

**PROTOCOL FOR SMALL SCALE GLUTATHIONE-S-TRANSFERASE(GST)
FUSION PROTEIN INDUCTION AND PURIFICATION**
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1. Subclone the chosen DNA fragment into the appropriate pGEX vector in the correct reading frame, transform competent E.coli cells(BL-21), and inoculate 2ml LB/Amp in a 15ml-tube. Grow the culture at 37°C on shaker overnight.
2. Use the 100µl of overnight culture to inoculate 1ml LB/Amp and grow the culture at 230rpm, 37°C to OD600 of 1.0 (usually about 2 hours).
3. Add IPTG to a final concentration of 0.1mM for inducing expression of the tac promoter-driven fusion gene; grow the culture another 4-6hrs.
4. Chill the cells/bacterias on ice 5-10min. (It is important to keep the cells/lysates at 4°C for the duration of the procedure).
5. Transfer the culture to 1.5ml-tube and spin down at top speed for 2 minutes at 4°C, and discard supernatants.(You can freeze the pellets at -80°C for couple of days or continue the step 6)
6. Resuspend the cell pellet in 500µl ice cold PBS-PI (containing proteinase inhibitor) and lyse the cells by sonication (power=10) 4-6 X 10 second bursts.
7. Add Triton X-100 to a final concentration of 1% and tumble the solution at 30rpm for 30 minutes at 4°C.
8. Spin down the cell debris at the top speed for 5min at 4°C and remove the supernatant into a new 1.5ml-tube.
9. Add 15µl 50% slurry of glutathione-agarose beads and tumble the tubes at 30rpm for 30 minutes at 4°C. (Before using the glutathione-agarose beads, wash it with the same volume PBS-PI once)
10. Spin down the beads at top speed for 3 minutes at 4°C.
11. Resuspend the beads with 300µl ice-cold PBS-PI and recover the beads at top speed for 3 minutes at 4°C.
12. Wash the beads with PBS-PI again.
13. Add 100µl of elution buffer into each tube and tumble the tubes @ 4°C, 20-30min.
14. Spin down the beads @ top speed for 3 minutes @ 4°C.
15. Transfer the supernatant into tubes and store @ -80°C.

Recipes

PBS-PI

1 tablet protease inhibitor cocktail dissolved in 10ml PBS

Elution buffer

50mM Tris-HCl pH8.0, 100mM NaCl, 10mM reduced glutathione