

Whole cell extract preparation

1. Pellet cells in a clinical centrifuge at 1000rpm for 5 minutes at 4°C
2. Wash cells once with cold 1X PBS
3. Pellet cells in a clinical centrifuge at 100rpm for 5 minutes at 4°C
4. Remove PBS and resuspend cells in small residual PBS volume
5. Spin gently in a microcentrifuge (about 2500rpm) for 30 seconds
6. Remove the remaining PBS
7. Resuspend cells in 100µl WCE Lysis Buffer per 10⁶ cells
8. Place on ice for 20 minutes. Vortex every 5 minutes during this incubation
9. Centrifuge at maximum velocity in a microcentrifuge for 10 min at 4°C
10. Collect and save supernatant. Discard Pellet
11. Determine protein concentration by Bradford Assay
12. Store at -80°C

WCE LYSIS BUFFER

0.5% Triton X-100 or NP40

50mM Tris-HCl pH8.0

150mM NaCl

1mM EDTA

protease inhibitors