Purification of GST-Fusion Protein

Materials:

PBS:	140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 (pH 7.3)
PBST:	PBS + 0.5% Triton X-100
Glutathione Resin:	Glutathione Sepharose 4B (Pharmacia 17-07556-01)
Elution Buffer:	100 mM Tris (pH 8.5-9.0), 20 mM reduced glutathione (aliquot and store @ -20 deg C)
0.5 M IPTG	prepare in H_2O and freeze in aliquots
Protease Inhibitors	add to solutions immediately before use

Procedure:

1. Induce expression of GST-fusion protein
   - grow 5 ml O/N culture of E. coli strain harboring pGEX plasmid in LB + Carb (50 ug/ml)
   - innoculate 500 mls LB+Carb media with 5 mls saturated O/N culture
   - grow culture at 30 degrees until mid-log phase (OD_{600} ~ 0.6 - 0.7)
     - typically 2.5-3.5 hours for TG1 host strains
     - collect 100 ul cells for analysis and resuspend in 10 ul PBS
   - induce fusion protein expression by adding IPTG to 0.1 mM
     - grow cells for 3 additional hours at 30 degrees (final OD_{600} ~ 1.2 - 1.3)
     - collect 100 ul cells for analysis and resuspend in 20 ul PBS buffer
   - harvest cells by centrifugation 3000 x g_{av} for 8 min (5K rpm in SLA-1500 rotor)
   - resuspend cells in 20 mls PBS
   - concentrate cells by centrifugation 3000 x g for 8 min and resuspend in final volume of PBS no greater than 5 mls
   - transfer to 50 ml conical tube
2. Prepare extract (all work done @ 4 degrees C)
   - freeze cells at -80 degrees 1 hr - O/N
   - thaw cells and add protease inhibitor cocktail
   - lyse cells by sonication: 4 - 6 x 10 seconds with 45 second rests on ice between bursts
     - (Fisher 550 Sonic Dismembranator @ setting 4.5 with microtip)
     - monitor cell lysis by examination with compound microscope
   - add Triton X-100 to final concentration of 1% and rock gently for 20 minutes
   - clarify extract by centrifugation
     - transfer lysate to centrifuge tube
     - wash and collect residual with 5 mls GST buffer and combine with lysate
     - spin down insoluble material 10 min @ 8000 x g_{av} (10K rpm in SS-34 rotor)
   - transfer supernatant to clean tube
     - save 10 ul for analysis
   - resupend pellet in 10 mls PBST and save 10 ul for analysis
3. Prepare glutathione resin
   - add 1.33 mls 75% glutathione sepharose slurry to 15 ml conical tube (= 1 ml bed volume)
   - spin down resin 2 min @ 500 x g (2K rpm in IEC tabletop centrifuge) and remove supernatant
   - wash 2x with cold PBS

- wash 1x with cold PBST

4. Bind GST-fusion protein to GSH-resin
   - transfer clarified extract to prepared glutathione resin
   - rock gently @ 4 degrees for 2 hours to allow binding
   - remove unbound material by centrifugation 2 min @ 500 x g and removal of supernatant
   - wash resin 2x with cold PBST
   - wash resin 1x with cold PBS
5. Elute bound protein from resin
   - transfer resin to empty column and allow resin to settle
   - elute protein from resin in 10 x 250 ul fractions
   - determine fractions containing protein by Bradford assay
     - GST-fusion proteins typically eluted in fractions 3-6
   - asses protein quality by SDS-PAGE of protein containing fractions
   - pool protein containing fractions and store at -80 degrees

Notes & Misc:

- Often a 70 kD protein co-purifies with the GST-fusion protein. This is likely the chaperonin DnaK and can sometimes be removed by treating the clarified lysate with 2 mM ATP, 10 mM $MgSO_4$ before binding the GST-fusion to the resin.
- Use 25-30 degrees instead of 37 degrees for cultures to improve solubility of GST-fusion proteins
- 1 ml bed volume of resin is reported to bind approx 5-8 mg protein

References:

- GST Gene Fusion System (Pharmacia Biotech)
- solubilization from inclusion bodies: Frangioni, J.V. and B.G. Neel (1993). Anal. Biochem. 210:179-187.

protocol compiled by Chad Rappleye

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