

Freezing (-80⁰c) E.coli protocol

Materials: Bacterial culture, LB medium, 80% glycerol in water, appropriate antibiotic

Note: Cells grown overnight in the presence of appropriate antibiotics (15-16 hr) before freezing. All materials and tubes kept on ice throughout the process.

Protocol:

1. Spin for 10 minutes at 2,500-3,000 rpm
2. Discard supernatant without disturbing the pellet, while leaving sufficient volume for the desired number of samples. (~1ml for every sample)
3. Resuspend the pellet in the remaining supernatant.
4. Label the vials with the name of the E. coli strain, the plasmid name (if applicable), the desired protein and the date.
5. Add 900µl cell culture and 300µl sterile 80% glycerol, to a final glycerol concentration of 20%.
6. Two phases will be created.
Mix by gentle inversion of the tube (several times) until a homogeneous solution appears.
7. Store in -80⁰c