ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Zymoprep™-96 Yeast Plasmid Miniprep

Catalog Nos. **D2005, D2006,** & **D2007**

Highlights

- Innovative spin-plate format eliminates the need for glass beads, phenol, vortexing, or isopropanol precipitation.
- The fastest and simplest high-throughput procedure for purifying the highest quality plasmid DNA from yeast.
- Ideal for low copy and hard-to-isolate plasmids.

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Note: 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™-96 Yeast Plasmid Miniprep (Kit Size)	**D2005** (2x 96 preps)	**D2006** (4x 96 preps)	**D2007** (8x 96 preps)	**Storage Temperature**
Solution 1	45 ml	90 ml	2x 90 ml	Room Temp.
Solution 2 (Green)	45 ml	90 ml	2x 90 ml	Room Temp.
Solution 3 (Yellow)	90 ml	180 ml	2x 180 ml	Room Temp.
DNA Wash Buffer† (concentrate)	24 ml	48 ml	2x 48 ml	Room Temp.
Zymolyase†	2x 2,000 Units	4x 2,000 Units	8x 2,000 Units	- 20°C
Storage Buffer†	2x 500 µl	4x 500 µl	8x 500 µl	- 20°C
96-Well Block	2	4	8	-
Collection Plate	2	4	8	-
Zymo-Spin™ I-96 Plate	2	4	8	-
Elution Plate	2	4	8	-
Air-Permeable Sealing Cover	2	4	8	-
96-Well Plate Cover Foil	8	16	32	-
Instruction Manual	1	1	1	-

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

†Buffers require preparation prior to use as described on page 2.

Specifications

- **Specificity:** *S. cerevisiae, C. albicans*, *S. pombe* and other fungi species sensitive to yeast lytic enzymatic digestion (**Zymolyase™**).
- **DNA Purity:** Eluted plasmid DNA is well suited for downstream applications such as PCR, transformation, hybridization and other sensitive applications requiring pure DNA. $Abs_{260/280}$ is $\geq$ 1.8.
- **Plasmid DNA Yield:** Typically between 0.01 - 0.3 ng for most 2 µ based plasmid from 1.5 ml overnight cultures.
- **Plasmid DNA Size:** Up to 25 kb.
- **Recovery Volume:** ≥ 10 µl per well.
- **Procedure:** Performed at room temperature (15-30 °C) using a centrifuge with micro plate carriers.

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended 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.

Product Description

The Zymoprep™-96 Yeast Plasmid Miniprep kit provides all the necessary reagents for high-throughput plasmid isolation from *S. cerevisiae, C. albicans*, *S. pombe* and any fungi whose cell walls are susceptible to yeast lytic enzyme lysis. **The Zymoprep™-96** is a simple and efficient yeast plasmid miniprep kit that is based on the classic *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 isolated plasmid can be used directly for *E. coli* transformation, PCR, and Southern blot analysis. The **Zymoprep™-96** incorporates our **Zymoprep™ II** kit into a 96-well format resulting in a high-throughput method that recovers about 5 fold more plasmid DNA compared to **Zymoprep™ I** and eliminates the isopropanol precipitation step. This system is ideal for low-copy number and hard to isolate plasmids and the plasmid DNA can be eluted directly in water or TE buffer.

Two protocols are provided with the **Zymoprep™-96** kit. The first protocol is for yeast plasmid recovery from colonies or patches and the second protocol is for liquid culture. Although both protocols work equally well, it is much easier to use colonies or patches to process large numbers of samples.

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

Buffer Preparation:

1. Add 96 ml of 100% ethanol (104 ml of 95% ethanol) to the 24 ml **Wash Buffer Concentrate** (D2005), or 192 ml of 100 % ethanol (208 ml of 95 % ethanol) to the 48 ml **Wash Buffer Concentrate** (D2006 & D2007) before use.

2. Reconstitute the **Zymolyase™** by adding 400 ul of the supplied **Storage Buffer** to the lyophilized **Zymolyase™**. Mix the solution until the enzyme is completely dissolved and spin briefly in a microcentrifuge. Store the reconstituted **Zymolyase™** at -20°C.

3. Incubate **Solution 3** on ice for 30 minutes before use.

Culturing Yeast in the 96-Well Block:

1. Dispense up to 1.5 ml of YPD or selective medium into each well of the provided **96-Well Block**.

   *Make sure to use a **96-Well Block** and not a **Collection Plate**.*

2. Inoculate each well from either a glycerol stock, culture plate, or pre-culture (2-3 µl) using a 96-pin device or other method.

3. Seal the block using an **Air-Permeable Sealing Cover**. Incubate cultures in an incubator/shaker at 30 °C with constant shaking at 250-300 rpm until the cells reach early log phase (OD_{600}: 0.2-0.6).

Protocol For Colonies or Patches

The following procedure should be done at room temperature.

Ensure that buffers have been prepared according to the instructions on page 2.

1. Add 330 µl of **Zymolyase™** to 22 ml of **Solution 1** and mix well. Dispense 200 µl of the **Solution 1/Zymolyase™** mixture into each well of the supplied **96-Well Block**.

2. Using a tooth pick or pipette tip, pick roughly 2-10 µl volume of yeast colonies or patches from plates and dispense into the wells of the 96-Well Block.

3. Seal the block with a **96-Well Plate Cover Foil**[1] and incubate at 37°C for 15-60 minutes[2].

4. Remove the foil and add 200 µl **Solution 2** to each well of the block. Seal the block with a **96-Well Plate Cover Foil** and invert 4-6 times[3].

   *After addition of **Solution 2** and mixing, the solution should change from opaque to clear green, indicating yeast cell lysis is complete.*

5. Pierce foil to add 400 µl of ice cold **Solution 3** (yellow) to each well and dry the top of the block thoroughly with a paper towel. Seal the block with a second **96-Well Plate Cover Foil**[1] and invert gently 4-6 times[3] until the lysate is completely neutralized.

   The sample will turn yellow when neutralization is complete and a yellowish precipitate will form.

6. Centrifuge the block for 10 min at 3,000 - 5,000 *x g*. Pierce (or remove) foil and transfer 550 µl of the supernatants to the wells of a **Zymo-Spin™ I-96 Plate** on a **Collection Plate**. Pipette only to a depth of ~75% of the volume of each well so as to not disturb the pelleted debris.

7. Centrifuge the **Zymo-Spin™ I-96/Collection Plate Combo** for 5 min at 3,000 - 5,000 *x g*. Discard the flow through from the **Collection Plate**.

8. Re-place onto the **Collection Plate** and add 550 µl of **Wash Buffer** to each well of the **Zymo-Spin™ I-96 Plate**. Centrifuge for 5 min at 3,000 - 5,000 *x g*.

9. Discard the flow through from the **Collection Plate** and centrifuge the combo again for 5 min at 3,000 - 5,000 *x g* to remove any residual **Wash Buffer**.

10. Add 10 µl of water or TE buffer[4] directly to each well of the **Zymo-Spin™ I-96 Plate** on an **Elution Plate**. Let stand at room temperature 1-2 minutes and then centrifuge for 3 minutes at 3,000 - 5,000 *x g* to elute the plasmid DNA.

    *The eluted DNA can be used immediately for molecular based applications or stored ≤-20 °C for future use. Use the **96-Well Plate Cover Foil** to prevent evaporation.*

Notes:

[1] The foil should be completely sealed on the sides of the block and the outline of each individual well clearly defined.

[2] 15 minutes is the minimal incubation time. A longer incubation is optional and is suggested for stationary phase or older cells.

[3] Inverting the block too many times may result in cross-contamination and/or genomic DNA inclusion in the eluted plasmid DNA.

[4] The DNA yield can be increased by pre-warming the water or TE buffer to 50ºC and/or increasing the incubation period up to 5 minutes prior to centrifugation.

Protocol For Liquid Culture

The following procedure should be done at room temperature.

Ensure that buffers have been prepared according to the instructions on page 2.

1. Remove the **96-Well Block** from the incubator and discard the **Air-Permeable Sealing Cover**. Spin the cells down at 600 *x g* for 2 minutes and discard the supernatant.

   Ideally cells should be harvested in early log phase (OD_{600}: 0.2-0.6).

2. Add 330 µl of **Zymolyase™** to 22 ml of **Solution 1** and mix well. Dispense 200 µl of the **Solution 1/Zymolyase™ mixture** into each well of the **96-Well Block**.

3. Completely resuspend the cell pellets by mild vortexing or pipetting up and down. Seal the block with a **96-Well Plate Cover Foil**[1] and incubate at 37°C for 15-60 minutes[2].

4. Remove the foil and add 200 µl **Solution 2** to each well of the block. Seal the block with a **96-Well Plate Cover Foil** and invert 4-6 times[3].

   *After addition of **Solution 2** and mixing, the solution should change from opaque to clear green, indicating yeast cell lysis is complete.*

5. Pierce foil to add 400 µl of ice cold **Solution 3** (yellow) to each well and dry the top of the block thoroughly with a paper towel. Seal the block with a second **96-Well Plate Cover Foil**[1] and invert gently 4-6 times[3] until the lysate is completely neutralized.

   The sample will turn yellow when neutralization is complete and a yellowish precipitate will form.

6. Centrifuge the block for 10 min at 3,000 - 5,000 *x g*. Pierce (or remove) foil and transfer 550 µl of the supernatants to the wells of a **Zymo-Spin™ I-96 Plate** on a **Collection Plate**. Pipette only to a depth of ~75% of the volume of each well so as to not disturb the pelleted debris.

7. Centrifuge the **Zymo-Spin™ I-96/Collection Plate Combo** for 5 min at 3,000 - 5,000 *x g*. Discard the flow through from the **Collection Plate**.

8. Re-place onto the **Collection Plate** and add 550 µl of **Wash Buffer** to each well of the **Zymo-Spin™ I-96 Plate**. Centrifuge for 5 min at 3,000 - 5,000 *x g*.

9. Discard the flow through from the **Collection Plate** and centrifuge the combo again for 5 min at 3,000 - 5,000 *x g* to remove any residual **Wash Buffer**.

10. Add 10 µl of water or TE buffer[4] directly to each well of the **Zymo-Spin™ I-96 Plate** on an **Elution Plate**. Let stand at room temperature 1-2 minutes and then centrifuge for 3 minutes at 3,000 - 5,000 *x g* to elute the plasmid DNA.

    *The eluted DNA can be used immediately for molecular based applications or stored ≤-20 °C for future use. Use the **96-Well Plate Cover Foil** to prevent evaporation.*

Notes:

[1] The foil should be completely sealed on the sides of the block and the outline of each individual well clearly defined.

[2] 15 minutes is the minimal incubation time. A longer incubation is optional and is suggested for stationary phase or older cells.

[3] Inverting the block too many times may result in cross-contamination and/or genomic DNA inclusion in the eluted plasmid DNA.

[4] The DNA yield can be increased by pre-warming the water or TE buffer to 50ºC and/or increasing the incubation period up to 5 minutes prior to centrifugation.

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	
Culture growth conditions	• An overgrown/under-grown or contaminated culture or omission of selectable marker from the growth medium. Use a fresh culture for optimal performance.
Procedural errors	• Incomplete lysis: After addition of **Solution 2** the solution should change from opaque to clear blue, indicating complete lysis. Different yeast strains often require different growth conditions and may vary in their susceptibility to alkaline lysis. • Incomplete neutralization: Cell debris will float to the surface after centrifugation and the pellet may appear "puffy". Make sure the neutralization is complete prior to centrifugation. Invert the block an additional 2-3 times after the sample turns yellow following the addition of **Solution 3**.
Solution 2 (precipitation)	• **Solution 2** may have precipitated during shipping: To completely resuspend the buffer, incubate the bottle at 30-37 ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.
Wash Buffer	• Ensure that ethanol has been added to the wash buffer.
DNA elution	• Incomplete elution: For large size plasmids (>10 kb), incubate the plate for 5-10 minutes before centrifugation. Also, pre-warm the water or TE buffer to 50 ºC prior to elution.
Low DNA Quality	
DNA does not perform well	• Incomplete neutralization: Incomplete neutralization generates poor quality supernatant and results in loading too much cell debris into the wells of the plate. Ensure that neutralization is complete by inverting the sample an additional 2-3 times after the addition of **Solution 3**. • The **Zymo-Spin™ I-96** plate tips are contaminated with wash buffer flow through: Avoid tilting the **Collection Plate** after the last wash step to ensure that the plate tips do not contact the flow through. Empty the **Collection Plate** when recommended in the protocol. • Insufficient centrifugation: make sure that all centrifugation steps are performed between 3,000-5,000 x *g*. If a lower centrifuge speed is used, extend the centrifugation time to compensate.

Ordering Information:

Product Description	Kit Size	Catalog No.
Zymoprep™-96 Yeast Plasmid Miniprep	2x 96 preps. 4x 96 preps. 8x 96 preps.	D2005 D2006 D2007

For Individual Sale	Amount	Catalog No.
Solution 1	10 ml 45 ml 90 ml	D2004-1-10 D2004-1-45 D2004-1-90
Solution 2 (Green)	10 ml 45 ml 90 ml	D2004-2-10 D2004-2-45 D2004-2-90
Solution 3 (Yellow)	20 ml 90 ml 180 ml	D2004-3-20 D2004-3-90 D2004-3-180
DNA Wash Buffer (concentrate)	24 ml 48 ml	D4003-2-24 D4003-2-48
Zymolyase™ (500 µl storage buffer)	1,000 Units 2,000 Units	E1004 E1005
96-Well Block	2 10	P1001-2 P1001-10
Collection Plate	2	C2002
Elution Plate	2	C2003
Air-Permeable Sealing Cover	2 4 8	C2011-2-2 C2011-2-4 C2011-2-8
96-Well Plate Cover Foil	6 8 12 24	C2007-6 C2007-8 C2007-12 C2007-24

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**