ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Zymoprep™ Yeast Plasmid Miniprep I

Catalog No. **D2001**

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.

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

Product Contents

Zymoprep™ Yeast Plasmid Miniprep I (Kit Size)	**D2001** (100 preps)	**Storage Temperature**
Solution 1, Digestion Buffer	15 ml	RT
Solution 2, Lysis Buffer	15 ml	RT
Solution 3, Neutralizing Buffer	15 ml	RT
Zymolyase™ and Storage Buffer*	1,000 units	-20°C
Instruction Manual	1	-

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

*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.

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

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 x 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 for 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.	Kit Size
Zymoprep™ Yeast Plasmid Miniprep I	D2001	100 preps.
Zymoprep™ Yeast Plasmid Miniprep II	D2004	50 preps.

For Individual Sale	Catalog No.	Amount
Zymolyase™ and Storage Buffer (lyophilized)	E1004	1000 units
	E1005	2000 units

Other Popular Yeast Purification Products from Zymo Research

Product	Format	Kit Size	Cat No.
	Yeast Growth & Transformation		
Frozen-EZ Yeast Transformation II Kit™	Transformation efficiency 10^5-10^6 CFU/µg	120 rxns	**T2001**
YPD Plus™	Increases yeast transformation efficiency >50%	50 ml 100 ml	**Y1003-50** **Y1003-100**
	Yeast Specialty Products		
Yeast Protein Kit™	Efficient lysis of yeast for downstream protein and DNA analyses	200 preps.	**Y1002**
5-Fluorootic Acid (5-FOA)	Yeast genetic counter selection	1 g. 5 g. 250 ml (2X SC) 10 ml (100X)	**F9001-1** **F9001-5** **F9002** **F9003**
α-Factor Mating Pheromone	Optimized for yeast mating induction	240 ul	**Y1001**
Zymolyase-Yeast Lytic Enzyme	Efficient digestion of yeast and fungal walls	1,000 U 2,000 U	**E1004** **E1005**
	Yeast DNA/RNA Purification		
ZymoPrep™ Yeast Plasmid Miniprep I	Isopropanol precipitation Format, elution ≥ 35 µl	100 preps.	**D2001**
ZymoPrep™ Yeast Plasmid Miniprep II	Spin Column Format (up to 5 µg/prep.)	50 preps.	**D2004**
YeaStar™ Genomic DNA Kit	Spin Column Format (up to 20 µg/prep.)	40 preps.	**D2002**
ZR Soil Microbe DNA MiniPrep™	Bead Bashing, Spin Column Format (up to 25 µg/prep.)	50 preps.	**D6001**
ZR Fungal/Bacterial DNA MiniPrep™	Bead Bashing, Spin Column Format (up to 25 µg/prep.)	50 preps.	**D6005**
ZR Fungal/Bacterial RNA MiniPrep™	Bead Bashing, Spin Column Format (up to 25 µg/prep.)	50 preps.	**R2014**
YeaStar™ Genomic RNA Kit	Spin Column Format (up to 25 µg/prep.)	40 preps.	**R1002**