

Protocol 106_OmpC protein extraction

Protocol adapted from Forst et al. JB 1988.

- Transfer 5 ml of overnight *E. coli* culture to 500 ml fresh LB broth
- Grow up to O.D of approximately 0.5
- Harvest bacterial cells by centrifuging at 4,000 rpm for 10 mins
- Resuspend into 5 ml of 20mM sodium phosphate buffer
- Sonicate for at least 6 cycles (1 cycle: 30s sonication and 30s cooling on ice)
- Add sodium N-lauryl sarcosinate added at a final concentration of 0.5%
- Incubate at room temperature for 35 mins
- Centrifuge at 3,000g for 1 min
- Collect supernatant and centrifuge at 393,000g for 14 min in the ultracentrifuge
- Ultracentrifuge is on F-floor. Please ask Hans before use.
- Adjust the temperature of the ultracentrifuge to 4°C and let it cool at least 30 mins before use
- Discard supernatant and solubize pellet in 20mM sodium phosphate buffer containing 1% SDS.
- Run proteins on SDS