

## **Talin His-T196-889 Amplification and Purification (no tag cleavage)**

Vector - pET151D-TOPO  
Molecular Weight about 78.1KD w/His Tag  
Theoretical pI = 6.02  
Ext Coef = 0.463

### **Amplification:**

- 1) Grow 200ml o/n culture in Tuner/LB/carb.
- 2) Inoculate 2X700ml LB/carb 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.
- 7) Run SDS PAGE to check expression.

### **Purification:**

- 1) Quick thaw cell pellets. Add protease inhibitors. Lyse cells with Homogenizer.
- 2) Spin: 16K, 30min, 30ml tubes.
- 3) Meantime, wash Ni-NTA beads with binding buffer. Do a **10ml column**.
- 4) Load sup on column. Save flow through.
- 5) **Wash** 100ml binding buffer.
- 6) **Elute** with 300mM Imidazole buffer.
- 7) Immediately dialyze (20mM Tris pH7.9/250mM NaCl/5mM EDTA).
- 8) Concentrate Protein to at least 10mg/ml for ITC.
- 9) Run on S200 large column.
- 10) Dialyze into Dialysis Buffer 2

#### **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

#### **Dialysis Buffer 1**

20mM Tris-HCl, pH 8.0  
250mM NaCl  
5mM EDTA  
1mM DTT

#### **Dialysis Buffer 2**

20mM PIPES pH 7.0  
50mM NaCl  
1mM MgCl<sub>2</sub>