Purification of GPI-GST

Day 1
1. Thaw the bacterial stab.
2. Culture bacteria in 5-10 ml of LB (w/ 50-100 ug/ml of ampicillin) in 50 ml tube at 37C over night with stirring.

Day 2
1. Transfer 5 ml of cultured bacteria into 500 ml of LB (w/ ampicillin)
2. Incubate at 37C with stirring.
3. Check OD600 frequently after 2 hours of incubation.
4. When OD600 reached 0.5-0.6, add 0.5 ml of IPTG (100 mM).
5. Culture O/N.

Day 3
1. Transfer the culture media into 250 ml Sovall bottles.
2. Centrifuge 3500 rpm x 10 min
3. Decant and discard supernatant.
4. Add 1 x PBS (adequate volume) to the pellet and mix by pipetting.
5. Transfer them into Nalgen 30 ml tubes
6. Centrifuge at 3500 rpm x 10 min.
7. Discard supernatant.
8. Add Buffer A* containing 1 mM PMSF and 0.1 mM DTT (30 ml for 500 ml LB product) and Mix.
9. Freeze and Thaw once.
10. Add 10 mg/ml of Lysozyme (3.3 ml for 30 ml BufferA).
11. Incubate on ice for 1 hour.
12. Freeze and Thaw one-two times.
13. Add 0.5M EDTA and 10% NP40 (33 ul and 1.15 ml for for 33 ml Buffer A / Lysozyme, respectively).
14. Sonicate on ice. 10 sec. x 5 times.
15. Centrifuge 10,000 rpm x 30 min at 4C.
16. Take supernatant and store at 4C.

Make a glutathione column
1. Add 1 ml of glutathione sepharose 4B into a column.
2. After dripping stops, add 10 ml of PBS for washing.
3. After dripping stops, add 5 ml of Buffer A.
4. After dripping stops, apply the sample (it takes 1-6 hours).
5. Wash with 10 ml of PBS
6. Set a new conical tube under the column.
7. Apply 5 ml of Elution buffer**.

Day 4.
1. Dialyse by centricon.
2. Check the concentration by OD280 (A280 1.0 ≈ 0.5 mg/ml).
**Buffer A**
50 mM Tris-HCl, pH 8.0
0.5 mM EDTA
0.4 M NaCl
5 mM MgCl2
5 % (v/v) glycerol

**Elution Buffer**
5 mM Glutathione
50 mM Tris-HCl, pH 8.0