Various Lysis Methods

Glass beads

1. Harvest cells in Falkon tubes
2. Centrifuge (3800 g, 5 min)
3. Discard supernatant
4. Resuspend pellet in 250 µl citrate-phosphate buffer
5. Transfer to a fresh Eppendorf tube
6. Add 100 µl glass beads
7. Vortex thoroughly (at least 3 x 10 s)
8. Centrifuge (17000 g, 8 min)
9. Transfer supernatant to fresh Eppendorf tube
10. Centrifuge (17000 g, 8 min)
11. Transfer supernatant to Eppendorf tube from step 9

Sonication

1. Harvest cells in Falkon tubes
2. Centrifuge (3800 g, 5 min)
3. Resuspend pellet in 300 µl citrate-phosphate buffer
4. Transfer to a fresh Eppendorf tube
5. Put samples on ice
6. Lyse using an ultrasonic homogenizer (10 s, 6 cycles, 10 % power)
7. Centrifuge (16000 g, 1 min, 4 °C)
8. Transfer supernatant to fresh Eppendorf tube

Freeze and Thaw

TE-Buffer: 10 mM Tris pH 7 and 1 mM EDTA pH 8

1. Harvest cells in Falcon tubes (amount according to expected abundance of the protein of interest; about 50 – 100 ml)
2. Centrifuge (2 min, 4000 g)
3. Discard supernatant
4. Resuspend pellet in remaining supernatant
5. Transfer to 1.5 ml Eppendorf tubes
6. Repeat steps 2 and 3

7. Resuspend pellet in 400 µl buffer (TE or another buffer depending on assay performed afterwards)
8. Add 2 % (v/v) protease inhibitor
9. Freeze at -80 °C
10. Thaw at room temperature
11. Repeat steps 9 and 10 4 times (5 times freezing and thawing in total)
12. Take 10 µl of the sample for later SDS-Page (whole protein)
13. Centrifuge (10 min, 12000 g)
14. Take supernatant off; take 10 µl of the supernatant for later SDS-Page (soluble), do assays with the rest
15. Resuspend pellet in 400 µl Triton (1 % in Buffer)
16. Vortex thoroughly (at least 30 s for each sample)
17. Incubate for 10 min at RT while inverting
18. Centrifuge (30 min, 12000 g)
19. Take supernatant off; take 10 µl of the supernatant for later SDS-Page (membrane)
20. Resuspend pellet in 400 µl 1x SDS-sample buffer (insoluble)
21. Add 10 µl of 1x SDS-sample buffer to each sample except the insoluble one
22. Heat each sample for SDS-page (10 min, 95 °C)
23. Perform SDS-page