Purification of 6xHis epitope tagged proteins by Ni-NTA-Agarose

Buffer composition from Sarah Duellman (Burgess Lab), remainder is from manufacturer

Low ("Low") Imidazole Buffer	0.5L
100mM Imidizole	3.4g
5% glycerol	25ml 100% glycerol
50mM Tris-HCl (pH 7.9)	50ml 0.5M Tris-HCl (pH 7.9)
0.1% Tween-20	0.5M 100% Tween-20
500mM NaCl	50ml 5M NaCl
dH_2O	Fill to 0.5L (start w/ 350ml)

High ("High") Imidazole Buffer	0.5L
500mM Imidizole	17.0g
5% glycerol	25ml 100% glycerol
50mM Tris-HCl (pH 7.9)	50ml 0.5M Tris-HCl (pH 7.9)
0.1% Tween-20	0.5M 100% Tween-20
500mM NaCl	50ml 5M NaCl
dH_2O	Fill to 0.5L (start w/ 350ml)

Sonication and Solubility Test:

1. Resuspend 1L worth of bacterial pellet in 30ml Low Buffer
- Take 30ul pre-sonication sample
2. Sonicate 3 pulses at 80% power with 7th floor sonicator, 2 min on ice between pulses (NOTE: do not let sonicator tip touch side of tube to reduce frothing)
- Take 30ul crude sonicated sample
3. Dispense into 1.5 ml eppendorf tubes, spin 14k rpm, 4^{o}C, 30 min
4. Combine soluble fractions into 1 tube
- Take 30ul soluble fraction as post-sonication soluble sample
5. Test solubility of target protein by SDS-PAGE/Coomassie Blue Stain
- Add 10ul 4x Sample Buffer and 2ul β-Mercaptoethanol to each 30ul sample
- Add an equal volume of 4x Sample Buffer to one insoluble pellet, add β-Mercaptoethanol to a final concentration of 5%.
- Load 14ul (equals 10ul of fraction) on an appropriate concentration SDS-PAGE gel, electrophorese to separate bands well
- Stain with Coomassie Blue for 30 min @ RT with rocking, destain with shreds of brown paper towel to aid in removal of dye from gel

Purification of Soluble Target Protein:

1.