Small-Scale Purification of His- and GST- Tagged Proteins

2004-06-30 (Jim Blonde, derived from Christ Udell's protocol)

1. Innoculate a single colony of BL21(DE3) carrying the appropriate plasmid into 2.5 mL Terrific Broth (TB) containing 100 µg/mL ampicilin and culture at 37 ^{0}C for 4 hours.

2. Add IPTG to a final concentration of 0.4 mM and culture at 37 ^{o}C for an additional 2 hrs.

3. Pellet the cells from 1.5 mL of culture (spin at max. speed for 1 minute). Discard supernatant and store pellet at -20 ^{o}C overnight.

4. Resuspend the frozen pellet in **0.4 mL** of lysis buffer + TX100 + PMSF:

   - For His-tagged protein: lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM immidazole, pH 8.0) & 0.1 % Triton X-100 and 2 mM PMSF

   - For GST-tagged protein: PBS & 0.1 % Triton X-100 and 1mM PMSF.

5. Lyse cells by sonication with micro-tip using 15 sec pulses, separated by 30 sec intervals. 2-3 pulses are enough (use Cote lab sonicator).

6. Centrifuge lysate for 10 min in a microfuge at maximum speed and at 4°C.

7. While centrifuging lysate, wash Ni-NTA agarose (or glutathione sepharose) resin 3 times with lysis buffer (or PBS for glutathione sepharose). (To pellet the resins, spinning at max speed for 30sec is enough). *20 µL bed volume resin will be required for each sample, which corresponds to about 40 µL of a 50% slurry stock. 200 µL of slurry is therefore plenty for about 3 purifications. When doing more than one purification, resins for all purifications can be pooled and washed together.*

8. Save 20µL of supernatant, which is the **soluble fraction**. Transfer the rest of the supernatant to a fresh tube and add 20 µL (bed volume) washed Ni-NTA agarose resin (QIAGEN, for His-tagged protein) or glutathione sepharose (Pharmacia) for GST-tagged proteins. Incubate suspension at 4 ^{o}C for 30 min with mixing. *Resuspend pellet in 40-80 µL SDS sample buffer—this is the* ***insoluble pellet****. Add 20 µL SDS sample buffer to the saved 20 µL of supernatant —this is the* ***soluble fraction****.*

9. Pellet resin (1 min spin at max. is more than enough time) and wash 2-3 times with lysis buffer (or PBS for GST-tagged proteins) (no triton, no PMSF).

10. For pilot expression experiments, both types of fusions can be eluted by adding an equal volume of 2X SDS-PAGE sample buffer and boiling (95-100 ^{o}C) for 5 minutes. This is the **eluate.**

11. After boiling the **insoluble pellet, the soluble fraction**, and the **eluate,** load 20 µL of each directly on to an SDS-PAGE gel. *NOTE: the insoluble pellet maybe be very thick and goopy and boiling—you can improve this situation by passing the sample repeated through a 27 gauge needle, although this is usually not necessary if you're careful with your loading).*