Purification of a 6-liter prep expression of TEV Protease, DMH

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Background: The plasmid for producing the Tobacco Etch Virus (TEV) protease was obtained from Reba Howard of Dan Minor's lab at UCSF. The vector is amp^R and called pTEV. The vector apparently encodes Maltose Binding protein (MBP) separated by a TEV recognition site followed by a mutant TEV protease (and 6×His-tag) that resists autoproteolysis. During expression, TEV cleaves off its MBP tag so that the only protein that binds to the 6×His-affinity column is TEV. TEV is not very stable and needs to be stored frozen at –80°C. TEV needs a reducing agent to ensure activity and prefers low ionic strength buffer. A good buffer is 50 mM Tris, pH 8.0, 1 mM DTT.

Reagents:

- **2×YT** medium. 250 mL medium and 6 × 1 L medium
- Stock **ampicillin** (100 mg/mL in 50% ethanol)
- Stock **chloramphenicol** (34 mg/mL in ethanol)
- One-shot **BL21(DE3) pLysE** *E. coli* cells (Invitrogen)
- Stock **IPTG** (1 M in water)
- **Benzonase** (a non-specific nuclease from Novagen)
- A 5 mL **Hi-Trap** chelating column (Amersham-Pharmacia)
- **Lysis buffer** (50 mM Tris, 500 mM KCl, 1 mM PMSF, pH 7.4)
- **Charge buffer** (100 mM $NiSO_4$)
- **5×Bind buffer** (50 mM NaPO4, 2.5 M NaCl, 25 mM imidazole, pH 7.4)
- **Elution buffer** (PBS, 150 mM imidazole, pH 7.4)
- **GuHCl** (5 M stock)
- **Triton X-100**
- **Glycerol**
- **Strip buffer** (50 mM EDTA).

Expression:

1. Transform pTEV into Invitrogen One-shot **BL21(DE3) pLysE** *E. coli* cells. Incubate for 1 h at 37°C according to the instructions.
2. Add entire transformation reaction into 250 mL **2×YT** medium + 100 µg/mL **ampicillin** + 34 µg/mL **chloramphenicol** in a 500 mL–1 L flask and grow overnight. Note: the cells will only induce protein in a DE3 strain of *E. coli*. *pLysE is a plasmid encoding T4 lysozyme, which suppresses basal expression. Chloramphenicol is needed to maintain the presence of pLysE.*
3. In the morning, aliquot 10 mL overnight culture each into 6 × 1 L flasks of **2×YT** medium + 100 µg/mL **ampicillin** + 34 µg/mL **chloramphenicol**. Incubate in shaker at 37°C until OD_{600} = 0.6 (*about 3.5 h*).

4. Express protein by adding 400 µL IPTG to each flask. Grow for 3 h.
5. Spin down cells at 3,000 rpm, 20 min, and 4°C in the Beckman J-6B centrifuge (or similar).
6. Resuspend cells in minimum volume of lysis buffer. *Use ~5 mL per liter of expression.*
7. Freeze cells at –20°C.

Purification:

8. Thaw cells, add 50 µL benzonase, and sonicate on ice using medium-sized probe, max power for 2 min (50% oscillation). *The pLysE results in spontaneous lysis after freezing due to the expressed lysozyme.*
9. Spin lysate at 17,000 rpm in the Sorval SS34 rotor for 30 min at 4°C (or similar). Keep supernatant and discard pellet.
10. Filter lysate through a 0.45 µM syringe filter.
11. Add **5×Bind buffer** to a final 1× concentration.
12. In the meantime, prepare the **Hi-Trap** chelating column. Wash with ~20 mL water using the peristaltic pump (flow rate at 600 — green ended tubes on Rabbit peristaltic pump). Wash ~20 mL with **charge buffer**. Wash ~20 mL with **water**. Wash ~50 mL **1×Bind buffer**.
13. Load lysate onto the column (flow at 600).
14. Wash column with ~25 mL **1×Bind buffer** (flow at 600).
15. Elute bound protein with 50 mL **elution buffer**. Discard the first 5 mL, keep the next 30 mL.
16. ***Important: Move quickly here and when freezing to stabilize protein from aggregation***. Immediately add Triton X-100 to 0.1% (0.05 g, *which is about one small drop*), and glycerol to 50 mL. *This will slow down aggregation significantly. The final buffer concentration of reagents will be ~(40% glycerol (v/v), 0.1% Triton X-100, 3/5× PBS, 90 mM imidazole). The final protein nominal concentration should be thus about 15 OD_{280}.*
17. Snap-freeze 1 mL aliquots in a dry ice/ethanol or liquid nitrogen bath. Store protein at –80°C. Aliquots can be later thawed and re-aliquot in 50 µL amounts to more efficiently store enzyme.
18. Run an SDS-PAGE gel (*e.g.,* 10–20% Tris-glycine) gel to check protein purity. It should be above 90% pure, with an expected mass of about 27 kDa.
1. Run 5 µL of protease on gel
2. Also run 5 µg BSA as a mass standard (optional)
19. Check protease concentration with a BioRad assay.
20. When using the protease be aware of the following points:
    1. The protease aggregates fairly fast so aim to not expect significant cleavage after the first few hours.
    2. The enzyme is most active at 30°C but cuts well at room temperature (and 4°C).

3. A dilution of 1/20 cuts the CThx1 fusion completely within 1 h in 50 mM Tris, pH 8.0, 1 mM DTT at fusion concentrations of about 1 to 3 mg/mL. *This fusion is thioredoxin-TEVsite-Huntintin(46Q)-Cerulean.*
4. The enzyme theoretically needs a reducing environment to cut well but I have found it to cut OK in just in PBS with no reducing agent.
5. The protease is highly specific and should not produce unwanted cleavage (so you can use heaps of enzyme to cut protein with no adverse effects).