CMC – Structure Initiative Protocols
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Talin Full Length Expression and Purification

pET30a-Talin Full Length
MW: 271.7KD
Ext Coef: 0.334
pI 5.8
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