

Yeast Lysis by Liquid Nitrogen Grinding

Cell Preparation

1. Inoculate a 100 ml culture and allow to grow overnight at 30 degrees
2. On the next day, inoculate a 1L culture to an OD600 of .3
3. Grow cultures at 30 degrees until OD600 reaches 1.0
4. Pellet cells by spinning 5 min. 5K at 4 degrees
5. Wash cells in 250 ml ice cold water, repeat spin
6. Wash cells in 250 ml 1X Extraction Buffer, repeat spin
7. Wash cells in 250 ml Complete Extraction Buffer, repeat spin
8. Resuspend pellet in 5 ml Complete Extraction Buffer and transfer to 50 ml conical tube with tiny hole in the bottom (made with a 20 gauge needle).
Hold the conical tube over a dewar of liquid nitrogen, since cells will begin to drip through
9. Using the piston portion of a 60cc syringe (*BD Luer-Lok syringes, Fisher #13-89-8*), extrude the cell lysate into liquid nitrogen
10. When cells are completely frozen, remove them by straining the liquid nitrogen through a small kitchen colander
11. Store cells at -80 degrees

Grinding (*Retsch Mixer Mill MM 400*)

10. Fill several dewars and the cooling box (*Retsch Cryo Kit #22.354.0001*) with liquid nitrogen
11. Label and then immerse two steel grinding jars (*Retsch 50ml stainless steel jars #01.462.0216*) and grinding balls into liquid nitrogen until boiling stops
12. Remove jar with tongs and dump out excess liquid N₂
13. Transfer frozen cells to larger half of grinding jar, place grinding ball on top of cells, and screw on lid
14. Resubmerge jar in liquid nitrogen until boiling stops

15. Place grinding jars into mixer mill and tighten according to manufacturer instructions (you must use two jars even if you have only one sample)
16. Grind samples 5 times for 3 minutes at 30 Hz. In between each grinding, submerge grinding chambers in liquid N₂ for 1-2 min.
17. After 5 grindings, transfer a small amount of cell powder to a slide and check breakage—90% of cells should be broken or damaged
18. Carefully unscrew grinding jars and scrape powder into a new conical tube submerged in a bath of liquid nitrogen- it is helpful to use a funnel to help get the powder into the tube and catch the grinding ball
19. Ground cell powder can be stored at -80 degrees

Cell Lysis and IP

20. Weigh out 5 g of powder into new 50 ml conical tube
21. Thaw cells at room temperature for a few minutes
22. Add 9 ml Noodle Lysis Buffer
23. Vortex each sample for 1 minute at room temperature
24. Homogenize for 30 seconds on setting 5 using Polytron (*Kinematica PT 10-35*)
25. Transfer lysate to a tube and spin 10 minutes, 3000 rpm at 4 degrees
26. Immediately remove supe to a 15 ml conical; save 100 microliters as “load”
27. Add 100 microliters of appropriate affinity beads that have been washed several times in Noodle Lysis Buffer
28. Incubate at 4 degrees with end-over-end rotation for 2-4 hours
29. Pellet beads by spinning for 5 minutes ~2000rpm
30. Pour off supe (save 100 microliters as flow-through)
31. Transfer beads to 1.5 ml siliconized tube; rinse out conical and combine
32. Wash beads 5X with 1 ml extraction buffer
33. Elute protein with 200ul 2X SDS Sample Buffer, or with appropriate peptide

Extraction Buffer

20 mM Hepes-NaOH, pH 7.5
300 mM NaCl
5 mM EGTA
1 mM EDTA
50 mM NaF
50 mM β -glycerophosphate

Complete Extraction Buffer

Extraction Buffer plus 1 mM DTT, 1 mM PMSF, protease inhibitors

1000 ml 5X Extraction Buffer

100 ml 1 M Hepes-NaOH, pH 7.5
300 ml 5 M NaCl (or 87.6 g)
50 ml 500 mM EGTA
10 ml 500 mM EDTA
10.5 g NaF
54.01 g β -glycerophosphate

Water up to 1000 ml

0.2 micron filter and store at 4 degrees

1X Extraction Buffer

100 ml 5X Extraction Buffer
400 ml water

1X Complete Extraction Buffer

120 ml 5X Extraction Buffer
0.6 ml 1 M DTT
0.6 ml 1 M PMSF
12 complete protease inhibitor tabs (*Roche #11836170001*)
Water up to 600 ml

1X Noodle Lysis Buffer (Complete Extraction Buffer plus 0.5% TritonX-100)

10 ml 5X Extraction Buffer
2.5 ml 10% TritonX-100
50 microliters 1 M DTT
50 microliters 1 M PMSF
1 complete protease inhibitor tabs
Water up to 50ml water

