Growing up of cells

Streak out His-tagged Xis strain from frozen supply
The next day pick a well isolated colony and start a 5 ml overnight per 500 ml to be prepared (2L suggested)
Early the next day, subculture overnight 1:100 into 500 ml LB plus appropriate antibiotic
Grow to mid to late log at 37C OD600=0.5-0.6 (takes about 4 hours)
Induce with a final IPTG concentration of 0.5 mM
Shake at room temperature for four more hours
Spin cells at least at 5000rpm for 20 minutes
Decant all LB
Freeze pellet overnight

Cell extracts

all steps performed on ice
Thaw pellet on ice for 30-60 minutes
Resuspend pellet with 2ml of extraction buffer
    50 mM Tris, pH 7.4, 10% sucrose, 0.8M KCl
Transfer to large homogenizer (25 mL capacity)
Add protease inhibitors
    5ul PMSF 50mM in EtOH - made fresh
    4ul leupeptin 5mg/ml
    2ul pepstatin A 25 mg/ml in EtOH
    1ul soybean trypsin inhibitor 100mg/ml
Add 1/20 total volume freshly made lysozyme (10 mg/ml in 0.25M Tris, pH 7.4)
Homogenize until even, watery consistency about 5 – 10 minutes
Incubate on ice 45 minutes
Spin at 45,000 rpm for 45 minutes at 4C
Transfer supernatant to a new eppendorf tube, take an aliquot, and freeze both in an ethanol/dry ice bath or liquid nitrogen
Transfer pellet back into homogenizer and add 0.5 mL extraction buffer – rehomogenize and spin again
Save the second supernatant and an aliquot and quickfreeze both

Protein purification

Thaw supernatants on ice, combine all together and determine volume
Use 200ul of Talon metal affinity resin slurry per 600ul of cell extract
Wash resin 5 times with 3 volumes of binding buffer, spin at lowest speed for 30 seconds for each wash
In cold room, combine resin and cell extract (about 400ul resin to 1.2 ml extract in a 2 mL epi)
Rotate for at least 2 hours to let protein bind to resin
Spin at lowest setting for 30 seconds and collect supernatant S1 (unbound proteins)
Wash the resin of contaminants and unbound/loosely bound proteins
    500ul of extraction buffer, rotate 30 minutes
Spin for 1 minute and collect supernatant S2

Repeat wash and spin, collect S3
Add 250ul of elution buffer with 0.2 M imidizole
Rotate for 30 minutes, spin and collect S4
Add 250ul of elution buffer with 0.4M imidizole
Rotate 30 minutes, spin and save S5
Add 250ul of elution buffer with 0.6M imidizole
Rotate 30 minutes, spin and save S6
Add 250ul of elution buffer with 1.0M imidizole
Rotate 30 minutes, spin and save S7

Xis assay

Run a protein gel and do a coomassie stain on the four xis containing fractions to determine purity. Combine clean fractions and titrate activity with an excision assay with an array of xis dilutions.

Buffers

Binding Buffer

- 50 mM Tris-HCl pH8
- 300 mM Kcl
- 10% glycerol

Elution Buffer

- 50 mM Tris-HCl pH 7.6
- 375 mM Kcl
- 10% glycerol
- 0.2 to 1.0 M Imidizole