User Manual

Easy Yeast Plasmid Isolation Kit User Manual

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I. Introduction

This kit provides an easy and efficient method to rescue plasmids from *Saccharomyces cerevisiae*. The key first step is highly efficient enzymatic disruption of the yeast cell walls using Zymolyase. This is followed by SDS/alkaline lysis of the resulting spheroplasts to generate a crude lysate. The lysate is neutralized with Y3 Neutralization Buffer which allows the plasmid DNA to bind the silica membrane of the Yeast Plasmid Spin Columns. Contaminants such as salts, metabolites, and soluble cellular components are then removed by a single washing step using ethanolic Y4 Wash Buffer. Plasmid DNA is eluted from the columns using a low ionic strength, slightly alkaline YE Elution Buffer (5 mM Tris/HCl, pH 8.5). The final step is to rescue and amplify the purified plasmid using a general purpose cloning strain of *E. coli.*

- Step 1: Disruption of yeast cell walls with Zymolyase enzyme
- Step 2: SDS/alkaline lysis of spheroplasts
- Step 3: Spin column purification of plasmid DNA
- Step 4: Rescue/amplification of plasmid DNA in *E. coli*

II. Additional Materials Required

The following materials are required, but not supplied with the kit.

- Isolated yeast colonies growing on the appropriate synthetic defined (SD) minimal media which selects for the relevant plasmid
- 96–100% ethanol
- 30º C shaker
- 30º C incubator
- 250 ml Erlenmeyer flask for shaking microtubes (optional)
- Stellar™ Competent Cells (Cat. No. 636763), or equivalent transformation-competent *E. coli* with efficiency of $\geq 2 \times 10^8$ cfu/µg.

III. Plasmid Isolation Protocol

A. Before using the kit for the first time:

1. Add 1 ml of Y1 Resuspension Buffer to the vial containing RNase A and mix by vortexing. Transfer the entire 1 ml of the dissolved RNase A into the Y1 Resuspension Buffer bottle and mix thoroughly. Indicate the date the Y1 Buffer/RNase A was made and store the solution at 4°C. The solution is stable at 4ºC for up to six months.
2. Add 24 ml of 96–100% ethanol to Y4 Wash Buffer. Store at RT.

B. Disruption of yeast cell walls.

1. Pick well-isolated yeast colonies growing on the selective medium appropriate for the strain and plasmid (e.g., SD/-Leu if using a Matchmaker™ pGADT7 vector). Patch/spread each colony on a fresh plate of selective agar medium to create a 1 cm x 1 cm square. Incubate the plate at 30ºC for 3 days to grow the patches.
2. Use a clean pipet tip or sterile toothpick to scoop all the cells from half of each patch (no more than 10 mg of cells) and resuspend them in a 1.5 ml microfuge tube containing 500 µl of the supplied 10 mM EDTA.

   NOTE: Using ≥ 10 mg of cells will reduce the efficiency of the plasmid isolation.
3. Pellet the cells in the microcentrifuge at 11,000 x g for 1 min. Remove and discard the supernatant.
4. Resuspend each washed yeast cell pellet in 200 µl of ZYM Buffer. Be sure the suspension is homogeneous.
5. Add 20 µl of the Zymolyase suspension. Be sure to invert the vial 2–3 times before pipetting to ensure a uniform suspension of Zymolyase. Close the microfuge tube and vortex gently to mix.
6. Incubate with gentle shaking at 30ºC for 1 hr (i.e., place the sealed microcentrifuge tubes into the bottom of a 250 ml Erlenmeyer flask and place in a shaking incubator).
7. Pellet the spheroplasts at 2000 x g for 10 min. Remove and discard the supernatant.

C. SDS/alkaline lysis of spheroplasts and spin column purification of DNA.

1. Resuspend the spheroplasts in 250 µl of Y1 Buffer/RNase A solution (see Section A.1).

   NOTE: The pellet may be viscous, but it will eventually resuspend completely.
2. Add 250 µl of Y2 Lysis Buffer. Sodium dodecyl sulfate (SDS) in Y2 Lysis Buffer may precipitate if stored at temperatures below 20ºC. If a precipitate is observed in the Y2 Lysis Buffer, incubate the bottle at 30–40ºC for several minutes and mix well. Mix gently by inverting the tube 6–8 times. Do not vortex. Incubate at RT for a maximum of 5 min.

   NOTE: Vortexing will contaminate the suspension with yeast chromosomal DNA.
3. Add 300 µl of Y3 Neutralization Buffer. Mix gently by inverting the tube 6–8 times. Do not vortex.
4. Clarify the lysate by centrifuging at 11,000 x g for 5 min at RT. Transfer the supernatant to a clean microfuge tube and repeat the centrifugation once more.
5. Place a Yeast Plasmid Spin Column inside a 2 ml collection tube and load the supernatant (clarified lysate) from step 4 onto the column. Centrifuge at 11,000 x g for 1 min. Discard the column flow-through.
6. Place the spin column back into the 2 ml collection tube and add 450 µl of Y4 Wash Buffer (previously diluted with ethanol, see Section A.2). Centrifuge at 11,000 x g for 3 min. Discard the column flow-through and repeat the centrifugation once more to completely remove any residual Wash Buffer.

III. Plasmid Isolation Protocol continued

7. Place the spin column in a 1.5 ml microcentrifuge tube and add 50 µl of YE Elution Buffer. Incubate at RT for 1 min. Centrifuge at 11, 000 x g for 1 min.

D. Rescue and amplification of plasmid DNA.

1. Transform Stellar Competent Cells (Cat. No. 636763), or other competent *E. coli*, using 3 µl of purified yeast plasmid DNA. Recommendation: plate 1/10th of the transformation directly onto one plate, then plate the remainder of the transformation on another plate after spinning down the cells and removing some of the outgrowth medium.
2. Propagate the cells and isolate plasmid DNA from *E. coli* using standard methods. For high yield and purity, we recommend NucleoBond Xtra Midi and Maxi columns (see **www.clontech.com**).