

## Production of N-His<sub>6</sub>-tagged TEV protease (1-liter preparation)

### Construct

N-terminal His<sub>6</sub>-tagged TEV protease gene in a pET-24d(+) vector (kanamycin resistance).

### Protocol

1. Freshly transform the construct into *E. coli* BL21 (DE3) pLysS (chloramphenicol resistance). Plate on LB-agar + Kan + Cam. Incubate overnight at 37°C.
2. Pick 2 colonies to inoculate 2 x 4 ml LB + Kan + Cam. Incubate overnight at 37°C.
3. Use the overnight cultures to inoculate 2 x 0.5 L LB + Kan + Cam (in 2-L flasks). Grow the cultures until OD<sub>600</sub> is approx. 0.6. Then cool the culture on ice to 20°C and induce by the addition of 0.2 mM IPTG.
4. Incubate the culture overnight at 20°C. Harvest the cells and store the pellet at -20°C.
5. Resuspend the pellet in 30 ml lysis buffer.

*Upon thawing the cells lyse because of the lysosyme produced by this strain. Add 40 µl DNase I (1 mg/ml).*

6. Sonicate (3 min at 30% duty cycles on ice) to improve cell lysis.
7. Spin the cell lysate in the SS-34 rotor (Sorvall) for 60 min at 18,000 rpm.
8. Add imidazole (2 M) to the supernatant to a final concentration of 20 mM.
9. Apply the supernatant to a 5-ml Chelating Sepharose column (GE Healthcare), charged with 100 mM NiSO<sub>4</sub> and equilibrated with binding buffer.
10. Wash the column with binding buffer until no protein elutes anymore (monitored by the absorbance at 280 nm).
11. Repeat step 10 with binding buffer containing 50 mM imidazole (including 10% elution buffer).
12. Elute the protein with elution buffer. Collect the eluate in 2-ml fractions.

*TEV protease is not stable in buffers containing a high concentration of imidazole. Therefore, you need to exchange the buffer of the protein solution immediately after elution from the Chelating Sepharose column.*

13. Apply the pooled fraction to a 50-ml HiTrap 26/10 Desalting column (GE Healthcare), equilibrated with desalting buffer. Collect the eluate in 2.5-ml fraction.

14. Pool the fractions that contain protein and determine the protein concentration.

*The amount of TEV protease can be determined by measuring the absorbance at 280 nm of the protein solution against the elution buffer. The concentration can be calculated using a specific extinction coefficient of 1.19 (a TEV solution of 1 mg/ml gives an  $A_{280}$  of 1.19).*

15. Dilute the preparation to a concentration of 1 mg/ml by the addition of pure glycerol to a final concentration of 50% (v/v) and desalting buffer. Store the final solution in 0.5-ml aliquots at  $-80^{\circ}\text{C}$ .

*Alternatively, the Chelating Sepharose eluate can be dialyzed overnight against 1 L of storage buffer. This has the additional advantage that the protein is concentrated by a factor of 2-3. Store the final solution in 0.5-ml aliquots at  $-80^{\circ}\text{C}$ .*

Arie Geerlof

30 April 2007

#### **Lysis buffer**

50 mM Tris-HCl pH 8.0  
300 mM NaCl  
0.2% (v/v) NP-40  
0.02% (v/v) 1-thioglycerol

#### **Desalting buffer**

50 mM Tris-HCl pH 8.0  
150 mM NaCl  
0.01% (v/v) 1-thioglycerol  
20% (v/v) glycerol

#### **Binding buffer**

50 mM Tris-HCl pH 8.0  
300 mM NaCl  
20 mM imidazole  
0.01% (v/v) 1-thioglycerol  
20% (v/v) glycerol

#### **Elution buffer**

50 mM Tris-HCl pH 8.0  
300 mM NaCl  
300 mM imidazole  
0.01% (v/v) 1-thioglycerol  
20% (v/v) glycerol

#### **Storage buffer**

50 mM Tris-HCl pH 8.0  
150 mM NaCl  
0.01% (v/v) 1-thioglycerol  
50% (v/v) glycerol