Instructions

Zymoprep™ Yeast Plasmid Miniprep II

Catalog Number D2004

Highlights

- Simple spin column format for plasmid rescue from yeast.
- Highly efficient.
- No glass beads, no phenol, no vortexing, no precipitation.
- Ideal for low copy and hard to isolate plasmids.

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The Beauty of Science is to Make Things Simple

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GENERAL INFORMATION

Specificity:

S. cerevisiae, C. albicans and *S. pombe*, and other fungi species sensitive to yeast lytic enzymatic digestion (**Zymolyase™**).

- **Plasmid Purity:** Plasmid is recovered in water or TE.
- **Recovery Volume:** 10 ul
- **Plasmid Size:** Up to 23 Kb (For plasmids > 15 Kb add elution water or TE and incubate for up to 5 minute before centrifugation).
- **DNA Binding Capacity:** 5 ug
- **Stability:** Quality is guaranteed for 1 year from the purchase date.

Kit Contents:

Products	Qty./Size	Storage	Catalog
Zymoprep™ II For 50 plasmid rescues.			**D2004**
Solution 1	10 ml	Room Temp.	D2004-1-10
Solution 2	10 ml	Room Temp.	D2004-2-10
Solution 3	20 ml	Room Temp.	D2004-3-20
Wash Buffer[1] (Concentrate)	6 ml	Room Temp	D4003-2-6
Zymolyase, (Resuspend the lyophilized enzyme by adding 200 ul **Storage Buffer** to make 5 units/ul)	1,000 Units[2] (lyophilized) Storage Buffer 500 ul	-20°C	E1004
Zymo-Spin I Columns™	Pack of 50	Room Temp.	C1003-50
Collection Tubes	Pack of 50	Room Temp.	C1001-50
Instruction Sheet	1	-	-
Additional Products			
Zymolyase, lyophilized	2,000 Units Storage Buffer 500 ul	-20°C	E1005

™ Zymoprep and Zymo-Spin I Column are trademarks of Zymo Research Corp.
1 Ethanol is not provided
2 This reagent contains beta-mercaptoethanol

For research use only. Always wear protective gloves and eye protection. These reagents are intended for use by trained professionals. Further precautions should be taken according to your own company's regulations.

GENERAL DESCRIPTION

Zymoprep™ II provides all the necessary reagents for plasmid isolation from *S. cerevisiae, C. albicans* and *S. pombe*, and any fungi whose cell walls are susceptible to yeast lytic enzyme lysis. **Zymoprep™ II** is a simple and efficient yeast plasmid miniprep kit 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, or phenol. Reliably recover your plasmid from yeast cells every time, whether you use colonies, patches on plates, or liquid cultures. The plasmid isolated by the kit via **Zymo-Spin column I** can be used directly for *E. coli* transformation, PCR, and Southern blot analysis. The plasmid recovery for **Zymoprep™ II** is about 5 fold more compared to **Zymoprep™ I** and eliminates the isopropanol precipitation step. The recovered plasmid is in water or TE buffer. This system is ideal for low-copy number and hard to isolate plasmids.
Two Protocols are provided. The first protocol is for yeast plasmid recovery from colonies or patches; the second protocol is for liquid culture. Although both protocols work equally well, it is much easy to use colonies or patches to process large number samples in our hands.

Before Starting

Prepare Wash Buffer: Add 24ml of 100% ethanol (or 26ml of 95% ethanol) to the **Wash Buffer Concentrate** to make the final working **Wash Buffer.**

Reconstitute the Zymolyase™: 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.

Unless stated otherwise, the following procedures are accomplished at room temperature. Grow yeast cells at 30°C in YPD or selective medium.

Protocol For Colonies or Patches

1. Use tooth pick or pipette tip to pick roughly 2-10 ul volume of yeast colonies or patches from plates and dispense into 200 ul of **Solution 1,** add 3 ul of Zymolyase™ to each tube.

Note: For multiple sample process, add 15μl **Zymolyase™** for each ml of **Solution 1** to make a **Solution 1-enzyme mixture**. Use 200μl of this mixture to resuspend the pellet for each sample. Generally fresh colonies or patches (less than 5 days) give better plasmid recovery than old colonies or patches. Add more Zymolyase™ for old culture to ensure efficient lysis.

2. Incubate at 37°C for 15-60 minutes (15 minutes is the minimal incubation time. Longer incubation is optional, but is suggested for stationary phase or older cells).

3. Add 200 ul **Solution 2** to each tube. Mix well.

4. Add 400 ul **Solution 3** to each tube. Mix well.

5. Centrifuge at maximum speed for 3 minutes.

6. Transfer the supernatant to the **Zymo-Spin-I** column.

7. Spin the **Zymo-Spin I** column for 30 seconds.

8. Discard the flow-through in the collection tube. Make sure the liquid does not touch the bottom part of the column.

9. Add 550 ul of **Wash Buffer** onto the column with the collection tube and spin for 1-2 minutes. Discard the flow through. Place column into a new 1.5 ml microfuge tube (not provided).

10. Add 10 ul of water or TE and spin for 30 seconds-1 minute to elute plasmid off the column into a new 1.5 ml microfuge tube. For plasmids larger than $\geq$ 15 Kb, incubate the column and elution buffer (water or TE) for 5 minutes before centrifugation to increase plasmid yields.

Protocol For Liquid Culture

1. Aliquot 0.1-1.5 ml of the yeast cells into 1.5 ml microfuge tubes and spin down the cells at 600 x g for 2 minutes.

Note: For multiple sample process, add 15 ul **Zymolyase™** for each ml of **Solution 1** to make a **Solution 1-enzyme mixture**. Use 200µl of this mixture to resuspend the pellet for each sample. Generally fresh culture gives better plasmid recovery than old culture. Add more Zymolyase™ for old culture to ensure efficient lysis. Ideally cells should be harvested in early log phase (OD600: 0.2-0.6). If cells are harvested from stationary phase cultures or old culture is used, add more Zymolyase™ to ensure efficient lysis.

2. Add 200 ul **Solution 1** to each pellet.

3. Add 3 ul of **Zymolyase™** to each tube. Resuspend the pellet by flicking with finger or mild vortexing.

4. Incubate at 37°C for 15-60 minutes (15 minutes is the minimal incubation time. Longer incubation is optional, but is suggested for stationary phase or older cells).

5. Add 200 ul **Solution 2** to each tube. Mix well.

6. Add 400 ul **Solution 3** to each tube. Mix well.

7. Centrifuge at maximum speed for 3 minutes.

8. Transfer the supernatant to the **Zymo-Spin-I** column.

9. Spin the **Zymo-Spin I** column for 30 seconds.

10. Discard the flow-through in the collection tube. Make sure the liquid does not touch the bottom part of the column.

11. Add 550 ul of **Wash Buffer** (ethanol added) onto the column with the collection tube and spin for 1-2 minutes. Discard the wash buffer. Place column into a new 1.5 ml microfuge tube (not provided).

12. Add 10 ul of water or TE and spin for 30 seconds-1 minute to elute plasmid off the column into a new 1.5 ml microfuge tube. For plasmids larger than $\geq$ 15 Kb, incubate the column and elution buffer (water or TE) for 5 minutes before centrifugation to increase plasmid yields.

Other Yeast Related Products

Product	Description	Cat. No.	Size
YeaStar™ Genomic DNA Kit	Reagents provided are for 40 fungal genomic DNA preparations.	D2002	1 Kit
YeaStar™ RNA Kit	Reagents provided are for 40 fungal RNA preparations.	R1001	1 Kit
Yeast Protein Kit™	Reagents provided are for 200 protein analyses.	Y1002	1 Kit
Zymolyase™ Liquid format. Shipped on wet ice.	One lytic unit is defined as a 10% decrease in absorbance at A_{800} in 30 minutes.	E1001 E1002	1,000 units, 400 ul 2,000 units, 200 ul
Zymolyase™ Lyophilized format. Shipped at ambient temperature	One lytic unit is defined as a 10% decrease in absorbance at A_{800} in 30 minutes. (Storage Buffer is provided for reconstitution of the enzyme.)	E1004 E1005	1,000 units 2,000 units
Alpha-Factor Mating Pheromone	Concentration: 4 mg at 10 mM in 0.1 M sodium acetate pH 5.2.	Y1001	1 Kit
Zymoprep™ Yeast Plasmid Miniprep I	Reagents provided are for 100 yeast minipreps. Isopropanol precipitation method.	D2001	1 Kit
Frozen EZ™ Yeast Transformation Kit	Reagents provided are for 120 regular or 600 microscale transformation experiments.	T2001	1 Kit
5FOA	Powder, molecular biology grade	F9001-1 F9001-5	1 g 5 g
	2Xsynthetic complete/5FOA at 2 mg/ml	F9002	250 ml
	100X5FOA in DMSO, 100mg/ml	F9003	10 ml