

Tensin SH2-PTB (ScPc) Expression and Purification

pGEX4T1-GST-ScPc
30.3KD w/o GST-tag
pI = 8.5
Ext = 0.559

Expression:

- 1) 100ml o/n culture in LB/amp.
- 2) Seed 1L TB/amp w/50ml o/n culture.
- 3) Grow at 37°C until OD600 = 0.6-1.2.
- 4) Induce w/1mM IPTG.
- 5) Grow for 3hr, 37°C.
- 6) Harvest (8krpm, 4°C, 10min). Resuspend pellet in 20ml PBS.
- 7) LN2 snap freeze.

Purification:

- 1) To 1L cell pellet add 1 crushed PI tablet.
- 2) Lyse cells w/0.2mg/ml lysozyme, 15min rocking or until very thick.
- 3) Add 10mM MgCl₂.
- 4) Clear DNA w/40µg/ml DNaseI, 15min rocking or until runny.
- 5) Add 0.1% TX-100. Rock 15min.
- 6) Spin, 16krpm, 30min, 4°C.
- 7) Wash 2ml Glutathione Sepharose 4B with 50ml PBS.
- 8) Rock beads with supernatant for 1hr, 4°C.
- 9) Load onto a column and collect flow through.
- 10) Wash beads with 50ml PBS.
- 11) Wash beads with 50ml Cleavage Buffer.
- 12) Cleave protein off the beads with 100µl PreScission Protease in 2ml Cleavage Buffer. Rock overnight (16hr, 4°C).
- 13) Elute the next day with Cleavage Buffer.
- 14) Dialyze into Dialysis buffer. Do protein concentration. Snap Freeze.
- 15) SDS PAGE.
- 16) Can further purify on Superdex 75 (10/300) to get rid of upper MW band (do 500µl at a time).

Buffers:

Cleavage Buffer:

50mM Tris-HCl pH7.0 (at 25°C)
150mM NaCl
1mM EDTA
1mM DTT

Dialysis Buffer:

10mM Tris-HCl pH 7.4
50mM NaCl
0.5mM EGTA
1mM DTT

Chill cleavage buffer to 4°C before using.