

VINCULIN TAIL Vd1, Expression and Purification

Molecular Weight = 23.1KD w/His
PI = 9.4 (post thrombin cleavage)
Ext coef = 0.721

Amplification:

- 1) Scrap a few μ l of the frozen glycerol stock of transformed bacteria (Tuner(DE3)). Grow up an overnight culture in 100ml LB-AMP at 37°C.
- 2) Next day seed a 700ml LB w/overnight culture. Grow at 37°C.
- 3) Induce with **0.1 mM IPTG** at OD600=0.6-1.0. Grow for 3hr at 37°C.
- 4) Harvest: 8000rpm (**GS-3** rotor), **15 min**, 4°C. Resuspend cells in binding buffer-high salt & LN2 freeze.

Purification:

- 1) Thaw cells + PI w/o EDTA (1L culture volume). Use homogenizer to lyse cells.
- 2) Centrifuge at 16K rpm (**SS34** rotor) for 15 min at 4°C.
- 3) Wash 3ml NTA-His beads with binding buffer. Load supernatant onto beads.
- 4) Wash with **50ml binding buffer-high salt**.
- 5) Wash with **30ml binding buffer-low salt**.
- 6) Wash with **30ml wash buffer-low salt**.
- 7) Elute with **10ml 300mM Imidazole buffer-low salt**. Immediately **dilute into 50ml room temp binding buffer with stir bar/stir plate**. If you wait, your protein will precipitate out and you will end up with low quality soluble protein! Final dilution ends up being 100ml most of the time.
- 8) Dilute to 1mg/ml. Thrombin digest with 50 μ l biotinylated thrombin. Mix and load into dialysis tubing. Digest for 6hr RT while dialyzing in Thrombin Digest Buffer. Spin down dialyzed cleaved product SS34 rotor, 20krpm, 30min, 4°C. Concentrate to ~1ml.
- 9) Capture biotinylated thrombin with 2ml streptavidin agarose. Incubate at RT for 30min. Spin down in spin columns 500rcf, 5min. Dialyze o/n in end buffer. Concentrate protein if necessary for experiments (4-5mg/ml for DSC).
- 10) SDS PAGE on purification.

Buffers

1X Binding Buffer (High Salt)

5mM Imidazole
500mM NaCl
20mM Tris-HCl, pH 7.9

Thrombin Digest Buffer

150mM NaCl (Use 50mM for Dorit)
20mM Tris pH 7.5
0.5mM DTT (not for DSC)

1X Binding Buffer (Low Salt)

5mM Imidazole
150mM NaCl
20mM Tris-HCl, pH 7.9

End Buffer (DSC)

150mM NaCl
20mM Tris **pH 8.0**

1X Wash Buffer (Low Salt)

60mM Imidazole
150mM NaCl
20mM Tris-HCl, pH 7.9

Elution Buffer

300mM Imidazole
150mM NaCl
20mM Tris-HCl, pH 7.9