Purification of PolyHistidine Tagged Proteins

Prepare buffers and solutions:

Ni^{2+} -NTA Affinity resin

Buffer A (binding buffer)	50 mM Tris Cl 8.0 (4^0C) 60 mM NH_4Cl 07 mM $MgCl_2$ 07 mM β-mercaptoethanol 01 mM PMSF 50 µM GDP 300 mM KCl 10 mM Imidazole 15% Glycerin
Buffer B (wash buffer)	50 mM Tris Cl 8.0 (4^0C) 60 mM NH_4Cl 07 mM $MgCl_2$ 07 mM β-mercaptoethanol 01 mM PMSF 50 µM GDP 300 mM KCl 20 mM Imidazole 15% Glycerin
Buffer E (elution buffer)	50 mM Tris Cl 8.0 (4^0C) 60 mM NH_4Cl 07 mM $MgCl_2$ 07 mM β-mercaptoethanol 01 mM PMSF 50 µM GDP 300 mM KCl 250 mM Imidazole 15% Glycerin
TAKM7	50 mM Tris Cl 7.5 (4^0C) 70 mM NH_4Cl 30 mM KCl 07 mM $MgCl_2$

Method

1. Assemble chromatography column ~5 mL (in 50 mL falcon tube).
2. Degas all of the buffers.
3. Mix bottle of resin and carefully pour the appropriate volume of Ni^{2+} resin slurry into the column (remember that the slurry is a mixture of solid phase and EtOH) (Ni^{2+} affinity resins will typically bind 8-12 mg of protein per mL of resin). Avoid frothing, it will result in air bubbles being trapped in the resin.
4. Spin the column at 500g for 2 minutes. Remove the supernatant.
5. Wash the column with 3 column volumes of sterile water. (Gently mix the solid phase with the liquid to create slurry. Spin at 500g for 2 minutes)
6. Wash the column with 6 column volumes of binding buffer and divide into 4 falcon tubes. The column is now ready for binding.
7. Remove 50 µL of cell lysate and store in microfuge tube. Apply the cleared cell lysate (S30) to the 4 columns evenly and gently mix thoroughly. Incubate on ice for 30-60 minutes inverting periodically to bind protein to the column.
   ALL FURTHER STEPS ARE PERFORMED AT 4°C.
8. Transfer all the column material into 1 falcon tube by spinning 1 column at 500g for 2 minutes, removing the supernatant and filling with cleared lysate+resin from another tube. Repeat. Remove and store supernatant at 4°C. Take 50 µL sample and store at 4°C.
9. Wash the column 3 times with a full falcon tube volume of Buffer A. Pool the washes, store at 4°C and take a 50 µL sample.
10. Wash the column 4 times with a full falcon tube volume of Buffer B. Pool the washes, store at 4°C and take a 50 µL sample. This step removes the unspecific proteins bound to the column
11. Elute the protein 10 times using 90 % column volume of buffer E. Incubate elution buffer for 5-10 minutes. Do not pool the elutants. Take a 50uL sample from each elution step and store at 4°C. Store the elutants at 4°C.
12. Run an SDS-PAGE on the samples to ensure proper protein purification.
13. Fractions containing the target protein are pooled and dialyzed overnight (1:1000) against a storage buffer of choice (TAKM7) to remove Imidazole.
14. The column material can be reused and is regenerated by washing with 0.2 M acetic acid in 30% glycerol. It can be stored as 50% slurry in 30% ethanol to prevent microbial growth.