Purification of TEV protease (S219V)

BL21-RIL cells are the expression host. Extra tRNA genes for particular R,I,L codons are carried on a plasmid selected by chloramphenicol. I streak the freezer stock of this strain on a CAM plate then do colony transformation to an AMP/CAM plate or culture.

The TEV protease plasmid is pRK793, encoding a fusion of MBP- (TEV protease cleavage site) - 6xHis tag – TEV protease (S219V) – polyR. The MBP fusion keeps the protease soluble as it folds initially (it attains a better specific activity than when refolded from denatured state), but the active protease cleaves off the MBP tag leaving only the 6xHis tag for purification. The C-terminal polyR tail can be used as a second step purification if desired, but this isn't necessary with the S219V variant because the whole prep of protease stays full-length. Wild-type and even the previous S219N variant undergo autoproteolysis when stored at any reasonable concentration, but the S219V variant does not.
** Kapust RB …. Waugh DS. Protein Engineering 14:993-1000 (2001).**

For the prep, grow cells at 37oC in LB + 100 μg/ml ampicillin and 30 μg/ml chloramphenicol. When the cells reach mid-log phase (OD600 ~ 0.5), add IPTG to 1 mM and move the cells to shaking at 30oC. After 4 hrs of induction, harvest by centrifugation. After draining all media, I freeze the cells in their centrifuge bottle by placing them at –20oC.

Resuspended in 10 ml/L original culture using TGI buffer (20 mM Tris-HCl pH 7.5, 10% glycerol, 20 mM imidazole). Lyse thoroughly by sonication. Use 5 M NaCl to adjust sonicate to 250 mM add 25% ammonium sulfate (final concentration 1M), rotate end-over-end 15 min 4oC. Spin 20 min 12.5K in SS34.

To supernatant, add PEI (polyethyleneimine, Polymin P) to 0.1% final from 5% stock (adjusted to pH 8) in increments (e.g., add 1/4th of the total, mix well, repeat). Rotate end-over-end 20 min 4oC. Spin 20 min 12.5K in SS34. Filter through miracloth.

Add NiNTA resin washed into TGI buffer + 250 mM NaCl (beware, no DTT or EDTA). I bound in batch rotating end-over-end 4oC for 2 h while getting a snack. I used 2.5 ml resin/L original culture.

I transferred to column format to wash and elute. Wash well with TGI buffer + 250 mM NaCl. I didn't wash well enough, and as a result there is still MBP left in the TEV protease prep (MBP becomes the most abundant protein in the cell lysate). I eluted in three steps: final concentrations of 45, 220 and 520 mM imidazole (note the 20 mM in TGI buffer; supplemented using a 5M imidazole stock pHed to 7.5). My second elution was the peak.

Fractions were examined by SDS-PAGE. NOTE: the protease fusion is ~30 kD, so run a high percentage acrylamide gel. Dialyze into storage buffer (50 mM Tris-HCl pH 8.0, 50% glycerol, 0.1 mM EDTA) with 2 mM DTT. You will achieve concentration with the change in glycerol. Or dialyze into 2X storage buffer with 20% glycerol, add 1 volume 80% glycerol and extra DTT.

First run through 12/02 prep: 2 L in 4x500 ml cultures. Final concentration 5-10 mg/ml TEV protease. About 6 ml, aliquot to store long-term at –80oC with working stock at -20oC.