

## pET15b Alpha-actinin Expression and Purification

MW after purification 102,973 Da  
Extinction Coefficient 124,620

### **Expression:**

1. Transform BL21 cells with 1  $\mu$ l of plasmid. Plate transformed cells onto LB + Agar + 50  $\mu$ g/ml of carbenecillin plate. Grow colonies overnight at 37°C.
2. Inoculate 50 ml TB + 0.4% glucose + 50  $\mu$ g/ml of carbenecillin with a single colony.
3. Grow culture shaking at 37°C until A600 = 0.6 to 0.8. Store this starter culture at 4°C overnight
4. Inoculate 500 ml TB in a 2L flask, containing 0.4% glucose and 50  $\mu$ g/ml carbenecillin, with 10 ml of starter culture.
5. Grow culture, shaking at 37°C until A280 = 0.6 - 0.8.
6. Add 1 mM IPTG to induce protein expression and grow for 3 hrs, shaking at 37°C
7. Spin down cells at 8000 RPM for 10 min.
8. Resuspend pelleted cells in 20 ml binding buffer per liter of induced cells. Store resuspended pellets at – 80°C.

### **Purification:**

1. Thaw a frozen pellet from 1 L of cultured cells.
2. To lyse cells add 1 mg/ml lysozyme, EDTA-free protease inhibitor cocktail, 5 mM BME and lyse for 20 min at RT.
3. Break up lysed suspension by sonication -- 1 second on 2 seconds off for 5 minutes. Keep cells on ice during sonication. When cells are sonicated, the once viscous solution will no longer be viscous.
4. Spin out insoluble material -- 19 K RPM for 1 HR
5. Recover supernatant for Ni<sup>++</sup> column purification.
6. Prepare a 5 ml HiTrap Ni<sup>++</sup> chelating column (Pharmacia) for supernatant application Hook up column to a peristaltic pump
7. Rinse column with 25 ml H<sub>2</sub>O
8. Charge column with 25 ml 100 mM NiCl<sub>2</sub>
9. Rinse column with 50 ml H<sub>2</sub>O
10. Equilibrate column with 25 ml 10 mM Imidazole Wash Buffer.
11. Apply supernatant from lysed cells to charged and equilibrated column. Collect a sample of this flow through and each of the following washes, eluted protein and EDTA strip for SDS-PAGE analysis.
12. Wash column with 25 ml 10 mM Imidazole Wash Buffer.
13. Wash column two times with 25 ml of 25 mM Imidazole Wash Buffer.
14. Elute bound protein with 12 ml of 250 mM Imidazole Elution Buffer. Collect eluted protein solution.
15. Strip column with 25 ml of EDTA Strip Buffer.
16. Dialyze the 12 ml of eluted protein against 2L of EDTA Dialysis Buffer 1 for 3 or more hours at 4°C
17. Change dialysis buffer to Calcium Dialysis Buffer and dialyze for 3 or more hours at 4 C.
18. Purify monodisperse protein using an S200 column equilibrated with Calcium Dialysis Buffer.
19. Concentrate pooled fractions to 5 – 10 mg/ml. Aliquot protein, freeze in liquid N<sub>2</sub> and store at 80°C.

### **Buffers:**

#### **Binding Buffer:**

500 mM NaCl  
20 mM TRIS pH 8  
10 mM Imadazole pH 8

**10 mM Imidazole Wash Buffer:**

500 mM NaCl  
20 mM TRIS pH 8  
10 mM Imidazole pH 8

**25 mM Imidazole Wash Buffer:**

500 mM NaCl  
20 mM TRIS pH 8  
25 mM Imidazole pH 8

**250 mM Imidazole Elution Buffer:**

500 mM NaCl  
20 mM TRIS pH 8  
250 mM Imidazole pH 8

**EDTA Strip Buffer:**

500 mM NaCl  
50 mM EDTA pH 8  
20 mM TRIS pH 8

**EDTA Dialysis Buffer:**

150 mM NaCl  
20 mM TRIS pH 8  
5 mM EDTA pH 8  
5 mM BME

**Calcium Dialysis Buffer:**

150 mM NaCl  
20 mM TRIS pH 8  
5 mM CaCl<sub>2</sub>  
5 mM BME