

Talin 482-636 Expression and Purification (no tag cleavage)

Vector pET15b
Molecular Weight about 16.9 kDa w/His Tag

Expression:

- 1) Grow 110 ml o/n culture in LB/amp.
- 2) Inoculate 2X500ml LB/amp broth with 50ml o/n culture.
- 3) Grow until OD600=0.6-1.0.
- 4) Induce w/1mM IPTG.
- 5) Grow 3hr at 37°C.
- 6) Harvest. Resuspend pellets in 10ml 1XHis Binding Buffer. LN2 freeze.
- 7) Store at -80°C
- 8) Run SDS PAGE to check expression.

Purification:

- 1) Quick thaw 2x500ml cell pellets. Add 2mM PMSF. Lyse cells with 0.4mg/ml lysozyme. Rock for 15min 4°C until the mixture is very thick.
- 2) Add MgCl₂ to 10mM. Add DnaseI up to 40µg/ml. Rock for 15min at 4°C until the mixture is really runny. Save 10µl for running on gel.
- 3) Add 1% Tx100. Rock for 15min 4°C.
- 4) Spin: 16K, 15min, 30ml tubes. Save sup and pellet. Save 10µl of each for running on a gel.
- 5) Meantime, wash Ni-NTA beads with binding buffer. Do a 3ml column.
- 6) Load sup on column. Save flow through. Save 10µl for gel.
- 7) Wash 50ml binding buffer.
- 8) Wash 30ml wash buffer (30mM Imidazole)
- 9) Elute with 300mM Imidazole buffer. You can batch elute in 20ml.
- 10) Immediately dialyze (20mM Tris pH7.9/250mM NaCl).
- 11) Dialyze into final happy buffer.
- 12) Concentrate Protein.

1X Binding Buffer (High Salt)

5mM Imidazole
500mM NaCl
20mM Tris-HCl, pH 7.9

Elution Buffer (Med Salt)

300mM Imidazole
250mM NaCl
20mM Tris-HCl, pH 7.9

1X Wash Buffer (Med Salt)

30mM Imidazole
250mM NaCl
20mM Tris-HCl pH 7.9

Dialysis Buffer (Med Salt)

20mM Tris-HCl, pH 7.9
250mM NaCl