Purification of His-TEV Protease

NOTE 1: TEV from Invitrogen (or whatever they're called these days) is super expensive (Cat# 10127-017; 1000U; 10U/µl). Following is protocol from Jessica Dermody. Plasmid is pTPSN from Jennifer Doudna (Lucast *et al* 2001 *BioTechniques* **30:**544) (Buratowski lab F587/b2664. Keogh Lab MKF308/mb323). Expresses HIS-tagged TEV (Tobacco Etch Virus) NIa protease with S219N mutation that removes a self cleavage site. Most expressed protein is insoluble. See reference for construction and expression steps.

Induction: rTEV-HIS6 under regulation of a T7 Promoter (use: *E.coli* Strain: BL21/DE3 *pLysS* or suchlike). Standard growth / induction (@ OD_{600} 0.4, add 0.1mM IPTG / 4h, RT°C). Harvest and store at –80°C until ready to proceed.

1. Resuspend induced *E.coli* pellets in 5x volumes Ni-NTA lysis buffer
50mM Tris-HCl, pH 8.0
150mM NaCl
10mM Imidazole, pH 8.0
10mM βME
150μM PMSF

2. Sonicate to lyse cells: 3 x 30 sec on, 30 sec off, power 4
(don't try extra sonication pulses to increase yield – it gives higher backgrounds)

3. Spin down lysate for 30min at 15000rpm at 4°C in SS34 rotor.

4. Mix clear lysate with prewashed Ni-NTA resin (1-2mL of slurry per liter of culture).

5. Incubate lysate plus resin on rocker at 4°C for 30-60min.

6. Spin down lysate+resin for 5min at 1000pm and remove supernatant and save as flowthrough. Pour resin into small Biorad column. DO ALL THIS IN THE COLD ROOM!!!

7. Wash resin 5x column volume (cv) with wash buffer, and collect in 1 cv fractions.
50mM Tris-HCl, pH 8.0
150mM NaCl
20mM Imidazole, pH 8.0
10mM βME
150μM PMSF

8. Elute 5x column volume with elution buffer, and collect in 1 cv fractions.
50mM Tris-HCl, pH 8.0
150mM NaCl
250mM Imidazole, pH 8.0
10mM βME
150μM PMSF

9. Run 15% SDS-PAGE to determine which elution fractions contain the protease

10. Dialyze protease into:
20% glycerol
50mM Tris-HCl, pH 8.0
1mM EDTA, pH 8.0
5mM DTT

11. Store in 20µl aliquots at –80ºC

NOTE 2: You will have to determine the home-made TEV amount empirically (see MCK lab notebook pH13.56). It is handy to have some of the invitrogen stuff to hand when making the first batch to see how well you're doing. The enzyme is usually so active that four hours at 4ºC should go to completion. It is possible to determine this by αPAP (Sigma) reactivity in westerns – you're looking for the disappearance of the TAP-band. You can also use the αCBP antibody (although I'm not a huge fan of the latter). Below % substrate hydolysis data from Invitrogen.

Time	**4ºC**	**16ºC**	**21ºC**	**30ºC**
0.5h	34	58	56	77
1h	58	80	78	90
2h	71	99	99	99
3h	84	99	99	99

rTEV purified with this protocol was used in:

Keogh *et al* (2006) *Nature* **439:**497
Keogh *et al* (2006) *Genes Dev* **20:**660-5