Protein Purification by GST Binding

1. Inoculate 3 ml LB+Amp [100ug/ml] with a single GST colony from a freshly streaked plate. Incubate overnight at 37oC, 250 RPM.
2. The following morning, use 2.5ml of the overnight culture to inoculate 250 ml of LB + Amp [100ug/ml] (1:100 dilution). Incubate at 37oC, 250 RMP until O.D.600 = 0.6
3. Induce the translation of the GST protein by adding 0.5-1.0 mM IPTG (this quantity may vary depending on the fusion protein). Incubate at 37oC, 250 RPM for 4 hours.
4. Pellet cells with a 5000 RPM spin for 10 minutes at 4oC. Discard supernatant.
5. Place cell pellet on ice. It is important to keep cells cold from here on! Resuspend cells in 5ml STE buffer. Transfer cells to cold 30ml centrifuge tubes. Pellet cells again at 5000 RPM for 10 minutes at 4oC. Discard supernatant.
6. Resuspend cells in 1.5ml NETN + PMSF+Aprotinin+Pepstatin. Set on ice for 15 minutes.
7. Add 75ul 1M DTT and 900ul 10% Sarkosyl in STE. Sonicate cells for one minute at setting #3. Sonicate another minute at setting #3. Keep cells on ice during sonication.
8. Pellet cell debris with 10,000 RPM spin for 10 minutes at 4oC. Transfer supernatant to 6ml culture tube. Add 1.5ml 10% Triton-X 100 in STE and 100ul Glutathione Sepharose beads. Rotate at 4oC for 2 hours.
9. Transfer sample to a 2ml microcentrifuge tube. Pellet beads with 3000 RPM spin for 3 minutes at 4oC.
10. Wash beads 5 times in NETN + PMSF+Aprotinin+Pepstatin, aspirating the supernatant between washes. (Be careful not to aspirate out the beads!)
11. Resuspend beads in 250ul of NETN + PMSF+Aprotinin+Pepstatin.
12. Remove a sample of the beads (5ul) and place with Lysis loading buffer (10ul). Heat samples at 95oC for 10 minutes. Run a mini-SDS-PAGE with the appropriate percent acrylamide.

Recipes

STE, pH 7.5

	For 100ml:
100mM NaCl →	2ml 5M NaCl
10 mM Tris →	1ml 1M Tris, pH 7.5
1 mM EDTA→	0.2ml 0.5M EDTA

NETN, pH 8.0

	For 100ml:
0.5% NP40→	0.5ml NP40
20mM Tris→	2ml Tris, pH 8.0
1 mM EDTA→	200 ul 0.5M EDTA
100 mM NaCl	2 ml 5 M NaCl

Add protease Inhibitors to NETN:
For every 100 ml, add:
1 ml 100mM PMSF
50ul Pepstatin
50ul Aprotinin