

Talin 1647-2541 Expression and Purification

Vector pET15b
Molecular Weight about 96KD w/His Tag
pI = 5.56
Ext Coef: 0.254

Expression:

Use BL21 Star (DE3) e.coli to express.

- 1) Grow 210ml o/n culture in LB/carbenicillin (200µg/ml).
- 2) Inoculate 2X800ml LB/carb (200µg/ml) broth with 100ml 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. Store at –80°C.
- 7) Run SDS PAGE to check expression.

Purification:

- 1) Quick thaw 2x800ml cell pellets. Add PIs. Lyse cells with homogenizer.
- 2) Spin: 16K, 15min, 30ml tubes. Save sup and pellet. Save 10µl of each for running on a gel.
- 3) Meantime, wash Ni-NTA beads with binding buffer. Do a 10ml column.
- 4) Load sup on column. Save flow through. Save 10µl for gel.
- 5) Wash 50ml binding buffer.
- 6) Wash 30ml wash buffer (30mM Imidazole)
- 7) Elute with 300mM Imidazole buffer.
- 8) Immediately dialyze (20mM Tris pH7.9/250mM NaCl).
- 9) Dialyze into final happy buffer.
- 10) 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