**Purifying TEV Protease**

**Based on protocol from Hill Lab**

**Revised by Emily March 2010**

**Day 1**

1. Have freshly streaked plate of TEV in BL21 from glycerol stock on LB+Amp+Chloramphenicol (Cam).
2. Inoculate 3 mL 2XYT+Amp+Cam with colony and grow 6-8 hrs at 37ºC.
3. Inoculate 2 flasks of 25 mL 2XYT+Amp+Cam with 50 µL of starter culture and grow overnight at 37ºC.
4. Make and autoclave four baffled flasks of 500 mL LB broth (2 L total).

**Day 2**

1. Add Amp and Cam to each 500 mL flask and inoculate with 12 mL overnight culture. Grow at ~20ºC (leave top of shaker open).
2. Monitor A600 and induce with IPTG when it reaches 0.5. Will take ~6-7 hrs.
3. Grow overnight at 20ºC.

**Day 3**

1. Spin down growths in pre-weighed bottles in JA-10 rotor at 5000 rpm for 15 min.
2. Weigh bottles with pellets and determine weight of pellets.
3. Make buffers:

Buffer A

20 mM Sodium Phosphate (NaP) pH 8

500 mM NaCl

10% glycerol

50 mM imidazole

Buffer B

20 mM NaP pH 8

500 mM NaCl

10% glycerol

300 mM imidazole

1. Resuspend each pellet in 1:5 weight/volume Buffer A (for example, resuspend 5 g pellet in 25 mL buffer). Aliquot 25 mL into 50 mL conical tubes. *Cell suspensions may be frozen here*.
2. Add half a tablet of protease inhibitor to each 25 mL of cells.
3. French press each 25 mL tube 5 times.
4. Add 25 µL DNase (1 mg/ml stock) to each 25 mL.
5. Spin down crude homogenate in JA-10 rotor at 10,000 rpm for 40 min.
6. Pool supernatants and syringe filter with 0.45 µm filter.

**Purify His-tagged TEV on Ni column:**

1. Use 10 mL Ni-NTA column. Charge column periodically by running 100 mL (10 column volumes) of water, followed by 10 mL of 0.1 M NiSO4, then 50 mL of water.
2. Equilibrate column with 50 mL Buffer A.
3. Load lysate onto column. Collect flow-through.
4. Wash column with 50 mL of Buffer A twice (Wash 1 and Wash 2).
5. Elute TEV with 50 mL of Buffer B.
6. Wash column with 50 mL of water.
7. Store column in 20% ethanol.
8. Take wavelength scans and remove samples from each fraction for SDS-PAGE to verify presence of protein.
9. If precipitate forms in eluted TEV fraction, spin down and retain supernatant.
10. Dialyze TEV fraction overnight at RT into 20 mM NaP pH 8, 500 mM NaCl, 10% glycerol, 10 mM DTT.

**Day 4**

1. Recover TEV sample. Take wavelength scan to determine concentration (blank with dialysis buffer).
2. Aliquot into eppendorf tubes and snap freeze in dry ice/ethanol bath.
3. Store at -80ºC.

