Expression & Purification of His-TEV(S219V)-Arg

(S. Cherry, J. E. Tropea, D. S. Waugh)

- BL21-RIL cells containing pRK793 are grown at 37 °C in L-broth containing 100 µg/ml ampicillin and 30 µg/ml chloramphenicol.
- When the cells reach mid log phase (OD600~0.5), IPTG is added to a final concentration of 1 mM and the temperature is reduced to 30 °C.
- After 4 hrs of induction, the cells are collected by centrifugation.
- Dissolve the cell pellet in 10 ml of Lysis Buffer[1)] per 1 gram of wet cell paste.
- Lyse the cells. We use a Gaulin cell homogenizer @ 10,000-10,500 psi for 3 passes.
- Add 5% polyetheleneimine (adjusted to pH 7.9 with HCl) to a final concentration of 0.1%.
- Mix by inversion and then immediately centrifuge at 15,000 x g for 30 minutes.
- Apply the supernatant to a Ni-NTA column equilibrated with Lysis Buffer, using ~ 2 ml of resin per gram of wet cell paste.
- Wash the column with 7 volumes of Lysis Buffer, and then elute the TEV protease with a linear gradient of Lysis Buffer to Elution Buffer[2)] in 10 column volumes.
- Pool the appropriate fractions and then add EDTA and DTT to a final concentration of 1 mM each.
- Concentrate the sample in a stirred cell using a YM10 membrane.
- Load the sample onto an S-100 column equilibrated with GF Buffer [3)] (sample volume = 3% of the column volume).
- Pool the appropriate fractions.
- Concentrate the protease to ~ 1 mg/ml and flash freeze with liquid nitrogen.
- Store at –80 °C.

The final yield should be approximately 10 mg of protein per gram of wet cell paste (~ 30 mg/liter)

1)
50 mM PO4 (pH 8.0), 100 mM NaCl, 10% glycerol and 25 mM imidazole
2)
50 mM PO4 (pH 8.0), 100 mM NaCl, 10% glycerol and 200 mM Imidizole
3)
25 mM PO4 (pH 8.0), 200 mM NaCl, 10% glycerol, 2mM EDTA and 10 mM DTT