

### GST-Fusion Protein Purification and Pull Down Assay

1. Grow BL21 bacteria containing plasmid(from a single colony or -70°C stock,) in 5ml LB plus 50ug/ml ampicillin at 37°C shaker for overnight;
2. Inoculate 400ml of LB plus 50ug/ml ampicillin with 4ml o/n culture;
3. Grow for 2-3 hrs until OD<sub>600</sub> is 0.5;
4. Add IPTG to a final concentration of 0.1 and grow at 37°C for 2-3hrs(update, except inducing GST-Rhoteckin at 30°C for 3hrs because of its instability);
5. Spin 6k rpm for 15 min 4°C and resuspend the pellet in 10ml of PBS plus 1mM PMSF and 10mM benzamidine;
6. Sonicate 5x30sec, at maximum power; Incubate the sonicates in ice between sonications for 30sec;
7. Add Triton X-100 to 1% and DTT to 1mM (both final concentration) and incubate for 30min in ice;
8. Spin 7k rpm for 10min at 4°C. Take the supernatant for the next step and save the pellet for inclusion body purification.
9. Transfer the supernatant into a 50 ml conical tube, add 25ml in PBS plus 1mM PMSF and 10mM Benzamidine and 2ml 1:1 slurry of glutathione-agarose beads. (***If make GST fusion protein from 5ml culture, reduce the PBS volume to 1ml and add 50ul glutathione-agarose beads***) The beads need to be preswollen overnight in PBS and washed 4 times with PBS before use.
10. Incubate at 4°C on a rotator for overnight;

11. Wash the beads with PBS at 4°C for 5 times.
12. Batch elute with 1 column volume of 20mM glutathione in 20mM Tris, pH8.0 for 6 times.
13. Pool and dialyze against 4 liters of PBS o/n at 4°C.
14. The GST fusion protein can be estimated by measuring the absorbance at 280nm. For the GST affinity tag,  $1A_{280}=0.5\text{mg/ml}$ . Also, it can be determined by SDS-PAGE with standard BSA protein.
15. Incubate about 500mg cell lysate with 2-5ug GST fusion protein and 40ul Glutathione-agarose beads(if the GST fusion protein-binding beads are less than 40ul, use empty beads to 40ul) in a total 1ml volume(adjust to 1ml volume with RIPA buffer ) at 4°C on a rotator for at least 1hr. Briefly wash the beads with Modified RIPA buffer for 3 times.
16. Elute the protein into the SDS loading buffer, then run SDS-PAGE gel.

**Reagent:**

1. Glutathione-Agrose beads: G-4510 from Sigma.
2. Glutathione, reduced form: G-6529 from Sigma.
3. Benzamidine: B-6506 from Sigma, 1M stock solution.
4. PMSF(phenylmethyl-sulfonyl fluoride): P7626 from Sigma, 0.1M stock solution in isopropanol.
5. RIPA buffer: 150mM NaCl, 50mM Tris.HCl(pH7.5), 1%NP-40, 0.25% Sodium Deoxyclolate, 2mM EGTA(stock solution 0.4M add before use), 1mM NaVO<sub>4</sub>(200mM stock solution, add before use)
6. 6XSDS loading buffer: 0.35M Tris-HCl(pH6.8), 10.28(w/v)%SDS, 36%(v/v) glycerol, 0.6M dithiothreitol(or 5%β-mercaptoethanol), 0.012%(w/v)bromophenol blue. Store in 0.5ml aliquots at -80°C.