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ZYMO RESEARCH

DNA
Purification
MADE SIMPLE
Made Simple™

Zymoprep™ Yeast Plasmid Miniprep I

Plasmid DNA isolation from any fungi whose cell walls are susceptible to yeast lytic enzyme lysis.

Highlights

- Simple procedures for plasmid rescue from yeast.
- Ideal for low copy and hard to isolate plasmids.
- For isolation of plasmid DNA for downstream applications such as PCR, transformation, hybridization, etc.

Catalog Numbers:
D2001



Scan with your smart-phone camera to
view the online protocol/video.



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Product Contents

Zymoprep™ Yeast Plasmid Miniprep I	D2001 (100 Preps.)	Storage
Solution 1, Digestion Buffer	15 ml	Room Temp.
Solution 2, Lysis Buffer	15 ml	Room Temp.
Solution 3, Neutralizing Buffer	15 ml	Room Temp.
Zymolyase™ and Storage Buffer ¹	1,000 Units	-20°C
Instruction Manual	1	-

¹ The **Zymolyase™** is stable as shipped. Add 200 µl of supplied **Storage Buffer** to each **Zymolyase™** tube prior to use. The final concentration of **Zymolyase™** after the addition of the **Storage Buffer** is 5 units/µl.

Specifications

- **Sample Sources** – *S. cerevisiae*, *C. albicans* and *S. pombe*, and other fungi species sensitive to yeast lytic enzymatic digestion (Zymolyase™).
- **Format** – Isopropanol precipitation.
- **Plasmid Size** – DNA up to 23 kb.
- **Equipment Needed** – Incubator shaker, microcentrifuge

Product Description

The **Zymoprep™** is a simple and efficient yeast plasmid miniprep that is based on the *E. coli* alkaline lysis method but using **Zymolyase™** as the first solution. There is no need for glass beads, phenol, or vortexing. Instead, plasmid DNA is reliably recovered from yeast cells whether colonies, patches on plates, or liquid cultures are sampled. Plasmid yields are typically between 0.01-0.3 ng for most 2 μ based plasmids from 1.5 ml overnight cultures. This kit also works well with low copy number yeast plasmids. Recovered plasmid DNA is in TE buffer and can be used for *E. coli* transformation, Western blotting, PCR, etc.

Protocol

Reagent Preparation

- ✓ Add 200 μl of the supplied **Storage Buffer** to the lyophilized **Zymolyase™**. Mix to dissolve the enzyme completely and spin down briefly using a microcentrifuge. 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 steps in the procedure are performed at room temperature.

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

Note: For multiple sample processes, add 13 μl **Zymolyase** for each ml of **Solution 1** to make a **Solution 1-enzyme mixture**. Use 150 μl 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, although longer incubation is optional).
5. Add 150 μl **Solution 2** to each tube. Mix well.
6. Add 150 μl **Solution 3** to each tube. Mix well.
7. Centrifuge at maximum speed for 2 minutes.
8. Transfer the supernatant to new tubes. Add 400 μl isopropanol (2-propanol) to each tube. Mix well.
9. Centrifuge at maximum speed for 8 minutes. Aspirate supernatant. Spin briefly again and remove any residual supernatant.

10. Resuspend the plasmid pellet in 35 μl TE buffer. It is not necessary to dry the pellet before adding the TE. Sometimes the pellet requires repeated pipetting to be completely dissolved.

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

Protocol to use with colonies or patches

1. Use toothpick or pipette tip to pick roughly 5-15 μl volume of yeast colonies or patches from plates and dispense into 150 μl of **Solution 1-enzyme mixture** (add 13 μl **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, although longer incubation is optional).
3. Add 150 μl **Solution 2** to each tube. Mix well.
4. Add 150 μl **Solution 3** to each tube. Mix well.
5. Centrifuge at maximum speed for 2 minutes.
6. Transfer supernatant to new tubes. Add 400 μl isopropanol (2-propanol) to each tube. Mix well.
7. Centrifuge at maximum speed for 8 minutes. Aspirate supernatant. Spin briefly and remove any residual supernatant.
8. Resuspend the plasmid pellet in 35 μl TE buffer. It is not necessary to dry the pellet before adding TE. Sometimes the pellet needs to be pipette for complete dissolving.

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

Ordering Information

Product Description	Catalog No.	Size
Zymoprep™ Yeast Plasmid Miniprep I	D2001	100 Preps.
Zymoprep™ Yeast Plasmid Miniprep II	D2004	50 Preps.

Individual Kit Components	Catalog No.	Amount
Zymolyase™ and Storage Buffer (lyophilized)	E1004 E1005	1000 Units 2000 Units
Solution 1, Digestion Buffer	D2001-1-15	15 ml
Solution 2, Lysis Buffer	D2001-2-15	15 ml
Solution 3, Neutralizing Buffer	D2001-3-15	15 ml



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Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

Integrity of kit components is guaranteed for up to one year at 4°C from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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