

VINCULIN HEAD Domain 1 (Vd1 and Vd1 Y100E substitution) Amplification and Purification

Vector - pET15b-VD1
Molecular Weight = 30.8 KD
Ext = 10870 to get μM
Ext = 0.355 to get $\mu\text{g}/\mu\text{l}$

Amplification:

- 1) 100ml o/n LB/amp culture per 800ml culture the next day, 37°C.
- 2) Seed 800ml LB/amp culture with 100ml o/n culture. Grow at 37°C. until OD600 = 0.6-1.2. Induce with 1mM IPTG. Grow 3hr. Harvest.
- 3) Spin 8krpm, 15min, 4°C.
- 4) Resuspend in His Binding Buffer. Snap freeze.

Purification:

- 1) Lyse 2x800ml cell pellets with homogenizer.
- 2) Spin 16krpm, 30min, 4°C.
- 3) Load sup on to 3ml equilibrated Ni-NTA column.
- 4) Wash with 50ml binding buffer.
- 5) Wash with 10 column volumes wash buffer.
- 6) Elute with elution buffer.
- 7) Dialyze protein into dialysis buffer 1.
- 8) Concentrate VD1 to 20-30mg/ml.
- 9) Dialyze protein into dialysis buffer 2 or end use buffer.
- 10) SDS PAGE on purification.
- 121) Dialyze protein into appropriate experimental buffer.

Buffers:

Binding Buffer:

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

Wash Buffer:

30mM Imidazole
500mM NaCl
20mM Tris-HCl pH 7.9

Elution Buffer:

75mM Imidazole
500mM NaCl
20mM Tris-HCl pH 7.9

Dialysis Buffer 1:

25mM Tris-HCl pH 8.0
150mM NaCl
5mM EDTA
5mM βME

Dialysis Buffer 2:

25mM Tris-HCl pH 8.0
150mM NaCl
5mM βME

ITC Buffer:

20mM Tris-HCl pH 8.0
150mM NaCl