

Last Update: June 2013

Purification of GST-tagged Proteins
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Amended from A. Rakotondrafara's Protocol

Buffers: calculated for the purification of ONE protein. Scale up as needed.

Buffer X (20 mM Tris-HCl pH 7.5; 1M NaCl; 0.2mM EDTA)

For 1L: 20 mL 1M Tris pH 7.5
 200 mL 5M NaCl
 0.4 mL 0.5M EDTA

Elution Buffer (50 mM glutathione; 100 mM HEPES pH 8.0—pH with KOH; 1 mM DTT)

For 5mL: 77 mg glutathione (MW= 307.3)
 500 uL 1M HEPES pH 8.0
 5uL DTT
 4.5mL H₂O

Dialysis Buffer (Buffer D): (20 mM HEPES pH 8.0—pH with KOH; 20% glycerol; 0.2 mM EDTA; 1 mM DTT)

For 1L: 20 mL 1M HEPES-KpH 8.0
 200 mL glycerol
 400 uL 0.5 M EDTA
 1 mL 1M DTT

PBS (for washing of glutathione-agarose beads): (137 mM NaCl; 2.7 mM KCl; 10 mM Na₂HPO₄; 1.8 mM KH₂PO₄)

For 1L: 8g NaCl
 0.2 g KCl
 1.44 g Na₂HPO₄
 0.24 g KH₂PO₄

Protocol: For a 1L culture

1. Inoculate 100 mL 1.5xLB + Amp + 0.7% glucose with a glycerol stock of BL21 transformed with the pGEX-6P recombinant and grow overnight at 37 degrees.
2. Use 5mL of the overnight culture to inoculate a 1L culture of 1.5xLB + Amp + 0.7 % glucose. Grow 2-3 hr, until the OD₆₀₀ = 0.6-0.7
3. Take a 1mL aliquot of uninduced sample, centrifuge, pour off supernatant, and freeze at -80 degrees.
4. Add 1000 uL of 1M IPTG (1 mM IPTG), and incubate at 37 degrees for 3 hr
5. Take a 1mL aliquot of induced sample, centrifuge, pour off supernatant, and freeze at -80 degrees.
6. Centrifuge 1L culture at 4000 rpm for 20 minutes. Pour off supernatant. Freeze pellet until usage, or proceed to next step(s).

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7. Use a freeze-thaw protein extraction procedure to check the induction efficiency of the protein. Compare the two 1-mL aliquots. If induction is efficient, proceed to next step.
8. If pellet was frozen, remove from freezer and place on ice. Place 2-50mL Falcon tubes with 50 mL Buffer X on ice. Add 1M DTT to each of them, and one tablet of Roche cOMplete protease inhibitor cocktail to one of them.
9. Resuspend pellet in 20 mL of Buffer X + DTT+ protease inhibitor. Sonicate in small bursts (~2 seconds) for 15 minutes total.
10. Transfer lysates to SS34 rotor-compatible tubes. Centrifuge for 1 hour at 10K/ 4 degrees in the SS34 rotor.
11. Meanwhile, to wash glutathione-agarose beads: take 1 mL of beads and place in a 10 mL Falcon tube. Be gentle! Centrifuge at 4 degrees, 500 g, 5 minutes. Carefully decant the supernatant with a pipetteman. Add 5 mL of PBS (pH 7.3), carefully resuspend beads, and centrifuge at 4 degrees, 500 g, 5 minutes. Carefully remove supernatant by pipetting, and wash one more time in PBS. The beads are now ready for use.
12. Transfer the centrifuged lysate supernatant to a cold 50 mL Falcon tube and add 1mL of washed beads.
13. Rotate by inversion in the cold room for 1 hr.
14. Centrifuge for 5 minutes at 500g, 4 degrees. Carefully remove the supernatant. Wash in Buffer X + DTT + protease inhibitor. Centrifuge for 5 minutes at 500g, 4 degrees. Remove supernatant, and check protein concentration in supernatant. Wash until $OD_{280} \sim 0$. Resuspend beads in Buffer X + DTT + protease inhibitors
15. Place bead slurry on 10 mL gravity column. Wait until beads settle on the bottom of the column.
16. Let liquid flow through column. NEVER let the column run dry! Check the OD_{280} . If not close to 0.0, do another Buffer X + DTT wash.
17. Elute with 5 mL of elution buffer. Set up 10- 1.5 mL eppendorf tubes and collect a 500uL fraction into each as the protein is eluting.
18. Estimate protein concentration by Bradford assay. Take a 10uL sample of each fraction and add 90 uL of diluted Bradford assay dye. Take the highest concentrations.
19. Perform dialysis overnight in the cold room by dialyzing the protein against 1 L of dialysis buffer. Place a stir bar in a 1L graduated cylinder or beaker and place dialysis bag in the buffer. Stir overnight in the cold room.
20. Measure the dialyzed protein concentration. Aliquot dialyzed protein the next day into 10uL aliquots. Do this in the cold room! Store aliquoted protein in -80 degree freezer in a separate box, and write protein concentration on the box.