TEV Purification
CP1089  pTEV S219V amp′  TEV Protease/ T7 Promotor (P. Kaufman)

Buffers:

**Ni-NTA Lysis Buffer**

- 50mM Tris-HCL pH 8.0
- 150mM NaCl
- 10mM Imidazole pH 8.0
- 10 mM βME
- 150 µM PMSF

**Wash Buffer**

- 50mM Tris-HCL pH 8.0
- 150mM NaCl
- 20mM Imidazole pH 8.0
- 10mM βME
- 150µM PMSF

**Elution Buffer**

- 50mM Tris-HCL pH 8.0
- 150mM NaCl
- 250mM Imidazole pH 8.0
- 10mM βME
- 150µM PMSF

**Dialysis Buffer**

- 20 % glycerol
- 50mM Tris-HCL pH 8.0
- 1mM EDTA pH 8.0
- 5mM DTT
1. Streak glycerol stock and do a mini prep (alkaline lysis is fine) 
2. Transform into Rosetta DE3 pLysS competent cells 
3. Pick single colonies and inoculate 1 ml LBamp 
4. Grow approx. 6 hours and use to inoculatea 50ml 2XYT amp/chlor culture-Allow to grow O/N at 37°C 
5. In the morning, inoculate 2 x 1L 2XYT amp/chlor with 25 ml of starter culture (25 ml per liter). 
6. Grow to an OD$_{600}$ and induce with 0.1mM IPTG 
7. Allow to grow for 4 hours and harvest at 3K for 30’ 
8. Resuspend each pellet in 25 ml lysis buffer and freeze at -80°C 
9. Check sample on SDS-PAGE to make sure there was adequate induction. 
10. Thaw frozen pellets at 37°C with frequent mixing 
11. Sonicate 4 X 30 seconds, keeping on ice in between 
12. Clear lysate by centrifuge, 15K for 30’ at 4°C 
13. Prepare 4 ml of Ni resin slurry by washing with 20 ml of wash buffer. 
14. Incubate cleared lysate with washed Ni resin for 1 hr. at 4°C 
15. Centrifuge at 1K for 5’, pour off all but 10 ml and save as FT 
16. Mix remaining 10 ml with the resin and pour into a 10ml disposable Bio-Rad column (do this at 4°C) 
17. Wash with 5 X column volumes of was buffer 
18. Elute with 5 X column volumes of