酵母蛋白提取*

Materials

Protease-deficient yeast cells (BJ926, EJ101, or equivalent)
YPD medium
Zymolyase buffer, room temperature and ice-cold
Zymolyase 100T (ICN Immunobiologicals)
1 M sorbitol (optional)
Lysis buffer
Extraction buffer
Storage buffer
Liquid nitrogen
Sorvall GS-3 or GSA rotor (or equivalent)
Sorvall SS-34 or SA-600 rotor (or equivalent)
Beckman Type 45Ti rotor (or equivalent; large capacity)
30°C shaker platform
Rubber policeman
Dounce homogenizer
Rotating wheel or rocker

Grow the cells

1. Grow cells to mid-log phase in YPD medium with vigorous shaking or forced aeration.

The OD_{600} can vary with aeration conditions, but should be between 1 and 5. The procedure is appropriate for between 100-ml and 20-liter cultures, although it can be scaled up if necessary.

2. Harvest cells by centrifugation 5 min at 1500 × *g* (GS-3 or GSA rotor at ～3000 rpm),
4°C, in preweighed centrifuge bottles.

Prepare the spheroplasts

3. Determine the wet weight (in grams) of yeast cells in the pellet by the weight increase
over that of the preweighed bottle. This is approximately equal to the packed cell volume (in milliliters), and for all subsequent steps will be considered 1 vol.

One liter of BJ926 (a diploid strain) at OD_{600} = 1.0 yields a packed cell volume of 2 to 3 ml.

4. Resuspend cells in 2 to 4 vol ice-cold water and immediately centrifuge 5 min at 1500
× *g* (SS-34 or SA-600 rotor at 3500 rpm), 4°C. Discard the supernatant.

5. Resuspend the cells by adding 1 vol zymolyase buffer containing 30 mM DTT (see note in reagents and solutions), and incubate 15 min at room temperature.

This step facilitates subsequent zymolyase treatment and spheroplast lysis by breaking disulfide bonds.

6. Centrifuge 5 min at 1500 × *g*, 4°C, and resuspend in 3 vol zymolyase buffer. Add 2 mg (200 U) Zymolyase 100T per ml of original packed cell volume to the resuspended

cells. Incubate 40 min at 30°C on a shaker platform at ～50 rpm.

7. Determine if conversion to spheroplasts has been completed by the lysis in water technique. If spheroplasting is incomplete, continue incubation until complete.

Perform all procedures from this point on at 4°C.

8. Centrifuge spheroplasts 5 min at 1500 × *g*. Decant the supernatant carefully—the spheroplast pellet will not be as tight as the previous cell pellets.

For some procedures (e.g., extracts for in vitro transcription or translation), spheroplasts are resuspended in YPD medium containing 1 M sorbitol and incubated for 30 to 60 min at 30°C to allow metabolic recovery.

9. Wash the spheroplasts by gently resuspending the pellet in 2 vol ice-cold zymolyase buffer and centrifuging 5 min at 1500 × *g*. Repeat this step two more times.

Spheroplasts are sticky and difficult to resuspend. To facilitate resuspension, first resuspend the spheroplasts in a small volume with the aid of a rubber policeman and then add more buffer to achieve the correct final volume.

The washing step is important for removing proteases, phosphatases, and nucleases present in the zymolyase preparation. For some purposes, it may be necessary to carry out additional washes.

Lyse the spheroplasts

10. Gently resuspend the pellet in 2 vol lysis buffer. Do not try to achieve a homogeneous suspension; simply dislodge the pellet from the side of the centrifuge tube and gently swirl 10 to 20 times. Centrifuge spheroplasts 10 min at 1500 × *g*.

Extensive manipulation of the pellet may result in premature osmotic lysis.

11. Thoroughly resuspend spheroplast pellet with 1 vol lysis buffer using a glass rod.

At this point, tubes containing the resuspended spheroplasts can be quick-frozen in liquid nitrogen and stored at −80°C. Thaw frozen spheroplasts overnight on ice before proceeding.

12. Lyse spheroplasts with 15 to 20 strokes of a tight-fitting pestle (clearance 1 to 3 μm) in a Dounce homogenizer.

Extract proteins from lysate

13. Half-fill ultracentrifuge tubes with lysate. Add an equal volume of extraction buffer and seal the tubes. Gently invert tubes on rotating wheel or rocker for 15 to 30 min at 4°C.

The lysate and extraction buffer are not premixed because the resulting solution will immediately become quite viscous and hence difficult to pour if the ionic strength is above ～0.5 M.

14. Centrifuge 90 min at 100,000 × *g* (Type 45Ti rotor at 33,000 rpm), 4°C.

15. Collect supernatant and dialyze 2 to 4 hr against 100 vol storage buffer. Transfer dialysis bag to 100 vol fresh storage buffer; dialyze an additional 2 to 4 hr.

A flocculent precipitate may form during dialysis. These precipitates usually contain negligible amounts of most protein factors and can be discarded.

16. Remove a few microliters of the dialysate, dilute 1:1000 with water, and determine the conductivity. If it is equal to that of similarly diluted storage buffer or below some

acceptable value (usually 100 to 250 mM NaCl), proceed to step 17. If not, continue dialysis.

17. Centrifuge dialysate 10 min at 10,000 × *g* (SS-34 rotor at 9200 rpm or SA-600 rotor at 8500 rpm), 4°C. Collect the supernatant, freeze in small aliquots in liquid nitrogen, and store at −80°C.

This crude extract contains most DNA-binding proteins as well as transcription and replication factors. The pellet contains proteins that can be "salted in" by resuspending in storage buffer containing 0.5 to 1.0 M KCl, if desired

REAGENTS AND SOLUTIONS

YPD medium

Per liter:

10 g yeast extract
20 g peptone
20 g dextrose

Extraction buffer

Lysis buffer
0.8 M ammonium sulfate
20% glycerol

Lysis buffer

50 mM Tris~Cl, pH 7.5
10 mM $MgSO_4$
1 mM EDTA
10 mM potassium acetate
1 mM DTT
1× protease inhibitor mix
1 mM phenylmethylsulfonyl fluoride (PMSF)

Storage buffer

20 mM Tris~Cl, pH 7.5
0.1 mM EDTA
10% glycerol
100 mM KCl
1 mM DTT
1× protease inhibitor mix
1 mM PMSF

100× protease inhibitor mix

Listed below are representative protease inhibitors; different combinations may be more appropriate for individual applications.

10 μg/ml chymostatin

200 μg/ml aprotinin
100 μg/ml pepstatin A
110 μg/ml phosphoramidon
720 μg/ml E-64
50 μg/ml leupeptin
250 μg/ml antipain
10 mM benzamidine
10 mM sodium metabisulfite

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