

## **Talin 1843-1973 Expression and Purification**

Vector pET151-DTOPO  
MW = 14.7KD w/His Tag

### **Expression:**

Use BL21 Star (DE3) e.coli to express.

- 1) Grow 210ml o/n culture in LB/carbenicillin (200 $\mu$ g/ml).
- 2) Inoculate 2X700ml LB/carb (200 $\mu$ g/ml) broth with 100ml 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 in -80.
- 7) Run SDS PAGE to check expression.

### **Purification:**

- 1) Quick thaw 2x800ml cell pellets. Add PI tablet w/o EDTA. Lyse cells with homogenizer.
- 2) Spin: 16K, 15min, 30ml tubes.
- 3) Meantime, wash Ni-NTA beads with binding buffer. Do a 3ml column.
- 4) Load sup on column. Save flow through. Save 10 $\mu$ l for gel.
- 5) Wash 50ml binding buffer.
- 6) Wash 30ml wash buffer (30mM Imidazole)
- 7) Elute with 300mM Imidazole buffer. You can batch elute in 20ml.
- 8) Immediately dialyze (20mM Tris pH7.9/250mM NaCl).
- 9) Run SDS-PAGE. Concentrate Protein if needed.

#### **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