

Dialysis

Aims of the experiment

Dialyzing increases protein production yield of the cell extract by separating the small molecules out of the extract. The dialysis is proceed according Sun, Z. Z et al., 2013.

Materials

- 2L S30B buffer with DTT
- 10k MWCO Dialysis tubes
- Nylon string
- 2L beaker
- 1.5ml micro-centrifuge tubes
- 2ml micro-centrifuge tubes

Procedure

1. After incubating samples in bead beating tubes from the Sonication Protocol or Bead Beating Protocol, consolidate samples into individual reaction tubes and centrifuge at 12,000 rcf for 10 minutes at 4°C.
2. Wet the required amount of dialysis tubes in S30B buffer poured in a beaker, and tie up one end with nylon string tightly to avoid leakage. Always keep the dialysis tubes in buffer before and after adding supernatant.
3. Transfer supernatant into individual dialysis tubes, close the tubing with nylon string.
4. Use empty tubes as buoyancy chamber and mark the samples.
5. Add sterile magnetic stirrer into the beaker.
6. Incubate dialysis with stirring at 4°C for 3 hours.
7. Extract samples into sterile centrifuge tubes from dialysis tubing using 1ml syringe.
8. Aliquot required amount of each sample for later analysis use and flash freeze all samples in liquid nitrogen.
9. Store all the extracts at -80°C.