PROTOCOL FOR INDUCTION AND PURIFICATION OF GST FUSION PROTEIN

Lan Zhou 11/06/00, Edited by JYP 8/12/02

1. Use a bacterial colony containing the pGEX-derived construct to 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 an additional 4-6hrs.
4. Chill the cells/bacterias on ice (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.
6. Resuspend the cell pellet in 500μl ice cold PBS (containing proteinase inhibitor) and lyse the cells by sonication for 4-6 X 10 second bursts.
7. 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 decant 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.
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 again.
13. Add 100μl of elution buffer (50mM Tris-HC1 pH8.0, 100mM NaCl, 10mM glutathione) into each tube and tumble the tubes @ 4°C.
14. Spin down the beads @ top speed for 3 minutes @ 4°C.
15. Transfer the supernatant into tubes and store @ -80°C.