



INSTRUCTION MANUAL

ZymoPURE[™] II Plasmid Midiprep Kit

Catalog Nos. D4200 & D4201 (Patent Pending)

Highlights

- Fast, easy, reliable, ultra-pure transfection grade plasmid DNA Midiprep using a microcentrifuge spin-column.
- Innovative EndoZero[™] technology enables transfection in sensitive cells and *in vivo* research (≤ 0.025 EU/µg of plasmid DNA).
- State-of-the-art ZymoPURE binding technology guarantees highly concentrated plasmid DNA directly from a spin-column in ≤ 20 minutes. No gravity filtration or ethanol precipitation!

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For Research Use Only Version 1.2.0

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

Product Contents:

ZymoPURE [™] II Plasmid Midiprep Kit (Kit Size)	D4200 (25 preps.)	D4201 (50 preps.)	Storage Temperature
ZymoPURE [™] P1 ¹ (Red)	210 ml	410 ml	4°C
ZymoPURE [™] P2 ² (Green)	210 ml	410 ml	Room Temp.
ZymoPURE [™] P3 (Yellow)	210 ml	410 ml	Room Temp.
ZymoPURE [™] Binding Buffer	210 ml	410 ml	Room Temp.
ZymoPURE [™] Wash 1	55 ml	2x 55 ml	Room Temp.
ZymoPURE [™] Wash 2 (Concentrate)	23 ml	2x 23 ml	Room Temp.
ZymoPURE [™] Elution Buffer	6 ml	12 ml	Room Temp.
Zymo-Spin [™] III-P Column Assemblies ³	25	50	Room Temp.
ZymoPURE [™] Syringe Filters	25	50	Room Temp.
ZymoPURE [™] Syringe Plungers	25	50	Room Temp.
EndoZero [™] II Spin-Columns	25	50	
Collection Tubes	25	50	Room Temp.
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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 maximal performance and reliability.

Specifications:

- **DNA Purity:** Eluted DNA is ultrapure, endotoxin-free, and well suited for transfection, transformation, sequencing, restriction endonuclease digestion, *in vitro* transcription, *in vivo* studies, and other sensitive applications.
 - Typical Abs_{260/280} \geq 1.8 and Abs_{260/230} \geq 2.0
 - o Endotoxin levels: ≤ 1 EU/μg of plasmid DNA using the Standard Protocol

≤ 0.025 EU/µg of plasmid DNA with optional EndoZero[™] II Spin-Column

- Plasmid DNA Yield: Up to 400 µg per preparation (Actual yield is dependent on the plasmid copy number, culture growth conditions, and strain of E. coli utilized)
- Plasmid DNA Size: Up to ~200 kb
- Recovery Volume: ≥ 100 µl of ZymoPURE[™] Elution Buffer or DNase-free water
- Required Equipment: Microcentrifuge and vacuum/vacuum manifold (recommended) or swinging bucket centrifuge.
- Processing Time: 20 min

Notes:

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.

™ Trademarks of Zymo Research Corporation.

Several ZymoPURE[™] product technologies are subject to U.S. and foreign patents or are patent pending.

 $pGL3^{\text{TM}}$ is a registered trademark of Promega Corporation.

¹ ZymoPURE[™] P1 contains RNase A (100 μg/ml) and is stable at room temperature without loss in RNase activity, however, for long-term storage the product should be stored at 4-8° C.

² Caution: ZymoPURE[™] P2 Buffer contains NaOH. Please use proper safety precautions.

³ The Zymo-Spin™ III-P, 15 ml Conical Reservoir and 50 ml Reservoir are pre-assembled as a single unit.

Product Description

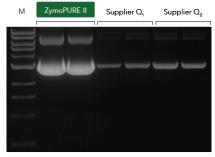
The **ZymoPURE**™ **II Plasmid Midiprep Kit** features a simple spin-column based method for the purification of up to 400 µg of transfection grade plasmid DNA in less than 20 minutes. The eluted plasmid DNA is EndoZero and ready for immediate use in the most sensitive applications. The unique ZymoPURE methodology removes the need for slow gravity flow anion-exchange columns, alcohol precipitations, lengthy endotoxin removal incubations, and time-consuming centrifugation steps.

ZymoPURE[™] technology uses a modified alkaline lysis method and features novel binding chemistry, which enables the highest yields and concentration of plasmid DNA (up to 3 µg/µl) directly from a spin-column. Coupling ZymoPURE with the innovative **EndoZero**[™] **II Spin-Columns**, to eliminate endotoxins, achieves EndoZero plasmid DNA (≤ 0.025 EU/µg of plasmid DNA), making it suitable for transfection, restriction endonuclease digestion, *in vivo* studies, bacterial transformation, PCR amplification, DNA sequencing, and other sensitive downstream applications.

As an added convenience, the **ZymoPURE**[™] **II Plasmid Midiprep Kit** contains colored buffers that permit error-free visualization and identification of complete bacterial cell lysis and neutralization. Syringe filters are included for rapid clearing of the lysate and the unique spin-column design allows the binding step to be performed using a vacuum or table top centrifuge.

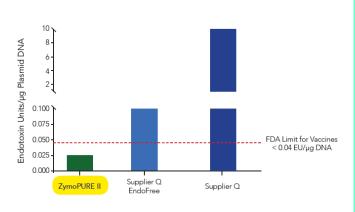
For **Technical Assistance**, please contact **Zymo** at 1-888-882-9682 or E-mail tech@zymoresearch.com.

Highest Recovery



Plasmid DNA concentration and yield from the ZymoPURE II Maxiprep kit compared to two separate kits from Supplier O. Plasmid DNA (pGL3®) was isolated from 150 ml of JM109 E. coli culture grown overnight following the manufacturer's suggested protocol (in duplicate). One (1) µI of eluted plasmid DNA was visualized post agarose gel electrophoresis. M, ZR 1 kb DNA Marker (Zymo Research).

Lowest Endotoxin Levels



Manufacturers' stated endotoxin for two separate Anion-Exchange kits from Supplier Q compared to ZymoPURE II.

Simplest Workflow



EZ-Load™ No Gravity Flow!



EZ-Elute™ No Alcohol Precipitation!



EndoZero™ Endotoxins < FDA limit for Vaccines

Procedure Overview:



Bacterial cells are resuspended in **ZymoPURE**[™] **P1** (red).



The solution will turn dark purple and viscous following the addition of **ZymoPURE**[™] **P2** (green) indicating bacterial lysis is complete.



The solution will turn yellow and a precipitate will form after adding **ZymoPURE**[™] **P3** (yellow) indicating neutralization is complete.



The neutralized lysate is loaded into the **ZymoPURE**[™] **Syringe Filter** and clarified into a new 50 ml conical tube.



ZymoPURE[™] **Binding Buffer** is added to the cleared lysate and mixed thoroughly.



The mixture is loaded into the **Zymo-Spin**[™] **III-P Column** using a vacuum manifold.



The **50 ml Reservoir** is removed and the **Zymo-Spin**[™] **III-P Column** is washed using a vacuum manifold.



Ultra-pure plasmid DNA is eluted from the **Zymo-Spin**™ **III-P Column** using a microcentrifuge.



The eluted plasmid DNA is passed through the **EndoZero™ II Column** using a microcentrifuge.

Buffer Preparation:

- ✓ Add 88 ml of 95% ethanol to the 23 ml **ZymoPURE**[™] **Wash 2 (Concentrate)** before use.
- ✓ The ZymoPURE[™] P2 and ZymoPURE[™] Binding Buffer may have precipitated. If this occurs, dissolve the precipitate by incubating the bottles at 30-37 °C for 10-20 minutes and mix by inversion. Do not microwave!

Before Starting:

✓ Centrifuge up to 50 ml of bacterial culture at $\ge 3,400 \times g$ for 10 minutes to pellet the cells in a 50 ml conical tube. Discard supernatant.

Protocol:

The following procedure should be performed at room temperature (15-30°C).

- Add 8 ml of ZymoPURE[™] P1 (Red) to the bacterial cell pellet and resuspend completely by vortexing or pipetting.
- 2. Add 8 ml of **ZymoPURE**[™] **P2 (Green)** and immediately mix by gently inverting the tube 6 times. Do not vortex! Let sit at room temperature for 2-3 minutes¹.

Cells are completely lysed when the solution appears clear, purple, and viscous.

3. Add 8 ml of **ZymoPURE**[™] **P3 (Yellow)** and mix gently but thoroughly by inversion. <u>Do not vortex!</u>

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

- 4. Ensure the plug is attached to the Luer Lock at the bottom of the ZymoPURE™ Syringe Filter. Place the syringe filter upright in a tube rack and load the lysate into the ZymoPURE™ Syringe Filter² and wait 5-8 minutes for the precipitate to float to the top.
- 5. Remove the Luer Lock plug from the bottom of the syringe and place it into a clean 50 ml conical tube. Place the plunger in the syringe and push the solution through the ZymoPURE[™] Syringe Filter in one continuous motion until approximately 20 ml of cleared lysate is recovered. Save the cleared lysate!
- 6. Add 8 ml **ZymoPURE**[™] **Binding Buffer** to the cleared lysate from step 5 and mix thoroughly by inverting the capped tube 8 times.

To continue processing the lysate using the recommended vacuum protocol, proceed to the next page. If a vacuum is not available, proceed to page 6 for an alternative centrifugation method.

Notes:

¹ Do not allow the lysis reaction to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA.

² If the precipitate has formed a homogenous layer at the surface of the neutralized lysate then invert the tube 3-4 times prior to loading the lysate into the Zymo PURE™ Syringe Filter.

Notes:

¹ To achieve optimal performance, the vacuum pump should be able to apply at least 400 mm Hg pressure. If less pressure is applied, centrifuge the column prior to washing to remove any residual lysate remaining in the matrix.

- ² The **ZymoPURE**[™] **Elution Buffer** contains 10 mM Tris-HCl, pH 8.5 & 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.
- ³ The DNA yield can be increased by pre-warming the **Zymo PURE**[™] **Elution Buffer** to 50 °C and/or increasing the incubation period up to 10 minutes prior to centrifugation.
- ⁴ For low copy number plasmids or if higher concentration is desired, the plasmid DNA can be eluted in as little as 100 µl.
- ⁵ This optional step will reduce endotoxin levels from ≤ 1 EU/μg of plasmid DNA to ≤ 0.025 EU/μg of plasmid DNA.

<u>Vacuum Protocol</u>: (Recommended)

This product is compatible with any conventional vacuum-based manifold. The vacuum pump should be a single or double-staged unit capable of producing up to 400 mm Hg pressure at the vacuum manifold¹.

- 7. Ensure the connections of the **Zymo-Spin**[™] **III-P Column Assembly** are fingertight and place onto a vacuum manifold. (If vacuum is not available, see page 6 for the centrifugation protocol.)
- 8. With the vacuum off, add the entire mixture from step 6 into the Zymo-Spin[™] III-P Column Assembly, and then turn on the vacuum¹ until all of the liquid has passed completely through the column.
- Remove and discard the 50 ml Reservoir from the top of the Zymo-Spin[™] III-P Column Assembly.
- 10. With the vacuum off, add 2 ml of **ZymoPURE**[™] Wash 1 to the Zymo-Spin III-P Column. Turn on the vacuum until all of the liquid has passed completely through the column.
- 11. With the vacuum off, add 2 ml of **ZymoPURE**[™] Wash 2 to the Zymo-Spin III-P Column. Turn on the vacuum until all of the liquid has passed completely through the column. Repeat this wash step.
- 12. Unscrew the purple Luer Lock cap from the top of the **Zymo-Spin**[™] **III-P Column** and discard the **15 ml Conical Reservoir**. Place the Zymo-Spin III-P Column in a **Collection Tube** and centrifuge at ≥ 10,000 x g for 1 minute, in a microcentrifuge, to remove any residual wash buffer.
- 13. Transfer the column into a clean 1.5 ml microcentrifuge tube and add 200 μ l of **ZymoPURE** Elution Buffer^{2,3,4} directly to the column matrix. Wait 2 minutes, and then centrifuge at \geq 10,000 x q for 1 minute in a microcentrifuge.
- 14. *Optional:* For EndoZero Plasmid DNA⁵, place the **EndoZero[™] II Spin-Column** in a clean 1.5 ml microcentrifuge tube. Add the entire eluate from step 13 into the EndoZero[™] II Spin-Column, wait 2 minutes, and then centrifuge at 10,000 x g for 1 minute in a microcentrifuge. Store the eluted plasmid DNA at \leq -20°C.

Centrifugation Protocol: (Alternative)

Perform steps 1-6 as indicated in the general protocol, see page 4.

- 7. Remove the **50 ml Reservoir** from the top of the **Zymo-Spin** III-P Column **Assembly**. Ensure the connection between the **15 ml Conical Reservoir** and **Zymo-Spin** III-P column is finger-tight and place the assembly into a 50 ml conical tube.
- 8. Add 10 ml of the mixture from step 6 into the **15 ml Conical Reservoir/Zymo- Spin** [™] **III-P Column** assembly, and centrifuge at 500 x g for 2 minutes. Empty the 50 ml conical tube and repeat this step until the entire sample has passed through the column.
- 9. Add 2 ml of **ZymoPURE**[™] **Wash 1** to the Zymo-Spin III-P column assembly and centrifuge the column at 500 x *g* for 2 minutes.
- 10. Add 2 ml of of **ZymoPURE**[™] **Wash 2** to the Zymo-Spin III-P column assembly and centrifuge the column for 2 minutes at 500 x g. Repeat the wash step.
- 11. Unscrew the purple Luer Lock cap from the top of the **Zymo-Spin**[™] **III-P Column** and discard the **15 ml Conical Reservoir**. Place the Zymo-Spin III-P Column in a **Collection Tube** and centrifuge at ≥ 10,000 x g for 1 minute, in a microcentrifuge, to remove any residual wash buffer.
- 12. Transfer the column into a clean 1.5 ml microcentrifuge tube and add 200 μ l of **ZymoPURE** Elution Buffer^{1,2,3} directly to the column matrix. Wait 2 minutes, and then centrifuge at \geq 10,000 x g for 1 minute in a microcentrifuge.
- 13. Optional: For EndoZero Plasmid DNA⁴, place the **EndoZero[™] II Spin-Column** in a clean 1.5 ml microcentrifuge tube. Add the entire eluate from step 12 into the EndoZero[™] Spin-Column, wait 2 minutes, and then centrifuge at 10,000 x g for 1 minute in a microcentrifuge. Store the eluted plasmid DNA at ≤ -20°C.

Notes:

- ¹ The **ZymoPURE**[™] **Elution Buffer** contains 10 mM Tris-HCl, pH 8.5 & 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.
- ²The DNA yield can be increased by pre-warming the **Zymo PURE™** Elution Buffer to 50 °C and/or increasing the incubation period up to 10 minutes prior to centrifugation.
- ³ For low copy number plasmids or if higher concentration is desired, the plasmid DNA can be eluted in as little as 100 µl.
- ⁴ This optional step will reduce endotoxin levels from ≤ 1 EU/μg of plasmid DNA to ≤ 0.025 EU/μg of plasmid DNA.

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	
Culture growth conditions	 Poor aeration of culture. The optimal culture volume to air volume ratio is 1:5 or less. For best aeration, use baffled culture flasks, or a vented or gas-permeable seal on the culture vessel. The culture was overgrown, undergrown, contaminated, or antibiotics were omitted from the growth medium. Use a fresh culture for optimal performance. An OD₆₀₀ of 0.2-0.35 is the optimal optical density of a tenfold dilution of the culture.
Cell density is too high	 Too much culture used. Lysis and neutralization will be incomplete and the Zymo PURE™ Syringe Filter may clog during filtration. More culture does not always equal more plasmid. Incomplete lysis and neutralization are two of the most common causes of failed plasmid preps and both are caused by too much culture being used. Incomplete lysis: After addition of ZymoPURE™ P2, the solution should change from opaque pink to a clear viscous purple, indicating complete lysis. Different <i>E. coli</i> strains often require different growth conditions and may vary in their susceptibility to alkaline lysis. Incomplete neutralization: The solution should not be viscous following neutralization and the yellowish precipitate should appear fluffy and readily float to the surface. Make sure the neutralization is complete prior to filtration. Invert the tube an additional 2-3 times after the sample turns yellow following the addition of ZymoPURE™ P3.
Lysate Clarification	 Less than 20 ml of cleared lysate was recovered from the ZymoPURE[™] Syringe Filter. For optimal performance, add 8 ml of ZymoPURE[™] Binding Buffer to approximately 20 ml of clarified lysate.
ZymoPURE P2 and ZymoPURE Binding Buffer precipitated	 Both buffers may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30-37 °C for 10 minutes and mix by inversion. DO NOT MICROWAVE.
Wash buffer	 Ensure that ethanol has been added to the ZymoPURE[™] Wash 2. Ensure that the bottle cap is screwed on tightly after each use to prevent evaporation of the ethanol.
DNA elution	 Incomplete elution: For large size plasmids (> 10 kb), add ZymoPURE[™] Elution Buffer and incubate the column for 5-10 minutes before centrifugation. Also, prewarm the ZymoPURE[™] Elution Buffer to 50 °C prior to elution.
Low DNA Quality	
DNA does not perform well	 Incomplete neutralization: Incomplete neutralization generates poor quality supernatant. Ensure that neutralization is complete by inverting the sample an additional 2-3 times after the addition of ZymoPURE™ P3 and extending the incubation. Ethanol contamination in eluate. Centrifuge the Zymo-Spin™ III-P column as indicated in the protocol prior to adding the ZymoPURE™ Elution Buffer.
RNA in eluate	 Ensure that ZymoPURE[™] P1 has been stored at 4°C. RNase A can be purchased separately if necessary.
Genomic DNA in eluate	 Improper handling (Sample was vortexed or handled too roughly). Genomic DNA contamination is usually caused by excessive mechanical shearing during the lysis and neutralization steps. Also, prolonged lysis or incomplete mixing of lysis or neutralization buffers may contribute to genomic DNA contamination in your sample. Overgrown culture. Overgrown or old cultures may contain more genomic DNA contamination than fresh cultures.

Ordering Information

Product Description	Kit Size	Catalog No.
ZymoPURE [™] II Plasmid Midiprep Kit	25 preps.	D4200
ZymoPURE [™] II Plasmid Midiprep Kit	50 preps.	D4201

For Individual Sale	Amount	Catalog No.
ZymoPURE [™] P1 (Red)	150 ml 210 ml 410 ml	D4200-1-150 D4200-1-210 D4200-1-410
ZymoPURE [™] P2 (Green)	150 ml 210 ml 410 ml	D4200-2-150 D4200-2-210 D4200-2-410
ZymoPURE [™] P3 (Yellow)	150 ml 210 ml 410 ml	D4200-3-150 D4200-3-210 D4200-3-410
ZymoPURE [™] Binding Buffer	150 ml 210 ml 410 ml	D4200-4-150 D4200-4-210 D4200-4-410
ZymoPURE [™] Wash 1	55 ml 410 ml	D4200-5-55 D4200-5-410
ZymoPURE [™] Wash 2 (Concentrate)	23 ml	D4200-6-23
ZymoPURE [™] Elution Buffer	6 ml 12 ml 30 ml	D4200-7-6 D4200-7-12 D4200-7-30
Zymo-Spin [™] III-P Column Assemby w/ 15 ml Conical and 50 ml Reservoir	5	C1040-5
15 ml Conical Reservoir	25	C1031-25
50 ml Reservoir	25	C1032-25
ZymoPURE [™] Syringe Filter	5	C1036-5
ZymoPURE [™] Syringe Plunger	5	C1037-5
EndoZero [™] II Spin-Columns	25	C1060-25
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000

DNA PURIFICATION

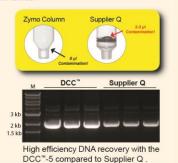


Purify DNA from PCR & other sources

DNA Clean & Concentrator™ (DCC™)

- ✓ Recovery of ultra-pure DNA that is free of salts and contaminants.
- ✓ Small (≥6 µl) elution volume.
- ✓ DNA is ideal for ligation, PCR, Next-Gen sequencing, etc.

Product	Size (Cat. No.)
DNA Clean & Concentrator™-5	50 Preps. (D4013) 200 Preps. (D4014)
ZR-96 DNA Clean & Concentrator™-5	2 x 96 Preps. (D4023) 4 x 96 Preps. (D4024)
Genomic DNA Clean & Concentrator™	25 Preps. (D4010) 100 Preps. (D4011)

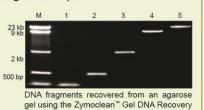


Boost DNA recoveries from agarose gels to >80%

Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in ≥6 μl.
- ✓ Ultra-pure DNA ideal for DNA ligation, sequencing, etc.
- √ Format also available for large DNA >20 kb.

Product	Size (Cat. No.)
Zymoclean™ Gel DNA Recovery Kit	50 Preps. (D4001) 200 Preps. (D4002)
Zymoclean™ Large Fragment DNA Recovery Kit	25 Preps. (D4045) 100 Preps. (D4046)



Kit. Lanes: M: DNA Ladder; 1-5: individual

ladder DNA fragments.

Recover transfection-quality plasmid DNA directly from culture

Zyppy™ Plasmid Prep Kits

- ✓ The fastest, simplest method available for purifying high quality plasmid DNA from *E. coli*.
- ✓ Pellet-Free™ procedure omits conventional cell-pelleting and resuspension steps.
- ✓ Transfection quality plasmid DNA directly from culture in under 15 minutes.



 Product
 Size (Cat. No.)

 Zyppy™ Plasmid Miniprep Kit
 50 Preps. (D4036) 100 Preps. (D4019) 400 Preps. (D4020) 800 Preps. (D4037)

OTHER INNOVATIVE PRODUCTS FROM ZYMO RESEARCH...

