MEMBRANE-FREE RIBOSOMES (mfrs)

The removal of endogenous vesicles also removes a large fraction of ribosomes, which must be added back to the reaction exogenously. The procedure for isolation of mfrs is as follows:

1. Inoculate 1.5L of TB + 0.2% glucose with 100ml saturated culture of MRE 600 cells.
2. Grow to OD600 of 1.0 at 30 C.
3. Pellet cells (5000 rpm, 15 minutes), and wash 1X with ddH2O.
4. Resuspend in buffer A at 0.5g/ml
5. Pass suspension through French pressure cell 3X at 4000psi
6. Centrifuge 30 min. at 40 00xg (19 000 rpm in 50.2).
7. Dilute supernated 10- fold in buffer B.
8. Centrifuge 2 hours at 150 000xg (37 000 rpm in Ti 50.2).
9. Resuspend pellet in 18 mL of buffer B (Note: use Dounce homogenizer with type A pestle). Incubate vernight with rotation at 4 C.
10. Centrifuge 2 hours at 150 000xg.
11. Resuspend in 20 mL of buffer C.
12. Centrifuge 2 hours at 150 000xg. Note: Pellet is almost clean, and has approximate consistency of "snot".
13. Resuspend pellet in 500(l buffer C.

    Note: Volume of mfrs required must be determined, but should be optimal in this case at about 0.1(l per 10(l reaction.