

Talin Rod Expression and Purification

pET30a-Talin Rod (kana)
MW: 218.9KD
Ext Coef: 0.221
pI 5.5
C-terminal His Tag (non cleavable)

Expression:

- 1) 100ml o/n culture (TB/kana).
- 2) Seed 1L TB/kana with o/n culture. Grow at 37°C until OD600 reaches 0.4-0.6. Transfer to 30°C. Induce with 0.2mM IPTG. Grow o/n at 30°C.
- 3) Harvest 8krpm, 10min. Resuspend in His Binding Buffer. LN2 freeze.

Purification:

- 1) Thaw cells (3L). Add 2mM PMSF. Add 1mM b-ME.
- 2) Lyse cells w/lysozyme. Rock 30min, 4°C.
- 3) Add 10mM MgCl₂. Digest DNA w/DNaseI. Rock 15min, 4°C.
- 4) Add 0.1% Tx-100. Rock 15min, 4°C.
- 5) Spin 18krpm, 1hr, 4°C.
- 6) Wash 3ml Ni-NTA column w/binding buffer.
- 7) Load supernatant onto column.
- 8) Wash column w/50ml binding buffer.
- 9) Wash column w/ 40ml wash buffer (hi salt).
- 10) Elute w/elution buffer.
- 11) Dialize into dialysis buffer.
- 12) Further clean with Hi Load 16/60 Superdex200 Prep Grade column.
- 13) LN2 freeze.

Buffers:

Binding Buffer:

20mM Tris HCl pH 7.9
500mM NaCl
5mM Imidazole
1mM β-ME

Elution Buffer:

20mM Tris HCl pH 7.9
500mM NaCl
200mM Imidazole
1mM β-ME

Wash Buffer:

20mM Tris HCl pH 7.9
500mM NaCl
30mM Imidazole
1mM β-ME

Dialysis Buffer:

20mM Tris HCl pH 7.4
150mM NaCl
1mM EDTA
5mM β-ME