

Talin MBP-F3 Expression and Purification

pMAL-CGX-F3.4
MBP tag
His tag
Factor Xa cleavage site to cut tag off.

Protein Expression

- 1) Overnight culture w/colony from amp plate (100ml o/n culture/500ml culture next day).
- 2) Seed 2X500ml LB/amp with 100ml o/n culture.
- 3) Grow at 37°C until OD600 = 0.6-1.0.
- 4) Induce w/ 1mM IPTG.
- 5) Grow 6hr.
- 6) Harvest. 8krpm, 4°C, 15min. Resuspend in His Bind Buffer.
- 7) LN2 freeze. Store in -80°C.

Protein Purification

- 1) Add 2mM PMSF.
- 2) Lyse cells: 0.2mg/ml lysozyme (10mg/ml stock). Rock 15min, 4°C.
- 3) Chop DNA: 10mM MgCl₂, 40ug/ml DNaseI (1mg/ml stock). Rock 15min, 4°C.
- 4) Detergent: 0.1% TX-100 (25% stock).
- 5) Centrifuge at 16000 rpm (**SS34** rotor) for 15 min at 4°C.
- 6) Equilibrate His Bind Beads with binding buffer.
- 7) Load lysate supernatant onto beads.
- 8) Wash w/50ml binding buffer. Wash with 50ml low salt binding buffer.
- 9) Wash w/10 x column volume of wash buffer.
- 10) Digest 6hr RT w/FactorXa (use 20units/1mg protein). Capture w/Xarrest Agarose beads (100ul slurry/4units enzyme. Incubate 5min w/beads. Put into spin columns (800ul total volume). Spin 1000x g for 5min to remove beads.
- 11) Dialize into NMR happy buffer. Run SDS PAGE and protein concentration.

Buffers

His Bind Buffer (hi salt)

5mM Imidazole
500mM NaCl
20mM Tris-HCl pH7.9
2mM DTT

His Wash Buffer (lo salt)

30mM Imidazole
100mM NaCl
20mM Tris-HCl pH 7.9
2mM DTT

His Bind Buffer (lo salt)

5mM Imidazole
100mM NaCl
20mM Tris-HCl pH7.9
2mM DTT

NMR Happy Buffer

50mM NaH₂PO₄ pH7.0
50mM NaCl
2mM βME
0.3mM PMSF