

Sonication

Aims of the experiment

Sonication can be used to lyse the cells for obtaining the cell extract. It is proceed according Sun, Z. Z et al., 2013.

Materials

- S30A buffer with DTT
- 2ml micro-centrifuge tubes
- 200µg/ml Lysozyme
- Bead beating tubes
- 15ml falcon tubes

Procedure

1. Thaw the frozen pellet prepared by the Harvesting and Washing Protocol on ice.
2. Resuspend pellets in 1*V S30A buffer by vortexing.
3. Aliquot cell suspension to 1ml in 2ml centrifuge tubes.
4. Prepare lysozyme if needed.
5. Add 5µl of lysozyme into corresponding tubes of the cell suspension aliquot.
6. Incubate lysozyme samples for 30 minutes on ice.
7. Keep cells in a cooler rack for sonication.
8. Carry out sonication and program depends on the machine used and desire.
9. After sonication, centrifuge samples at 12,000 rcf for 10 minutes at 4°C.
10. Transfer supernatant into empty bead beating tubes, and place in 15ml falcon tubes with the bead-beating tube cap placed up-side-down as support inside.
11. Incubate at 37°C for 80 minutes.
12. Continue with Dialysis Protocol for completing cell extract.