

## **Tensin-SH2 Expression and Purification**

pET15b-His-Tensin-SH2c (aa1514-1631)  
MW 13.6KD  
Ext Coef 0.801 (or 10930 to get  $\mu$ M)

### **Expression:**

- 1) 100ml o/n LB/amp culture
- 2) Next day seed 700ml LB/amp with 100ml o/n culture, 37°C.
- 3) Grow cells until OD600 = 0.6-1.2
- 4) Induce with 1mM IPTG. Grow for 4hr 37°C.
- 5) Harvest 8krpm, 4°C, 10min.
- 6) Resuspend 800ml cell culture pellet in 20ml Ni-NTA binding buffer. LN2 freeze.

### **Purification:**

- 1) Thaw 1L cells. Add PI tablet w/o EDTA. Add 2mM PMSF.
- 2) Add 0.4mg/ml lysozyme. Rock 15-30min until thick.
- 3) Add 10mM MgCl<sub>2</sub>. Add 0.4 $\mu$ g/ml DNaseI. Rock 15-30min until loose.
- 4) Add 0.1% Tx-100. Rock 15min. Save 10 $\mu$ l for SDS-PAGE.
- 5) Spin 16krpm, 4°C, 15min. Save some sup and pellet for gel.
- 6) Meanwhile, wash 2ml Ni-NTA beads (for 4L of SH2c cells) with binding buffer.
- 7) Load supernatant onto beads.
- 8) Wash with 50ml binding buffer.
- 9) Wash with 50ml wash buffer low salt.
- 10) Elute with elution buffer low salt.
- 11) Dialize into dialysis buffer1 while digesting off His tag with biotinylated thrombin. Digest for 6hr RT.  
A lot of protein will ppt out of solution. Spin 40k, 4°C, 20min. Capture biotinylated thrombin with streptavidin agarose.
- 12) Dialize flow through in FPLC Buffer A.
- 13) Run SDS PAGE on purification. Do a quick protein concentration check.

### **FPLC Purification:**

Use 1ml Qlinear 40mlCV FPLC program. This one get's around the computer bug that messes up the fraction collector.  
1ml SP-Cation Exchange column  
Load 2ml protein (~3mg/ml)  
Flow rate 1ml/min  
Gradient 0-50% FPLC Buffer B  
Elute 40min  
Run SDS PAGE  
Dialize pure peak into buffer2. Do concentration. LN2 freeze.  
(note: if separation of contaminants is not perfect lengthen the elution or shorten the gradient)

### **Buffers:**

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

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

#### **Dialysis Buffer1**

20mM Tris-HCl pH 7.9  
100mM NaCl  
1mM DTT

1mM DTT

**Wash Buffer (Low Salt)**

30mM Imidazole

100mM NaCl

20mM Tris-HCl pH 7.9

1mM DTT

**Elution Buffer (Low Salt)**

300mM Imidazole

100mM NaCl

20mM Tris-HCl pH 7.9

1mM DTT

**FPLC Buffer A**

50mM HEPES pH 7.6

1mM DTT

**FPLC Buffer B**

50mM HEPES pH 7.6

1M NaCl

1mM DTT

**Dialysis Buffer2**

20mM Tris-HCl pH 7.9

50mM NaCl

1mM DTT