

F2+3 Talin (209-400) Expression and Purification

Vector pET15b
Molecular Weight about 28KD w/His Tag

Expression:

- 1) Grow 110ml o/n culture in LB/amp.
- 2) Inoculate 2X500ml 2XYT/amp broth with 50ml o/n culture.
- 3) Grow until OD600=1.0.
- 4) Induce w/0.2mM IPTG.
- 5) Grow 3hr at 37°C.
- 6) Harvest. Resuspend pellets in 10ml 1%TX-100/1XHis Binding Buffer. LN2 freeze. Store in –80.
- 7) Run SDS PAGE to check expression.

Purification:

- 1) Quick thaw 1x500ml cell pellet. 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 4°C until the mixture is really runny. Save 10µl for running on gel.
- 3) Spin: 16K, 15min, 30ml tubes. Save sup and pellet. Save 10µl of each for running on a gel.
- 4) Meantime, wash Ni-NTA beads with binding buffer. Do a 3ml column.
- 5) Load sup on column. Save flow through. Save 10µl for gel.
- 6) Wash 50ml binding buffer.
- 7) Wash 50ml wash buffer (30mM Imidazole)
- 8) Elute with 100mM Imidazole buffer. You can batch elute in 20ml.
- 9) Immediately dialyze (20mM Tris pH7.5/500mM NaCl).
- 10) Digest w/50units of thrombin while dialyzing (high salt requires more thrombin to cut tag off with) to for 3-6hr RT.
- 11) Run column to get rid of thrombin.
- 12) Dialyze into final happy buffer. If NMR buffer then do in 2 steps.
- 13) Concentrate Protein.

1X Binding Buffer (High Salt)

5mM Imidazole
500mM NaCl
20mM Tris-HCl, pH 7.9
2mM DTT

Elution Buffer (High Salt)

100mM Imidazole
500mM NaCl
20mM Tris-HCl, pH 7.9
2mM DTT

NMR Buffer 1

50mM NaH₂PO₄ pH 7.0
100mM NaCl
2mM βME
0.3mM PMSF

1X Wash Buffer (High Salt)

30mM Imidazole
500mM NaCl
20mM Tris-HCl pH 7.9
2mM DTT

Dialysis Buffer (High Salt)

20mM Tris-HCl pH 7.5
500mM NaCl
2mM βME

NMR Buffer 2

50mM NaH₂PO₄ pH 7.0
50mM NaCl
2mM βME
0.3mM PMSF