

## **Talin 482-789 Expression and Purification**

Vector pET15b  
Molecular Weight about 32.6KD w/His Tag  
Ext coef = 0.221

### **Expression:**

- 1) Grow 110ml o/n culture in LB/amp.
- 2) Inoculate 2X500ml LB/amp broth with 50ml o/n culture.
- 3) Grow until OD<sub>600</sub>=0.6-1.0.
- 4) Induce w/1mM IPTG.
- 5) Grow 3hr at 37°C.
- 6) Harvest. Resuspend pellets in 10ml 1XHIS Binding Buffer. LN2 freeze. Store at -80°C.
- 7) Run SDS PAGE to check expression.

### **Purification:**

- 1) Quick thaw 2x500ml cell pellets. Add 2mM PMSF. Lyse cells with 0.4mg/ml lysozyme. Rock for 15min 4°C until the mixture is very thick.
- 2) Add MgCl<sub>2</sub> to 10mM. Add DnaseI up to 40µg/ml. Rock for 15min 4°C until the mixture is really runny. Save 10µl for running on gel.
- 3) Add 1% Tx100. Rock for 15min 4°C.
- 4) Spin: 16K, 15min, 30ml tubes. Save supernatant and pellet. Save 10µl of each for running on a gel.
- 5) Meantime, wash Ni-NTA beads with binding buffer. Do a 3ml column.
- 6) Load supernatant on column. Save flow through. Save 10µl for gel.
- 7) Wash 50ml binding buffer.
- 8) Wash 30ml wash buffer (30mM Imidazole)
- 9) Elute with 300mM Imidazole buffer. You can batch elute in 20ml.
- 10) Immediately dialize (20mM Tris pH7.9/250mM NaCl).
- 11) Dialize into final happy buffer.
- 12) Concentrate Protein.

#### **1X Binding Buffer (High Salt)**

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

#### **Elution Buffer (Med Salt)**

300mM Imidazole  
250mM NaCl  
20mM Tris-HCl, pH 7.9

#### **1X Wash Buffer (Med Salt)**

30mM Imidazole  
250mM NaCl  
20mM Tris-HCl pH 7.9

#### **Dialysis Buffer (Med Salt)**

20mM Tris-HCl, pH 7.9  
250mM NaCl