Protocol for Whole Cell Lysis of Yeast

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Bio-protocol LLC, Sunnyvale, CA 94087, USA
*For correspondence: team@ed.bio-protocol.org

[Abstract] This protocol describes how to perform lysis on whole yeast cell samples using NaOH. The lysed cells can then be used for downstream applications such as the extraction of total proteins.

Materials and Reagents

1. NaOH (Sigma-Aldrich)
2. Beta-mercaptoethanol (beta-ME) (Sigma-Aldrich)
3. 100% (w/v) trichloroacetic acid (TCA) (Sigma-Aldrich)
4. Pepstatin A (Sigma-Aldrich, catalog number: P5318)
5. Leupeptin (Sigma-Aldrich, catalog number: L2884)
6. PMSF (Sigma-Aldrich, catalog number: P7626)
7. Acetone (Sigma-Aldrich)
8. Protease inhibitors
9. DMSO
10. Glycerol
11. BPB
12. Ethanol
13. SDS lysis buffer
14. 7.5 ml 1 M Tris-HCl (pH 6.8) (see Recipes)
15. DTT stock (see Recipes)
16. 5x SDS-PAGE gel loading dye (see Recipes)
17. Protease inhibitors (see Recipes)

Procedure

1. Pellet 2 OD of cells. Wash 1x with 0.5 ml of water (cell pellets may be frozen in liquid nitrogen at this point).
2. Resuspend pellets in 100 µl of water (make sure pellets are fully resuspended).

3. Add 17 µl of 1.85 M *NaOH/7.4% (v/v) beta-ME and vortex. Incubate on ice for 10 min.
4. Add 8 µl of 100% (w/v) TCA, vortex, and incubate on ice for 10 min.
5. Spin extract for 10 min at 20,000 *x g* /14,000 rpm at 4 °C. Wash protein pellet with 500 µl of cold acetone (do not pipette up and down; add the solution and vortex; it is OK if the pellet is not completely re-suspended).
6. Re-spin sample for 5 min at 4 °C. Dry almost completely. If the pellet gets too dry, it will not dissolve well later.
7. Resuspend pellet in 100 µl of SDS lysis buffer + 1x protease inhibitors (pipette up and down, then vortex, then resuspend the pellet completely).
8. If doing a BCA assay, boil for 5 min and perform the assay.
9. Add 25 µl of 5x SDS-PAGE gel loading dye to 100 µl of sample.
10. Boil for 5 min and load 40 µg total proteins per lane.

Recipes

1. To make 1 ml of NaOH/beta-ME, mix 741 µl of water, 185 µl of 10 N NaOH (stock 10 N NaOH solution) and 74 µl of beta-ME stock. Always make fresh NaOH/beta-ME.
2. 5x SDS-PAGE gel loading dye
   150 mM Tris-HCl
   15% SDS
   25% glycerol
   0.02% BPB
   12.5% beta-ME
3. To make 7.5 ml 1 M Tris-HCl (pH 6.8)
   7.5 g SDS
   25 ml 50% glycerol
   10 ml H_2O
   20 mg BPB
   Bring final volume to 43.75 ml. Aliquot into 1 ml and add 125 µl of beta-ME fresh prior to use. SDS may come out of solution, but heating will fix this.
4. Protease Inhibitors
   Leupeptin/pepstatin A (1 mg/ml pepstatin A dissolved in DMSO; 10 mg/ml leupeptin dissolved in ddH_2O)
   Combine and aliquot 1 ml per tube and store at -20 °C.
   PMSF: 0.5 M dissolved in ethanol. Store in 1 ml aliquots at -20 °C. Supplied as 1,000x stock.
5. DTT stock (1 M)

Dissolve in ddH_2O, store in 1 ml aliquots at -20 °C. *I.e.* 1,000x stock.

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References

1. Horvath, A. and Riezman, H. (1994). Rapid protein extraction from *Saccharomyces cerevisiae*. *Yeast* 10(10): 1305-1310.

