffer until protein concentration is low by Bradford.

(Keep 20 ul as elution fraction.)

11. Run SDS-PAGE gel of: Insoluble lysate, soluble lysate , flowthrough, wash, elution fractions.

12. Concentrate elution to <15 ml, dialyze in 3L of 3.5-5 kDa dialysis tubing at 4C overnight.

(At this step, either dialyze into storage buffer, or buffer needed for additional purification steps. If protease is needed, add it in this step)

13. If no further steps are needed, measure protein concentration by Abs_{280}. Flash freeze in liquid nitrogen in small aliquots, store at -80.