

Protein Extraction from Bacteria

1. Take the bacterial broth from incubator and centrifuge it at 4000 Xg for 15 minutes.
2. Discard the supernatant, and wash the residue with 40 ml PBS, vortex it, and centrifuge it at 4000 Xg for 15 minutes. (Repeat this process 3× in total)
3. Discard the supernatant and add 10 ml of PBS and vortex it.
4. Take total 10, 1 ml tubes and add little amount of tiny glass beads.
5. Aliquot the 10 ml solution in **STEP 3**, into 1 ml tubes containing glass beads.
6. Homogenize the 1 ml tubes for 3 minutes at max speed.
7. Freeze the tubes at -20°C for at least 3 hours.
8. Thaw the tubes in ice for 1 hour.
9. Repeat **STEP 6,7,8**, in order, for 6× in total for **C. perfringens**.
10. Repeat **STEP 6,7,8**, in order, for 3× in total for **C. jejuni**.