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

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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 NaCl50ml 5M NaCl

dH2O 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 NaCl50ml 5M NaCl

dH2O Fill to 0.5L (start w/ 350ml)

Sonication and Solubility Test:

Resuspend 1L worth of bacterial pellet in 30ml Low Buffer

Take 30 µl pre-sonication sample

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 30 µl crude sonicated sample

Dispense into 1.5 ml eppendorf tubes,spin 14k rpm,4℃,30 min

Combine soluble fractions into 1 tube

Take 30 μl soluble fraction as post-sonication soluble sample

Test solubility of target protein by SDS-PAGE/Coomassie Blue Stain

Add 10 μl 4x Sample Buffer and 2 μl ?-Mercaptoethanol to each 30 μl sample

Add an equal volume of 4x Sample Buffer to one insoluble pellet,add

-Mercaptoethanol to a final concentration of 5%.

Load 14 μ l (equals 10 μ l 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