Recipe

- **SGI** (20g glucose, 3.4g Yeast Nitrogen Base without amino-acid, 5g Casein acid hydrolysate, 40mg Tryptophan) make up to 1 liter, autoclave.
- **SLI** (20g galactose, 3.4g Yeast Nitrogen Base without amino-acid, 5g Casein acid hydrolysate, 40mg Tryptophan) make up to 1 liter, autoclave.
- **TES** (50 mM Tris-HCL pH 7.5, 0.6 M Sorbitol, 1 mM EDTA)
- **TES-M** (50 mM Tris-HCL pH 7.5, 0.6 M Sorbitol, 1 mM EDTA, 10 mM 2-mercaptoethanol)
- **TES-E** (50 mM Tris-HCL pH 7.5, 0.6 M Sorbitol, 1 mM EDTA, 2 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride)
- **TES-G** (50 mM Tris-HCL pH 7.5, 20% glycerol, 1 mM EDTA, 1 mM 2-mercaptoethanol)

Important Notes

- Handling of yeast must be performed in a clean sterile lamina flow.
- All yeast culture glassware must be clean and autoclaved.
- Desk-top centrifuge and ultra-centrifuge must be pre-cooled for centrifugation at 4C.

Protocol

1. Streak a single colony of PCR confirmed recombinant yeast into 1 liter of SGI culture media.
2. Shake at 200 rpm at 30°C for 16 hours or until growth is in log phase.
3. Centrifuge the culture at 3000 x g for 15 minutes at room temperature. After centrifugation, there should be a large visible blob of yeast cells at the bottom, and the supernatant should be clear.
4. Resuspend the yeast blob in 1 liter of SLI induction media.
5. Shake the yeast at 200 rpm at 30°C for 16 hours for recombinant protein expression.
6. Centrifuge the culture at 3000 x g for 15 minutes at room temperature.
7. Resuspend the yeast blob in 50ml of TES buffer.
8. Centrifuge the culture at 3000 x g for 15 minutes at room temperature.
9. Resuspend the yeast blob in 50ml of TES-M buffer.
10. Leave the yeast resuspended in the TES-M buffer at room temperature for 10 minutes.
11. Pre-cool the Centrifuge Ultracentrifuge to 4°C, and TES-E in ice.
12. Centrifuge the culture at 3000 x g for 15 minutes at 4°C.
13. Resuspend the yeast blob in 10ml of pre-chilled TES-E and leave in ice.
14. Add 0.5mm glass beads until the final volume is 25ml.
15. Vortex vigorously for 30 seconds.
16. Leave in ice for 1 minute.
17. Repeat steps 15~16 for a total of 20 minutes per sample.
18. Add TES-E until final volume is 50ml.

19. Centrifuge at 10,000 x g for 30 minutes at 4°C.
20. Pour the supernatant into a new 50ml conical tube and centrifuge again at 10,000 x g for 30 minutes at 4°C.
21. Carefully pipette 5ml of supernatant into each ultracentrifuge tubes. Take care not to disturb the small pellet at the bottom. Leave in ice.
22. Balance the ultracentrifuge tubes.
23. Centrifuge at 100,000 x g for 2 hours at 4°C.
24. Pre-chill buffer TES-G in ice.
25. After centrifugation, there should be a visible pellet at the bottom. Pour off the supernatant, and wash the pellet twice with chilled TES-G.
26. Resuspend the pellet in 1ml of chilled TES-G using the protein suspension apparatus.
27. Aliquot and store at -80°C.