

Tensin-PTBc Expression and Purification

pET15b-His-Tensin-PTBc (aa1653-1786)
MW 15.5KD
Ext Coef 0.484 (or 7090 to get μM)

Expression:

- 1) 100ml o/n LB/amp culture
- 2) Next day seed 700ml LB/amp with 100ml o/n culture, 37°C.
- 3) Grow cells until OD600 = 0.6-1.2
- 4) Induce with 1mM IPTG. Grow for 3hr 37°C.
- 5) Harvest 8krpm, 4°C, 10min.
- 6) Resuspend 800ml cell culture pellet in 20ml Ni-NTA binding buffer. LN2 freeze.

Purification:

- 1) Thaw 1L cells. Add PI tablet w/o EDTA. Add 2mM PMSF.
- 2) Add 0.4mg/ml lysozyme. Rock 15-30min until thick.
- 3) Add 10mM MgCl₂. Add 0.4 $\mu\text{g/ml}$ DNaseI. Rock 15-30min until loose.
- 4) Add 0.1% Tx-100. Rock 15min. Save 10 μl for SDS-PAGE.
- 5) Spin 16krpm, 4°C, 15min. Save some supernatant and pellet for gel.
- 6) Meanwhile, wash 2ml Ni-NTA beads with binding buffer.
- 7) Load supernatant onto beads.
- 8) Wash with 50ml binding buffer.
- 9) Wash with 50ml wash buffer low salt.
- 10) Elute with elution buffer low salt.
- 11) Dialize into dialysis buffer while digesting off His tag with biotinylated thrombin. Digest for 6hr RT. Capture biotinylated thrombin with streptavidin agarose.
- 12) Run SDS PAGE on purification. Determine if need to do further purification on S75 or S200. Do a quick protein concentration check. Concentrate if necessary.

Buffers:

Binding Buffer (High Salt)

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

Wash Buffer (Low Salt)

30mM Imidazole
100mM NaCl
20mM Tris-HCl pH 7.9
1mM DTT

Elution Buffer (Low Salt)

300mM Imidazole
100mM NaCl
20mM Tris-HCl pH 7.9
1mM DTT

Dialysis Buffer

20mM Tris-HCl pH 7.9
100mM NaCl
1mM DTT