Purification of his-tagged TEV protease

Growth (6L)

1. Streak LB-amp plate with frozen glycerol stock or transform his-tev into BL21 cells and grow overnight.
2. Inoculate 8ml LB+amp with a single colony and grow overnight at 37°C.
3. Centrifuge culture (~10min at 4K rpm) and use cell pellet to inoculate 100ml LB-amp and grow to an OD_{600} of 0.5-0.7.
4. Centrifuge and resuspend in ~6ml (or 12ml) of LB and equally divide that into the 6 1L flasks (1 or 2ml/flask depending on total volume)
5. Grow the 1L culture at 37°C to and OD_{600} of 0.4-0.6 and then induce with 0.12g IPTG and grow overnight at 20°C.
6. Centrifuge culture using 1L plastic bottles for about 20-30min at 4000rpm.
7. Pour off supernatant and resuspend pellet in ~10ml buffer A per liter of culture (old buffer A or comparable buffer is fine, but no DTT containing buffer).
8. Put cells into 50ml conical tubes (cells from 2L can go into 1 tube).
9. Centrifuge the 50ml tubes to pellet cells, pour off super and store in -80 freezer.

Purification

1. Thaw 2L of cells in a water bath at room temp and resuspend in ~25ml fresh Buffer A.
2. Lyse cells by homogenation using cell disruptor or by sonication.
3. Centrifuge at 19,000rpm using the hard wall opaque tubes with white lid for 30min.
4. Filter supernatant and load into a superloop to inject over a Nickel column. (2L can be put over a 5-10ml column without seeing too much loss in the flow through)
5. After injecting, wait until A_{280} reaches zero (or baselines) and then wash with 3 column volumes of 8%B, a small peak should come off.
6. Then, increase to 100%B for 2-3 CV to bump off tev.
7. Concentrate to 5ml using a 50ml stirred cell concentrator then dilute with buffer A+10% glycerol 10-fold.
8. Concentrate to ~2mg/ml (which should be 20-30ml)
9. Put 1mg tev/tube and flash freeze in liquid nitrogen and store in -80.

Buffer A: 50mM Tris pH 8.0
5mM BME

Buffer B: 50mM Tris pH 8.0
300mM NaCl
250mM Imidazole

A_{280}=1 is 1.4mg/ml (ε=17.86mM^{-1} assuming MW=25kDa)

Make Buffer A fresh for purification, but Buffer B can be used for up to 6 months (BME degrades after a few days)

Updated by Liz Nichols 3/20/07