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Purification of “Super” TEV from Rosetta *E. coli*

Day 1- streak out colonies

1. Transform Rosetta *E. coli* with the plasmid encoding “Super” TEV (JS1524). Grow the colonies on fresh selective plates containing 75 g/mL carbenicillin and 30 g/mL chloramphenicol. Alternatively, streak bacteria from a glycerol stock onto a fresh selective plate.
2. Incubate the plate overnight at 37 C.

Day 2- initiate starter culture

1. The next morning, select a single colony and inoculate 3 mL of LB containing 100 g/mL ampicillin and 30 g/mL chloramphenicol. Grow the culture at 37 C with shaking at 240 rpm for ~8 hours.
2. Dilute the starter culture 1:500 – 1:1,000 into 100 mL of LB containing 100 g/mL ampicillin and 30 g/mL chloramphenicol.
3. Grow the culture overnight at 37 C with shaking at 240 rpm.

Day 3- induce protein expression

1. Spin down the overnight culture at 4 C for 10 minutes at 6,000 rpm in the JA-14 rotor. Resuspend the cell pellet in 100 mL of fresh LB.
	1. This step will remove -lactamase secreted into the culture medium and provide more selective pressure on the ampR plasmid encoding “Super” TEV.
2. Add 20 mL of overnight culture to 1 L of LB containing 100 g/mL ampicillin and 30 g/mL chloramphenicol. Inoculate 4 L total.
3. Grow the cells at 37 C with shaking at 225 rpm and monitor cell density until the OD600 reaches 0.6 – 0.8.
	1. Note that the presence of chloramphenicol will slow cell growth, so the cells will take several hours to reach the density required for induction.
4. Add IPTG to a final concentration of 0.1 mM, and grow the cells overnight at **18 C** with shaking at 225 rpm.

Day 4- harvest cells and purify “Super” TEV

**Preparation of cell lysate**

1. 16 – 20 hours post-induction, collect the cells in 1 L centrifuge bottles and spin at 4,000 rpm in the swinging bucket rotor for 25 minutes at 4 C. Decant the supernatant, and then invert the bottles to remove all traces of medium.
2. Resuspend the cells thoroughly in 200 mL of buffer N1.
3. Lyse the cells using the EmulsiFlex C5.
	1. Pass the lysate through the EmulsiFlex once at low pressure (~5,000 psi), and then three more times at high pressure (~15,000 – 20,000 psi).
	2. **Collect a total lysate sample.**
4. Centrifuge the lysate at 100,000*g* for 1 hour at 4 C (36,000 rpm in the Ti45 rotor).
5. Collect the supernatant and filter through a 0.45 m PES low protein-binding filtration device.
	1. **Collect a load sample.**
6. Resuspend the pellet from one centrifuge tube in PBS.
	1. **Collect a pellet sample.**

**Ni2+ column chromatography**

1. Apply the soluble lyate to a 5 mL HisTrap FF column equilibrated in buffer N1.
	1. **Collect a flow-through sample.**
2. Wash the column with 15 column volumes of 100% buffer N1.
3. Wash the column with 15 column volumes of 5% buffer N2.
4. Elute bound proteins with 10 column volumes of 30% buffer N2- collect 3 mL fractions.
	1. Elute protein into tubes containing 3 mL of dilution buffer- although “Super” TEV contains mutations that reduce autolysis, some self-cleavage and precipitation can occur if the protein concentration is too high.
5. Bump the column with 5 column volumes of 100% buffer N2 and re-equilibrate the column in buffer N1.
6. **Collect wash-through and elution fractions** and analyze all samples by SDS-PAGE.

**Dilute protein**

1. Collect the elution fractions containing “Super” TEV and measure the absorbance at 280 nm.
2. Adjust the protein concentration to 3.6 mg/mL using dilution buffer.
3. Add 0.8 volumes of 100% glycerol.
	1. **Note**: A final glycerol concentration of 50% is necessary to prevent precipitation of the “Super” TEV when it’s thawed for use.
	2. Final buffer composition: 20 mM HEPES (7.5), 200 mM NaCl, 2 mM EDTA, 2 mM DTT, 50% (v/v) glycerol. Final “Super” TEV concentration: 2 mg/mL.
4. Make 0.5 mL aliquots in purple tubes, snap-freeze the protein in liquid nitrogen, and store at -80 C until use.

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| --- | --- | --- |
| **buffer N1** | **stock** | **add (mL)** |
| 20 mM HEPES (7.5) | 1 M | 20 |
| 300 mM NaCl | 5 M | 60 |
| 10 mM imidazole | 3 M | 3.34 |
| 10% glycerol | 50% | 200 |
|  |  |  |
| ddH2O |  | to 1 L |

|  |  |  |
| --- | --- | --- |
| **buffer N2** | **stock** | **add (mL)** |
| 20 mM HEPES (7.5) | 1 M | 6 |
| 300 mM NaCl | 5 M | 18 |
| 1 M imidazole | 3 M | 100 |
| 10% glycerol | 50% | 60 |
|  |  |  |
| ddH2O |  | to 300 mL |

|  |  |  |
| --- | --- | --- |
| **dilution buffer** | **stock** | **add (mL)** |
| 36 mM HEPES (7.5) | 1 M | 18 |
| 360 mM NaCl | 5 M | 36 |
| 3.6 mM EDTA | 0.5 M | 3.6 |
| 3.6 mM DTT | 1 M | 1.8 |
| 10% glycerol | 50% | 100 |
|  |  |  |
| ddH2O |  | to 500 mL |

**Notes and references**

“Super” TEV molecular weight: 28,533 g/mole

“Super” TEV extinction coefficient: 31,970 M-1cm-1 at 280 nm

van den Berg *et al*. (2006) Improved solubility of TEV protease by directed evolution. *Journal of Biotechnology* 121, 291-298.

Kapust *et al*. (2001) Tobacco etch virus protease: mechanism of autolysis and rational design of stable mutants with wild-type catalytic proficiency. *Protein Engineering* 14, 993-1000.

