Enzymatic Activity Assays in Yeast Cell Extracts

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[Abstract] *Saccharomyces cerevisiae* (*S. cerevisiae*) (commonly known as baker's yeast) is a model organism that has a similar upstream base excision repair (BER) pathway for the repair of methylated bases as that in mammalian cells, and it is very easy to maintain in the laboratory environment. Here, we described a method to prepare cell extracts from yeast to investigate their enzymatic activities. This protocol is a quick and efficient way to make yeast cell extracts without using commercial kits.

Materials and Reagents

1. EDTA-free protease inhibitor cocktail tablet (Roche Diagnostics, catalog number: 11836170001)
2. Bio-Rad protein assay dye reagent concentrate (Bio-Rad Laboratories, catalog number: 500-0006)
3. Dextrose (Sigma-Aldrich, catalog number: D9434)
4. Bacto-peptone (Sigma-Aldrich, catalog number: P5905)
5. Bacto-yeast extract (Sigma-Aldrich, catalog number: Y1625)
6. Adenine sulfate (Sigma-Aldrich, catalog number: A3159)
7. Trizma base (Sigma-Aldrich, catalog number: T1503)
8. EDTA (Sigma-Aldrich, catalog number: 93283)
9. NaCl (Sigma-Aldrich, catalog number: S7653)
10. β-mercaptoethanol (Sigma-Aldrich, catalog number: B0126)
11. Formamide (Sigma-Aldrich, catalog number: F9037)
12. Bromophenol blue (Sigma-Aldrich, catalog number: B0126)
13. Xylene cyanol (Sigma-Aldrich, catalog number: X4126)
14. Dithiothreitol-DTT (Sigma-Aldrich, catalog number: D0632)
15. Sodium borohydride-NaBH4 (Sigma-Aldrich, catalog number: 247677)
16. Sterile water
17. Yeast peptone dextrose adenine (YPDA) medium (see Recipes)
18. Potassium phosphate buffer (see Recipes)

19. Lysis buffer (see Recipes)
20. 10x reaction buffer (see Recipes)
21. Gel loading buffer (see Recipes)

Equipment

1. 0.5 mm diameter glass beads (Bio Spec Products, catalog number: 11079105)
2. Eppendorf tubes
3. Table-top centrifuge
4. Table-top heat block

Procedure

A. Preparation of yeast cell extracts

1. The cell extracts from yeast *Saccharomyces cerevisiae* were prepared as described (Wang *et al.*, 1995; Jazwinski, 1990) with modifications as below.
2. Grow yeast culture in yeast peptone dextrose adenine (YPDA) medium at 30 °C overnight (O/N) with shaking.
3. Prepare a fresh cell culture by diluting 10-fold from O/N culture in 50 ml YPDA medium.
4. Grow the cells to stationary phase at 30 °C with vigorous shaking.
5. When the culture reached an absorbance of 0.5-1.0 at 600 nm, centrifuge cells at 2,000 rpm (300 rcf) for 5 min at 4 °C.
6. Wash the cell pellet with water and then with potassium phosphate buffer.
7. Centrifuge cells again at 2,000 rpm (300 rcf) for 5 min at 4 °C.
8. Resuspend the cell pellet in 500 µl of ice-cold lysis buffer.
9. Transfer the suspension to an Eppendorf tube prefilled with pre-chilled beads (1:1 ratio of beads to lysate), and keep on ice.
10. Disrupt the cells at 4 °C by vortexing the bead/lysate mixture: 10 cycles of 2 min vortex at maximum speed alternating with cycles of 1 min cooling on ice.
11. Centrifuge the bead/lysate mixture at full speed at 4 °C for 15 min to remove cell debris.
12. Transfer the supernatant fraction to a fresh Eppendorf tube. We recommend not collecting the entire supernatant fraction, instead leaving part of it to prevent touching pellet.
13. Determine the protein concentration of the extract prepared by Bradford assay using dye reagent.

B. Enzymatic activity assays in yeast cell extracts

1. Prepare 10 µl of reaction mixture including 1x reaction buffer and 140 nM DNA substrate. DNA substrate used includes an adenylated uracil base at the 5′ end of the 3′-FAM–labeled oligonucleotide (Caglayan *et al.*, 2014).
2. Start the reaction by addition of yeast cell extract prepared as above (to a final amount of 50 µg) to the reaction mixture.
3. Incubate the reaction mixture at 30 °C for 5, 15, and 30 min.
4. Stabilize the reaction products by addition of 1 M freshly prepared and ice-cold $NaBH_4$ to the final concentration of 100 mM.
5. Incubate the reaction samples on ice for 30 min.
6. Mix the reaction products with 10 µl of gel loading buffer.
7. The reaction products in the yeast cell extracts were separated on a 15% polyacrylamide gel, the gel was scanned and the data were analyzed as reported (Caglayan *et al.*, 2014). The result image for dRP lyase, FEN1 excision and DNA deadenylation enzymatic activities in yeast extracts has been reported (Caglayan *et al.*, 2014).

Representative data

The result image that shows dRP lyase, FEN1 excision and DNA deadenylation enzymatic activities in yeast extracts was published as Supplementary Figure 5 in Caglayan *et al.* (2014).

Notes

Enzymatic activity in yeast cell extracts may be lost after continuous vortexing if samples warm up. It might be necessary to extend the alternating cooling steps depending on the sample.

Recipes

1. Yeast peptone dextrose adenine (YPDA) medium (1 L)
   20 g dextrose
   20 g Bacto-peptone
   10 g Bacto-yeast extract
   2 ml 0.5% adenine sulfate
2. Potassium phosphate buffer
   25 mM KPO_4 (pH 8.0)
3. Lysis buffer

25 mM Tris-HCl (pH 7.5)
1 mM EDTA
100 mM NaCl
10 mM β-mercaptoethanol
A protease inhibitor tablet

4. 10x reaction buffer
   500 mM HEPES (pH 7.5)
   100 mM $MgCl_2$
   200 mM KCl
   5 mM EDTA
   20 mM DTT
5. Gel loading buffer
   95% formamide
   20 mM EDTA
   0.02% bromophenol blue
   0.02% xylene cyanol

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References

1. Caglayan, M., Batra, V. K., Sassa, A., Prasad, R. and Wilson, S. H. (2014). Role of polymerase beta in complementing aprataxin deficiency during abasic-site base excision repair. *Nat Struct Mol Biol* 21(5): 497-499.
2. Jazwinski, S. M. (1990). Preparation of extracts from yeast. *Methods Enzymol* 182: 154-174.
3. Wang, Z., Wu, X. and Friedberg, E. C. (1995). The detection and measurement of base and nucleotide excision repair in cell-free extracts of the yeast *Saccharomyces cerevisiae*. *Methods* 7(2): 177-186.

