

Protocol 109_Preparation of cytoplasmic and crude membrane proteins

Protocol adapted from Camilla Urbaniak MSc thesis

- Centrifuge overnight culture of bacteria at 3000 rpm for 10 mins
- Resuspend bacterial pellet in 25 ml PBS and centrifuge again at 3000 rpm for 10 mins.
- After the second centrifugation, resuspend the pellet in lysis buffer (50 mM Tris, 50 mM NaCl and 5% glycerol)
- Sonicate at 40 Hz for 30s followed by 30s incubation on ice
- Repeat sonication/incubation cycles 6 times
- Centrifuge for 20 mins at 10,000 rpm
- Aliquot supernatant containing cytoplasmic proteins and store at -20°C
- Resuspend pellet in lysis buffer and store at -20°C