Solubilization and Purification of Active GST-Fusion Proteins

modified from:

Frangioni J.V. & B.G. Neel **(1993)** Solubilization and purification of enzymatically active glutathione S-transferase (pGEX) fusion proteins. **Analytical Biochemistry. 210:** 179-187.

1. Induce your GST protein of choice and pellet the bacteria. Store in –80C until you want to purify.

2. Resuspend bacteria in STE buffer with 1X PMSF or complete protease inhibitors with 0.5 to 1.5% sarkosyl (N-laurylsarcosine). If in an eppendorph tube I usually use 1 mL as my resuspension volume. I also don't tend to go above 1% sarkosyl.

3. Lyse bacteria by sonication 3 times for 10 seconds at power setting 60 on sonicator and let sit 5 min on ice between each sonication. If a large volume is being lysed (i.e. 15 mL) lysis can be done using a French Press.

4. Spin out cellular debris by centrifugation at 10,000Xg for 10 min at 4C.

5. Transfer the supernatant to a new tube.

6. Dilute lysate 10 fold with STE buffer.

7. Then incubate lysate on glutathione agarose batch column in tube for 1 hr at 4C. If from an eppendorph tube pellet I usually use 40 μL of glutathione agarose.

8. Wash 5 times with ice cold STE buffer and let sit for 5 min on ice before pelleting glutathione agarose beads and removing supernatant. If I am using an eppendorph tube I use 1 mL washes.

9. Elute GST-fusion protein off of column with 25 mM glutathione in STE buffer with the pH adjusted to 7.0 with 10 M NaOH. If from eppendorph tube I use 50 μL of the 25 mM glutathione STE buffer and do 4 elutions. I do not pool the elutions until after SDS-PAGE analysis and Bradford protein assays to ensure that I am not diluting my protein stocks by combining the elutants.

10. Add glycerol to 5-10% to eluted proteins and take an aliquot for SDS-Page electrophoresis to confirm presence of purified protein. Bradford assays can also be done to determine the concentration of protein recovered.

Solutions and Notes:

STE buffer:

10 mM Tris-Cl, pH 8.0
150 mM NaCl
1 mM EDTA

Add DTT to 5 mM immediately prior to use.

Notes:

Some people put 100 μg/mL of lysozyme in their STE buffer to aid in lysis of the bacterial cells.

This method may be used to solubilize 6-His proteins, but a different buffer must be used. STE buffer contains EDTA which will strip nickel and cobalt columns. If purifying a 6-His protein use PBS or another non-EDTA containing buffer. Use the same non-EDTA containing buffer for making the different imidazole containing buffers for elution of 6-His proteins from the nickel or cobalt column. Most 6-His proteins elute off at 125 mM imidazole. We use washes of 5 mM, 25 mM, and 50 mM imidazole. Elution of protein is done with 250 mM imidazole containing buffers.