Purification of GST fusions from *Escherichia coli*

Optimal conditions for expression and purification of glutathione S-transferase (GST) fusions in E. coli vary widely, depending on the molecular weight and solubility of fusion protein, compatibility of coding sequence with codon usage of the host bacterial strain, extent of expression induction and lysis of cells, among other parameters. The following protocol is used in our lab for expression and purification of several fusions from Pharmacia's pGEX vectors in Stratagene's BL21(DE3) Codonplus hosts.

Procedure

Induce expression of culture at OD_{600} ~ 0.5 with 0.05-1 mM IPTG for 3 hrs at 28-37°C, 250 rpm. **Note:** in many cases, inducing expression with 0.05-0.1 mM IPTG at 30°C for 3 hours usually yields considerable amount of soluble protein that otherwise form inclusion body aggregates.

Pellet cells by spinning 15 min. at 5,000 x *g* at 4°C. All steps should be performed on ice or at 4°C from this point on.

Resuspend cells ***gently*** on 1x extraction buffer (3-5 ml per gram of pellet) by scraping pellet and swirling or pipetting up and down ***slowly*** with a glass pipette of wide tip. Lysis is more efficient when pellet is fully resuspended.

Add lysozyme to 1 mg/ml and 1:500 PMSF from a saturated stock. **Note:** many host strains used for protein expression have reduced protease activity. However, some proteins are particularly sensitive to degradation. Use of a protease inhibitor cocktail in addition to PMSF usually minimizes degradation.

Incubate on ice for 30-60 min. Mix by inversion occasionally.

Add sarcosyl to 1% from a 10% stock. Mix by inversion.

Sonicate on ice until solution becomes fluid and clear (multiple pulses of 10 to 30 sec. each). **Note:** extent of sonication for optimal yields of intact fusion protein must be determined empirically. Typically, for 25 ml-lysates of non-fused GST, 3 to 5 pulses (50 W, 20 kHz) are enough.

Add Triton X-100 to 1% from a 20% stock. Mix by inversion.

Spin 15 min at 15,000 x *g*.

Filter supernatant through 2-4 layers of cheesecloth.

Load supernatant onto a glutathione-agarose column or slurry previously equilibrated on extraction buffer. As a general rule, use 1 ml of swollen glutathione-agarose per liter of bacterial culture when expressing small fusions or non-fused GST, which usually yield large amounts of protein. **Note:** if making batch instead of column purification, incubate lysate with slurry for 30-60 min. on an end-to-end shaker.

Wash column with 10 bed volumes of extraction buffer with 0.5% Triton X-100 and PMSF. **Note:** if making batch purification, wash for 15-30 min. on an end-to-end shaker.

Wash column with 10 bed volumes of extraction buffer with 0.1% Triton X-100. **Note:** if making batch purification, wash for 15-30 min. on an end-to-end shaker.

Elute with 10 bed volumes of extraction buffer with 10 mM glutathione, collecting 10-12 fractions. The bulk of protein is usually eluted in the first few fractions. **Note:** if making batch purification, split elution into several steps of 1-3 bed volumes.

Solutions required for extraction and purification

5x extraction buffer
250 mM Tris-Cl pH 8.5, 500 mM NaCl, 5 mM EDTA. Add DTT fresh to 1 mM when making 1x solution.

PMSF (phenylmethylsulfonyl fluoride)
Saturated solution in isopropanol. Store at room temperature.

50 mg/ml lysozyme
Dissolve 500 mg of lysozyme in 10 ml of ddH_20. Make 200 *u*l aliquots and store at –20°C.

10% (w/v) sarcosyl
Dissolve 10 g of sarcosyl on 100 ml of 1x extraction buffer (without DTT) or ddH_20. Store at room temperature.

20% (v/v) Triton X-100
Add 20 ml of Triton X-100 to 70 ml of ddH_20. Allow it to homogenize for several hours, mixing by inversion occasionally. Adjust volume to 100 ml with ddH_20. Store at 4°C.

Crosslinked glutathione-agarose
Swell beads on ddH_20 at 4°C for a few hours or overnight. Wash extensively with ddH_20, and store on 1M NaCl at 4°C. Equilibrate on lysis buffer prior to use.

Glutathione, reduced
Dissolve fresh on 1x lysis buffer.