# TEV Purification

CP1089 pTEV S219V amp$^r$ TEV Protease/ T7 Promotor (P. Kaufman)

## Buffers:

**Ni-NTA Lysis Buffer**

50mM Tris-HCL pH8.0
150mM NaCl
10mM Imidazole pH8.0
10 mM ßME
150 µM PMSF

**Wash Buffer**

50mM Tris-HCL pH8.0
150mM NaCl
20mM Imidazole pH8.0
10mM ßME
150µM PMSF

**Elution Buffer**

50mM Tris-HCL pH 8.0
150mM NaCl
250mM Imidazole pH 8.0
10mM ßME
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 innocluate 1 ml LBamp
4. Grow appox. 6 hours and use to innoculatea 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