LARGE SCALE PRODUCTION OF CRUDE BACTERIAL LYSATE (500ml)

1. Place 200ml-2L of superbroth cell culture into a 500ml polypropylene cetrifuge bottle(s). Spin at 2800 x g (Beckman JA-10 rotor) for 10 min. Remove supernatant.
2. Resuspend in 50ml-500ml of Buffer A, spin at 2800 x g (Beckman JA-10 rotor) for 10 min. Remove supernatnat. Repeat.
3. Add 50ml-500ml of lysis buffer, reususpend cells. Leave on ice 30 min.
4. Spin for 1.5hours 200,000 x g (Beckman Ti 50.2 rotor) (45 000 rpm).
5. Place supernatant on ice or freeze at -80 degrees C.

Lysis Buffer	**100mL**
13% sucrose	13g
150mM NaCl	3.75mL 4M NaCl
10mM EDTA	2mL 0.5M EDTA
50mM Tris pH 8.0	2.5mL 2M Tris pH 8.0
1.0% Triton X-100	1mL Triton
1mM PMSF	*1mL 0.1M PMSF
0.25 mg/mL lysozyme	*25mg lysozyme

* add just prior to use