

Glass bead lysis of whole yeast cells

The following protocol is suitable for preparation of 5 OD₆₀₀ units of whole-cell extract at a final concentration of 0.5 OD₆₀₀ units per 20 μ L of SDS-PAGE reducing sample buffer.

1. Dilute an overnight culture of yeast cells to 0.2 ODU/mL in 10 mL medium.
2. When the culture reaches mid-logarithmic stage (0.5 – 0.8 ODU/mL), centrifuge 5 ODU of cells at 500 x *g* for 5 min at room temperature (RT).
3. Decant supernatant, resuspend cell pellet in 1 mL sterile water, and transfer entire volume to eppi tube.
4. Add 100 μ L of 100% trichloroacetic acid (TCA) and vortex immediately.
5. Incubate on ice \geq 20 min.
 - If the day is late, place a lid over the ice bucket and leave the bucket in the cold room until the next morning.
6. Centrifuge at 14,000 x *g* for 5 min at 4°C.
7. Aspirate supernatant; keep pellets on ice.
8. Add 1 mL ice-cold acetone.
 - Large volumes of acetone can be stored at -20°C.
9. Resuspend pellet completely using a water bath sonicator.
10. Repeat steps 5 – 9.
11. Incubate on ice \geq 20 min.
12. Centrifuge at 14,000 x *g* for 5 min at 4°C.
13. Aspirate supernatant.
14. Dry pellets completely by speed-vac for 10-15 min.
 - Pellets can also be dried by leaving the tubes with the lids open at RT.
15. Add 200 μ L of 1X SDS-PAGE sample buffer + 0.2% β -mercaptoethanol
 - 1X SDS-PAGE buffer:
(remember to add β -mercaptoethanol to a final concentration of 0.2%).
16. Add 100 μ L of sterile glass beads.
17. Secure eppi's with lid-locks.
18. Vortex 15 min at RT.
19. Heat at 90 – 100°C for 5 min.
20. Repeat steps 18 – 19.
21. Centrifuge 15 – 30 seconds at 14,000 x *g* at RT.
22. Load 20 μ L (equivalent to 0.5 OD₆₀₀ units) onto an SDS-polyacrylamide gel.