Instructions

Zymoprep I™
Yeast Plasmid Minipreparation Kit

Catalog No. **D2001**

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Highlights

- Simple method for yeast plasmid minipreparation.
- Highly efficient.
- No glass beads, no phenol, no vortexing.
- Achieves great results whether you use colony or liquid cultures.

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Is To Make Things Simple

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Zymoprep™ (D2001) Kit Contents:

Products	Qty./Size	Storage	Catalog
Solution 1, Digestion Buffer	15 ml	Room Temp.	D2001-1-15
Solution 2, Lysis Buffer	15 ml	Room Temp.	D2001-2-15
Solution 3, Neutralizing Buffer	15 ml	Room Temp.	D2001-3-15
Zymolyase, (Resuspend the lyophilized enzyme by adding 200 ul of supplied **Storage Buffer** to make 5 units/ul)	1,000 Units (lyophilized) Storage Buffer 500 ul	Shipped at room temperature. Store at -20°C upon arrival.	E1004
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Additional Products			
Zymolyase, lyophilized	2,000 Units Storage Buffer 500 ul	-20°C	E1005

Ordering Information:

Product	Cat. No.	Size
Zymoprep, Yeast Plasmid Miniprep™	D2001	1 set
Re-ordering information:		
Zymolyase™	E1004	1000 units
Zymolyase™	E1005	2000 units

Related Products:

Zymoprep™ II- Yeast Plasmid Minipreparation Kit (D2004) :

Zymoprep™II is made compatible with our Zymo-spin columns to improve efficiency for faster plasmid rescue. The Zymoprep™ II kit gives about 5 fold more recovery and eliminates the isopropanol precipitation step making plasmid rescue faster and more convenient. It is ideal for hard to isolate plasmids and low copy vectors. The Zymo-spin I column allows elution into a small 10 ul volume to further increase recovered plasmid concentration.

Zymoprep™ is the new, simple and efficient yeast plasmid miniprep that is based on the old E. *coli* alkaline lysis method with our Zymolyase added in the first solution. There is no need for glass beads, phenol, or vortexing. Reliably recover your plasmid from yeast cells every time whether you use colonies, patches on plates, or liquid cultures. The total plasmid yield is 0.01-0.3 ng for most 2u based plasmids from 1.5 ml overnight cultures. This kit also works well with low-copy number yeast plasmids. The recovered plasmid is in TE buffer and can be used for E. *coli* transformations, Western blots, PCR, etc.

Before Starting: Add 200 ul of the supplied **Storage Buffer** to the lyophilized **Zymolyase™.** Mix to dissolve the enzyme completely, spin briefly in a micro-centrifuge. Store the reconstituted **Zymolyase™** at -20°C.

Standard Protocol

Grow yeast cells at 30°C in YPD broth or selective medium.

Unless stated otherwise, the following procedures are accomplished at room temperature.

1. Aliquot 0.5-1.0 ml of the full-grown yeast cells into 1.5 ml microfuge tubes and spin down the cells at 600 x g for 2 minutes. Discard the supernatant.
2. Add 150 ul **Solution 1** to each pellet.
3. Add 2 ul of **Zymolyase™** to each tube. Resuspend the pellet by flicking with finger or vortexing.

   Note: For multiple sample process, add 13 ul **Zymolyase** for each ml of **Solution 1** to make a **Solution 1-enzyme mixture**. Use 150 ul of this mixture to re-suspend the pellet for each sample.
4. Incubate at 37°C for 15-60 minutes (15 minutes is the minimal incubation time. Longer incubation is optional).
5. Add 150 ul **Solution 2** to each tube. Mix well.
6. Add 150 ul **Solution 3** to each tube. Mix well.
7. Centrifuge at maximum speed for 2 minutes.
8. Transfer the supernatant to new tubes. Add 400 ul isopropanol (2-propanol) to each tube. Mix well.
9. Centrifuge at maximum speed for 8 minutes. Aspirate supernatant. Spin briefly again and take out all residue supernatant.

Resuspend the plasmid pellet in 35 ul TE buffer. It is not necessary to dry the pellet before adding TE. Sometimes the pellet needs to be pipetted for complete dissolving

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Use 3-5 ul of the plasmid DNA for E. Coli transformation experiment. For PCR reaction, dilute the recovered plasmid 1:50 with TE and use 2-4ul of the diluted plasmid as template for each PCR reaction (adding too much of the crude plasmid preparation may inhibit your PCR reaction).

Protocol For Use with Colonies or Patches

1. Use toothpick or pipette tip to pick roughly 5-15 ul volume of yeast colonies or patches from plates and dispense into 150 ul of **Solution 1-enzyme mixture** (add 13 ul **Zymolyase™** to each ml of Solution 1 to make Solution 1-enzyme mixture).
2. Incubate at 37°C for 15-60 minutes (15 minutes is the minimal incubation time. Longer incubation is optional).
3. Add 150 ul **Solution 2** to each tube. Mix well.
4. Add 150 ul **Solution 3** to each tube. Mix well.
5. Centrifuge at maximum speed for 2 minutes.
6. Transfer the supernatant to new tubes. Add 400ul isopropanol (2-propanol) to each tube. Mix well.
7. Centrifuge at maximum speed for 8 minutes. Aspirate supernatant. Spin briefly again and take out all residue supernatant.

Resuspend the plasmid pellet in 35 ul TE buffer. It is not necessary to dry the pellet before adding TE. Sometimes the pellet needs to be pipetted for complete dissolving.

Use 3-5 ul of the plasmid DNA for E. *coli* transformation experiments.