ZYMO RESEARCH

DNA Purification Made Simple™

ZymoPURE™ 96 Plasmid Miniprep Kit

High-throughput purification of transfection-grade plasmid DNA from up to 5 ml of overnight *E. coli* culture.

Highlights

- Perform 96 plasmid minipreps simultaneously using a vacuum manifold or centrifuge.
- Purify up to 100 µg of highly concentrated, ultra-pure, transfection-grade plasmid DNA per prep.
- Novel Wash Plate design significantly reduces cross-contamination between wells.

Catalog Numbers:
D4214, D4215

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

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Product Contents

ZymoPURE™ 96 Plasmid Miniprep Kit	D4214 (2 x 96 preps)	D4215 (4 x 96 preps)	Storage Temperature
ZymoPURE™ P1[1] (Red)	30 ml (2x)	30 ml (4x)	4°C
ZymoPURE™ P2[2,3] (Blue)	30 ml (2x)	30 ml (4x)	Room Temp.
ZymoPURE™ P3 (Yellow)	30 ml (2x)	30 ml (4x)	Room Temp.
ZymoPURE™ Binding Buffer[3]	30 ml (2x)	30 ml (4x)	Room Temp.
ZymoPURE™ Wash 1	80 ml (2x)	80 ml (4x)	Room Temp.
ZymoPURE™ Wash 2 (concentrate)[4]	23 ml (2x)	23 ml (4x)	Room Temp.
ZymoPURE™ Elution Buffer	30 ml	30 ml (2x)	Room Temp.
ZymoPURE™ Filter Plates	2	4	Room Temp.
Zymo-Spin P-96 Plates	2	4	Room Temp.
Wash Plates	2	4	Room Temp.
96-Well 2.0 mL Deep Well Plates	8	16	Room Temp.
Air Permeable Sealing Covers	2	4	Room Temp.
96-Well Plate Cover Foil	6	12	Room Temp.
Instruction Manual	1	1	-

[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 ZymoPURE™ P2 and ZymoPURE™ Binding Buffer may have precipitated. If this occurs, dissolve the precipitate by incubating the bottles at 30-37 ºC for 10-20 minutes and mix by inversion. Do not microwave!
[4] ZymoPURE™ Wash 2 included with D4214 and D4215 are supplied as a concentrate and require the addition of ethanol prior to use. See Buffer Preparation (page 5) for instructions.

Specifications

- **DNA Purity –** Eluted DNA is ultra-pure, endotoxin-free, and well suited for transfection, transformation, lentivirus production, adenovirus production, AAV production, CRISPR, genome editing, *in vivo* studies, sequencing, restriction endonuclease digestion, *in vitro* transcription/translation, PCR, and other sensitive applications.
    - Typical Abs260/280 ≥ 1.8 and Abs260/230 ≥ 2.0
    - Endotoxin levels: ≤ 1 EU/µg of plasmid DNA
    - Suitable for transfecting stable, primary, and sensitive cells lines.
- **Plasmid DNA Yield –** Up to 100 µg per preparation. Actual yield is dependent on the plasmid copy number, culture growth conditions, and strain of *E. coli* utilized. Typical yields for 5 ml of overnight culture grown in LB for high-copy plasmids are 20 – 40 µg.
- **Plasmid DNA Size –** Up to ~200 kb
- **Recovery Volume –** ≥ 125 µl of ZymoPURE™ Elution Buffer or DNase free water[1].
- **Processing Time –** ≤ 60 min
- **Required Equipment –** 96-well plate vacuum manifold (recommended Zymo Cat# S7003) or swinging bucket centrifuge with microplate adapters. The vacuum pump should be a single or double-staged unit capable of producing up to 300 mm Hg at the vacuum manifold.

[1] If higher concentration is desired, the plasmid DNA can be eluted in as little as 50 µl. Please refer to page 10 in the appendix regarding using elution volumes less than 125 µl.

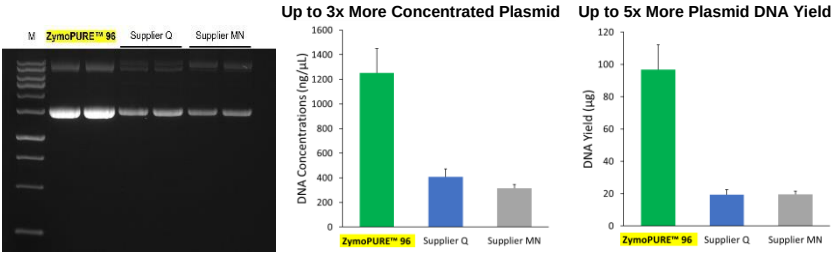
Product Description

The **ZymoPURE™ 96 Plasmid Miniprep Kit** features a high-throughput method for the purification of up to 100 µg of ultra-pure transfection-grade plasmid DNA using a vacuum manifold or centrifuge. The unique vacuum plate design provides zero buffer retention and low elution volumes. In addition, the provided wash plate also significantly reduces plasmid DNA cross-contamination between wells relative to other popular high-throughput plasmid prep kits.

ZymoPURE™ technology uses a modified alkaline lysis method and our patented binding chemistry, which enables the highest DNA binding capacity and rapid loading of the lysate and wash buffer, resulting in the purification of highly concentrated (up to 1 µg/µl) plasmid DNA directly from a 96-well vacuum plate. In addition, the wash regimen has been optimized to ensure the plasmid DNA is free of endotoxins, salt, protein, and RNA. The result is plasmid DNA suitable for transfection, transformation, lentivirus production, adenovirus production, AAV production, CRISPR, genome editing, sequencing, restriction endonuclease digestion, in vitro transcription/translation, PCR and other sensitive downstream applications.

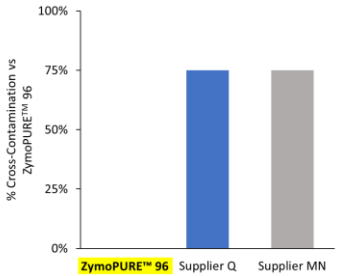
As an added convenience, the **ZymoPURE™ 96 Plasmid Miniprep Kit** contains colored buffers that permit error-free visualization and identification of complete bacterial cell lysis and neutralization. ZymoPURE™ Filter Plates are also included for rapid clearing of the lysate and the unique Zymo-Spin P-96 Plate design allows the binding step to be performed using a vacuum or centrifuge.

High-Throughput Purification of Highly Concentrated Plasmid DNA



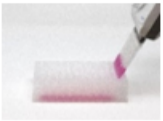
Concentration and yield for plasmid DNA using the ZymoPURE™ 96 Plasmid Miniprep kit compared to two popular 96 vacuum plate kits from Supplier Q and Supplier MN. Plasmid DNA (pGL3®) was isolated from 5 ml of JM109 *E. coli* culture grown overnight following the manufacturer's suggested protocol (in duplicate). One (1) µl of eluted plasmid DNA was visualized post agarose gel electrophoresis. M, ZR 1 kb DNA Marker (Zymo Research).

Innovative Wash Plate Design Reduces Plasmid Cross-Contamination

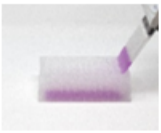


Percent cross-contamination of two popular 96 vacuum plate kits from Supplier Q and Supplier MN compared to ZymoPURE™ 96 Plasmid Miniprep kit. Plasmid DNA (pGL3®) was isolated from 1 ml of JM109 *E. coli* culture grown overnight following the manufacturer's suggested protocol (in replicates). Unused wells adjacent to sample-processed wells were eluted and quantified for the presence of pGL3® plasmid DNA through qPCR.

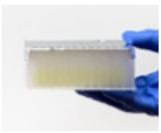
Procedure Overview



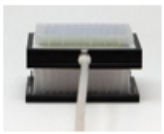
Bacterial cells are resuspended in **ZymoPURE™ P1** (red).



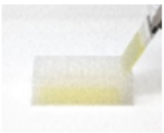
The solution will turn dark purple and viscous following the addition of **ZymoPURE™ P2** (blue) indicating bacterial lysis is complete.



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™ Filter Plate** and clarified into a new **Deep Well Plate**.



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

The mixture is loaded into the **Zymo-Spin P-96 Plate** using a vacuum manifold or centrifuge.

The **Zymo-Spin P-96 Plate** is washed using a vacuum manifold or centrifuge.

Ultra-pure Plasmid DNA is eluted from the **Zymo-Spin P-96 Plate** using a vacuum manifold or centrifuge.

Eluted plasmid DNA is ready for immediate use in the most sensitive applications.

Protocol

Buffer Preparation:

- ✓ Add 88 ml of 95% ethanol to **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 ºC for 10-20 minutes and mix by inversion. Do not microwave!

Cell Cultivation in a 96-Well 2.0 mL Deep Well Plate:

- ✓ Add 1.0-1.5 mL of growth medium with appropriate antibiotics to each well of the **96-Well 2.0 mL Deep Well Plate**. Do not exceed 1.5 mL culturing volume, as this may lead to spilling during shaking. Inoculate each well with a single bacterial colony, starter culture, or glycerol stock. Cover the Deep Well Plate with the Air Permeable Sealing Cover and incubate the cultures for 16-24 hours at 37 °C with shaking at 400 rpm.

Before Starting:

- ✓ Centrifuge up to 5 ml of bacterial culture at 3,200–5,000 *x g* for 10 minutes to pellet the cells in the wells of the provided Deep Well Plate[1]. Discard supernatant. Cell pellets can be collected in other vessels and transferred to the Deep Well Plate after resuspension in ZymoPURE™ P1 by pipetting or vortexing. For preparing an overnight culture in a flask, please refer to the Growing Overnight Culture section on page 10 in the appendix for optimal culture conditions.

Plasmid DNA Purification

The following procedure should be performed at room temperature (15-30 °C). Seal unused wells of the Zymo-Spin P-96 Plate with Cover Foil (Cat# C2007).

1. Add 250 µl of cold **ZymoPURE™ P1 (Red)** to the bacterial cell pellet and resuspend completely by pipetting.

2. Add 250 µl of **ZymoPURE™ P2 (Blue)** to each well and immediately mix by gently pipetting 8-10 times. Alternatively, the samples can be mixed by drying the top of the Deep Well Plate with a paper towel, sealing the plate securely[2] with a **96-Well Plate Cover Foil**, and inverting 8-10 times. Do not vortex! Let it sit at room temperature for 3 minutes[3]. Slowly remove the Cover Foil before proceeding to step 3. *Cells are completely lysed when the solution appears clear, purple, homogenous and viscous.*

3. Add 250 µl of **ZymoPURE™ P3 (Yellow)** to each well and mix by gently pipetting 8-10 times. Do not vortex! Alternatively, dry the top of the Deep Well Plate with a paper towel and apply a 96-Well Plate Cover Foil securely[2]. Mix by gentle inversion. Invert the plate an additional 5 times after the sample turns completely yellow. *The sample will turn yellow when the neutralization is complete, and a yellowish precipitate will form.*

   To continue processing the lysate using the recommended vacuum protocol, proceed to the next page. If a vacuum is not available, proceed to page 8 for an alternative centrifugation method.

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[1] The Deep Well Plate allows for a maximum of 1.8 ml of culture to be pelleted at a time per well. Preparing culture inputs larger than 1.8 ml will require multiple pelleting steps.
[2] Securely applying the aluminum seal is important, as a loose seal will results in samples spilling into adjacent wells. To seal the plate securely, scrape the top of the entire plate until a very distinct outline of the wells appears.
[3] Do not allow the lysis reaction to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA.

Vacuum Protocol:

This product is compatible with any commercially available 96-well plate vacuum manifold that can process stacked plates. We recommend our EZ-Vac 96 Vacuum Manifold (Zymo Cat# S7003). See the Vacuum Manifold Diagram on page 11 for more information regarding the set-up of the manifold. If you have any questions regarding the compatibility of your manifold, please contact technical support. The vacuum pump should be a single or double-staged unit capable of producing up to 300 mm Hg pressure at the vacuum manifold.

4. Place a new **96-Well 2.0 mL Well Deep Well Plate** on top of the Manifold Bed. Add the Manifold Collar on top of the Deep Well Plate and place a **ZymoPURE™ Filter Plate** on top of the Manifold Collar.

5. Pierce the aluminum seal on top of the Deep Well Plate from step 3 with a pipette and transfer the entire neutralized lysate, including the precipitates, into the wells of the ZymoPURE™ Filter Plate on the assembled vacuum manifold. Incubate the neutralized lysate at room temperature for 5 minutes.

6. Apply vacuum at 300 mm Hg for 5 minutes to collect the cleared lysate in the Deep Well Plate. Disassemble the manifold and remove the Deep Well Plate. Discard the ZymoPURE™ Filter Plate**.**

7.

≤ 3 mL Culture Input Volume	**> 3 mL Culture Input Volume**
Add 220 µl of **ZymoPURE™ Binding Buffer** to the cleared lysates in each well of the Deep Well Plate from Step 6 and mix thoroughly by pipetting 8-10 times.	Add 190 µl of **ZymoPURE™ Binding Buffer** to the cleared lysates in each well of the Deep Well Plate from Step 6 and mix thoroughly by pipetting 8-10 times.

8. Place a **Wash Plate** on top of the Manifold Base. Add the Manifold Collar on top of the Wash Plate and place a **Zymo-Spin P-96 Plate** on top of the Manifold Collar.

9. Transfer the samples from step 7 to the wells of the Zymo-Spin P-96 Plate on the assembled vacuum manifold. Incubate at room temperature for 2 minutes and then apply vacuum at 300 mm Hg until the liquid in each well has passed completely through.

10. With the vacuum off, add 800 µl of **ZymoPURE™ Wash 1** to each well of the Zymo-Spin P-96 Plate. Apply vacuum at 300 mm Hg until all of the liquid has passed completely through the wells[1].

11. With the vacuum off, add 800 µl of **ZymoPURE™ Wash 2** to each well of the Zymo-Spin P-96 Plate. Apply vacuum at 300 mm Hg until all of the liquid has passed completely through the wells.

12. With the vacuum off, add 200 µl of ZymoPURE™ Wash 2 to each well of the Zymo-Spin P-96 Plate. Apply vacuum at 300 mm Hg until all of the liquid has passed completely through the plate.

[1]The matrix bed inside the wells of the Zymo-Spin P-96 Plate will potentially become a pinkish/purple color after this step depending on the amount of plasmid DNA that was loaded onto the plate. This is normal and not an issue as long as the binding capacity of the Zymo-Spin P-96 Plate is not exceeded.

13. To dry the membrane, apply maximum vacuum (recommended > 600 mm Hg) for 10 minutes. Switch off the vacuum and slowly ventilate the vacuum manifold. Remove the Zymo-Spin P-96 Plate from the vacuum manifold and blot the nozzles of the plate with clean absorbent paper until no more drops come out. Disassemble the manifold and discard the Wash Plate.

14. Place a new **96-Well 2.0 mL Well Deep Well Plate** on top of the Manifold Bed. Add the Manifold Collar on top of the Deep Well Plate and place the Zymo-Spin P-96 Plate from step 13 on top of the Manifold Collar.

15. Add 125 µl of **ZymoPURE™ Elution Buffer**[1,2,3] directly to the center of the column matrix of each well in the Zymo-Spin P-96 Plate and incubate at room temperature for 2 minutes.

16. Apply maximum vacuum pressure (recommended > 600 mm Hg) for 30 seconds. Disassemble the vacuum manifold and remove the Deep Well Plate. Seal the top of the Deep Well Plate with a **96-Well Plate Cover Foil**. Store the eluted plasmid DNA at ≤ -20°C.

[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.
[2] The DNA yield can be increased by pre-warming the **ZymoPURE™ Elution Buffer** to 50 ºC and/or increasing the incubation period up to 10 minutes prior to centrifugation.
[3] If higher concentration is desired, the plasmid DNA can be eluted in as little as 50 µl. Please refer to page 10 in the appendix regarding using less than 125 µl of ZymoPURE™ Elution Buffer.

Centrifugation Protocol:

Perform steps 1-3 as indicated in the general protocol on page 5 and continue with the protocol below using a swinging-bucket centrifuge. Ensure that the buckets on the rotor have sufficient clearance to accommodate the 96-Well 2.0 mL Deep Well Plate and Zymo-Spin P-96 Plate combo (128 mm x 86 mm x 76 mm) before starting the centrifuge.

4. Pierce the aluminum seal on top of the Deep Well Plate from step 3 with a pipette and transfer the entire neutralized lysate, including the precipitates, from step 3 into the wells of the **ZymoPURE™ Filter Plate** on a new **96-Well 2.0 mL Well Deep Well Plate**. Incubate the neutralized lysate at room temperature for 5 minutes.

5. Centrifuge for 3 min at 2,000-4,000 x *g*[1].

6. Add 240 µl of **ZymoPURE™ Binding Buffer** to the cleared lysates in each well of the Deep Well Plate from step 5 and mix thoroughly be pipetting 8-10 times.

7. Place the **Zymo-Spin P-96 Plate** on top of a new Deep Well Plate. Transfer the entire mixture from step 6 into the wells of the Zymo-Spin P-96 Plate. Incubate at room temperature for 2 minutes.

8. Centrifuge for 3 min at 2,000-4,000 x *g*[1]. Discard the flow through from the Deep Well Plate. Dry the top of the Deep Well Plate with a clean absorbent paper.

9. Place the Zymo-Spin P-96 Plate back on top of the same Deep Well Plate. Add 800 µl of **ZymoPURE™ Wash 1** to each well of the Zymo-Spin P-96 Plate. Centrifuge for 3 minutes at 2,000-4,000 x *g*[1]. Discard flow through and dry the top of the Deep Well Plate[2].

10. Place the Zymo-Spin P-96 Plate back on top of the same Deep Well Plate. Add 800 µl of **ZymoPURE™ Wash 2** to each well of the Zymo-Spin P-96 Plate. Centrifuge for 3 minutes at 2,000-4,000 x *g*[1]. Discard flow through and dry the top of the Deep Well Plate.

11. Place the Zymo-Spin P-96 Plate back on top of the same Deep Well Plate. Add 200 µl of ZymoPURE™ Wash 2 to each well of the Zymo-Spin P-96 Plate. Centrifuge for 3 minutes at 2,000-4,000 x *g*[1]. Discard flow through and dry the top of the Deep Well Plate.

12. Place the Zymo-Spin P-96 Plate back on top of the same Deep Well Plate and centrifuge the combo again for 3 minutes at 2,000-4,000 x *g*[1] to remove any residual wash buffer.

13. Place the Zymo-Spin P-96 Plate on top of a new Deep Well Plate. Add 100 µl of **ZymoPURE™ Elution Buffer**[3,4,5] directly to the column matrix of each well in the Zymo-Spin P-96 Plate and incubate at room temperature for 2 minutes. Centrifuge for 3 minutes at 2,000-4,000 x *g*[1]. Seal the top of the Deep Well Plate with a **96-Well Plate Cover Foil**. Store the eluted plasmid DNA at ≤ -20°C.

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[1] It is not necessary to cover the ZymoPURE Filter Plate and Zymo-Spin P-96 plate during centrifugation. If you desire to, please use an Air-Permeable Sealing Cover (Cat # C2011-8) to avoid damaging the plate.
[2] The matrix bed inside the wells of the Zymo-Spin P-96 Plate will potentially become a Pinkish/Purple color after this step depending on the amount of plasmid DNA that was loaded onto the plate. This is normal and not an issue as long as the binding capacity of the Zymo-Spin P-96 Plate is not exceeded.
[3] 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.
[4] The DNA yield can be increased by pre-warming the **ZymoPURE™ Elution Buffer** to 50 ºC and/or increasing the incubation period up to 10 minutes prior to centrifugation.
[5] If higher concentration is desired, the plasmid DNA can be eluted in as little as 50 µl.

Appendices

Gram-Positive Bacteria Protocol

It is possible to isolate plasmid DNA from Gram-Positive species with the ZymoPURE™ 96 Plasmid Miniprep Kit. However, the cell walls of the bacteria must be digested with a lytic enzyme prior to alkaline lysis. The protocol below is for Gram-Positive strains that are sensitive to the lytic enzyme Lysozyme.

Before Starting:

✓ Centrifuge up to 5 ml of bacterial culture at 3,200–5,000 *x g* for 10 minutes to pellet the cells in the wells of the provided Deep Well Plate[1]. Discard supernatant. Cell pellets can be collected in other vessels and transferred to the Deep Well Plate after resuspension by pipetting or vortexing. For preparing an overnight culture in a flask, please refer to the Growing Overnight Culture section on page 10 in the appendix for optimal culture conditions.

Plasmid DNA Purification

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

1. Add 250 µl of **ZymoPURE™ P1 (Red)** containing lysozyme[2] at a final concentration of 1 mg/ml to the bacterial cell pellet and resuspend completely by pipetting. Incubate the resuspended cell pellet at 37 °C for 15-60 minutes[3].

2. Add 250 µl of **ZymoPURE™ P2 (Blue)** to each well and immediately mix by gently pipetting 8-10 times. Alternatively, the samples can be mixed by drying the top of the Deep Well Plate with a paper towel, sealing the plate securely[4] with a **96-Well Plate Cover Foil**, and inverting 4-6 times. Do not vortex! Let it sit at room temperature for 3 minutes[5]. To securely seal the plate, scrape along the top of the entire plate until a very distinct outline of the wells appears. Slowly remove the Cover Foil before proceeding to step 3. *Cells are completely lysed when the solution appears clear, purple, homogenous and viscous.*

3. Add 250 µl of **ZymoPURE™ P3 (Yellow)** to each well. Do not vortex! Dry the top of the Deep Well Plate with a paper towel and apply a 96-Well Plate Cover Foil securely[4]. Mix thoroughly by gentle inversion. Invert the plate an additional 5 times after the sample turns completely yellow. *The sample will turn yellow when the neutralization is complete, and a yellowish precipitate will form.*

   To continue processing the lysate using the recommended vacuum protocol, proceed to the next page. If a vacuum is not available, proceed to page 8 for an alternative centrifugation method.

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[1] The Deep Well Plate allows for a maximum of 1.8 ml of culture to be pelleted at a time per well. Preparing culture inputs larger than 1.8 ml will require multiple pelleting steps.

[2] Lytic enzymes other than lysozyme will require optimization and validation in the **ZymoPURE™ P1** buffer prior to use.

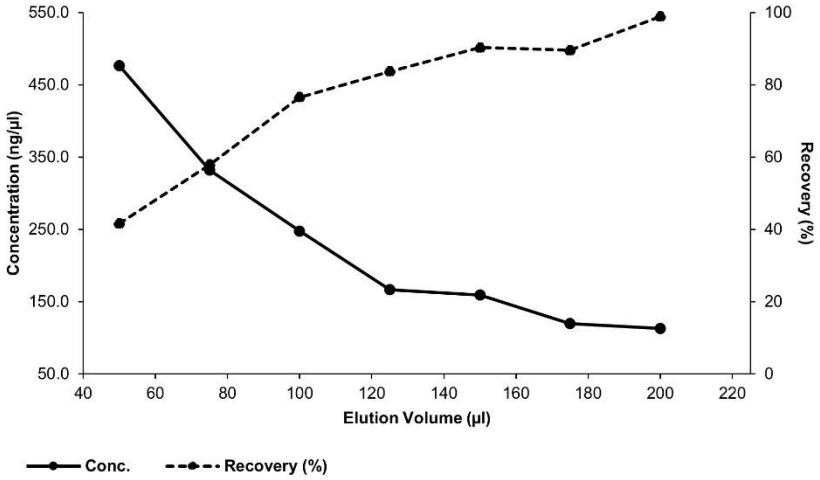
[3] Incubation times will vary depending on the culture volume, cell density, and age of cells. Harvesting the cells at early log phase is recommended for optimal cell wall digestion.

[4] Securely applying the aluminum seal is important, as a loose seal will result in samples spilling into adjacent wells. To seal the plate securely, scrape along the top of the entire plate until a very distinct outline of the wells appears.

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

Elution Parameters

The recommended volume to elute the plasmid DNA is 125 µl. However, it is possible to use less volume for the elution step if a more concentrated sample is desired. See the following figure for correlation between the elution buffer volume and the typical sample concentrations and percent recovery following the standard vacuum protocol.



Elution volume versus DNA concentration and percent recovery. Using the ZymoPURE™ 96 Plasmid Miniprep Kit protocol, 16 µg of pGL3® Plasmid DNA was purified and eluted with the indicated volumes of ZymoPURE™ Elution Buffer. To achieve a combination of high yield and high concentration, the standard protocol recommends 125 µl of ZymoPURE™ Elution Buffer for elution. However, higher yields can be obtained by increasing the elution volume. Alternatively, higher concentrations can be achieved by decreasing the elution volume.

Growing Overnight Culture

The plasmid purification protocol has been optimized for Luria-Bertani (LB) media. Enriched culture medias such as Terrific Broth or Super Broth can result in reduced performance and column clogging depending on the cell density and plasmid DNA copy number. Therefore, it might be necessary to reduce the volume of culture being processed when working with enriched culture media.

For overnight culture volumes greater than 10 ml, we recommend using a starter culture for optimal growth. This is accomplished by inoculating 10 ml or less of LB with the appropriate antibiotic using a colony on a plate or glycerol stock and shaking at 37 °C for 8 hours. After 8 hours, prepare the larger overnight culture by diluting the starter culture 1:500 to 1:1000 with LB containing the appropriate antibiotic.

The size of the culture vessel is also critical for proper aeration of the overnight culture. The optimal culture volume to air volume ratio is 1:5 or less (Example: Use a 250 ml flask for 50 ml of culture). For best aeration, use baffled culture flasks and a vented or gas-permeable seal on the culture vessel and shake at 200 – 300 rpm.

Vacuum Manifold Diagram

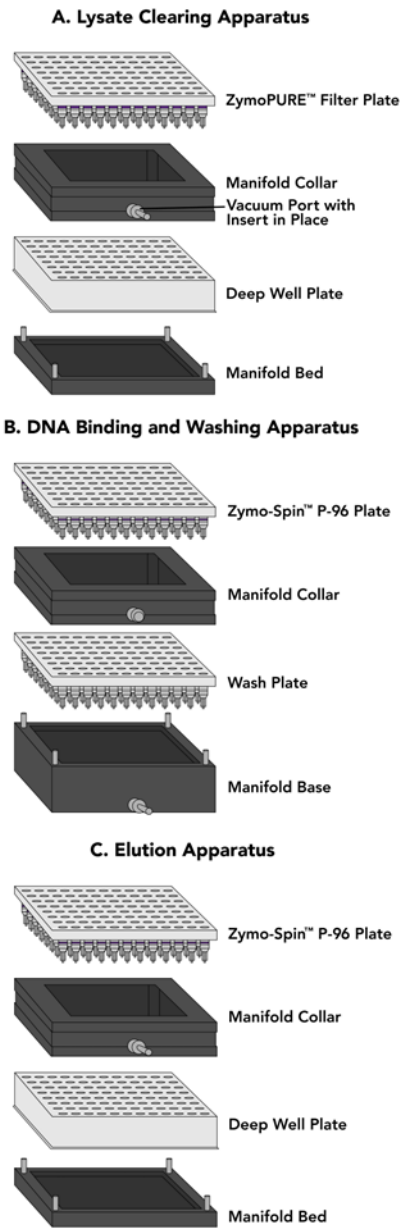


Diagram of the EZ-Vac 96 Vacuum Manifold setup with the ZymoPURE™ 96 Plasmid Miniprep Kit components. Refer to sections A, B, and C for the vacuum manifold and plate assembly necessary for lysate clearing, DNA binding and washing, and elution, respectively.

Troubleshooting

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	**Poor aeration of culture.** The optimal culture volume to air volume ratio is 1:5 or less. For best aeration, use baffled culture flasks and a vented or gas-permeable seal on the culture vessel and shake at 200–300 rpm. **The culture was overgrown, undergrown, contaminated, or antibiotics were omitted from the growth medium.** Use a fresh culture for optimal performance. An OD_{600} of 0.2-0.35 is the optimal optical density of a tenfold dilution of the culture. **Too much culture used.** Lysis and neutralization will be incomplete resulting in poor lysate clarification. 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 *E. coli* 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 centrifugation. Invert the plate an additional 3-4 times after the sample turns yellow following the addition of ZymoPURE™ P3. **ZymoPURE™ P2 and/or ZymoPURE™ Binding Buffer may have precipitated during shipping.** To completely resuspend the buffers, incubate the bottles at 30-37 ºC for 10 minutes and mix by inversion. DO NOT MICROWAVE. **ZymoPURE™ Wash 2:** Ensure that the correct volume of ethanol was added to the ZymoPURE™ Wash 2 prior to use. Also, ensure that the bottle cap is screwed on tightly after each use to prevent evaporation of the ethanol.

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	**Incorrect volume of binding buffer was added:** The ratio of binding buffer to lysate is critical for optimal plasmid DNA binding to the Zymo-Spin P-96 columns. Plasmid DNA yield will be significantly reduced if the incorrect volume of binding buffer was added relative to the volume of overnight culture being processed (i.e., 220 µl of Binding Buffer for ≤ 3 mL of Culture Input Volume and 190 µl of Binding Buffer for > 3 mL of Culture Input Volume). **Incorrect column washing:** Using less wash volume than stated to in the protocol, skipping a wash step, or performing the wash steps out of order can result in reduced plasmid DNA yields. **Incomplete elution:** For large size plasmids (> 10 kb), add ZymoPURE™ Elution Buffer and incubate the column for 5-10 minutes before centrifugation. Also, pre-warm the ZymoPURE™ Elution Buffer to 50 ºC prior to elution. **Low copy-number plasmid:** Increase the overnight culture processing volume up to 5 ml.
Low DNA Quality	**Incomplete neutralization:** Incomplete neutralization generates poor quality supernatant. Ensure that neutralization is complete by inverting the sample an additional 5 times after the sample turns yellow following the addition of ZymoPURE™ P3. **Insufficient centrifugation:** Make sure that all centrifugation steps are performed at the indicated speed and time. If a lower centrifuge speed is used, then extend the centrifugation time to compensate.
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, incomplete lysis or neutralization may contribute to genomic DNA contamination in your eluate. **Overgrown culture.** Overgrown or old cultures may contain more genomic DNA contamination than fresh cultures.

RNA in eluate	**ZymoPURE P1:** Ensure that ZymoPURE™ P1 has been stored at 4°C. RNase A can be purchased separately. **Too much culture used.** Using more than the recommended culture volume or using enriched culture media can cause incomplete lysis and the RNase A being overwhelmed by too many cells. Reduce the volume of culture being processed or switch to LB media. **Incorrect Ratio of Lysate to Binding Buffer:** Mixing too little ZymoPURE Binding Buffer with the lysate will result in degraded RNA also binding to the Zymo-Spin P-96 Plate. Ensure the correct volume of ZymoPURE Binding Buffer is used.
Pinkish/Purple Eluate	**Incorrect Column Washing:** Using less wash volume than stated to in the protocol, skipping a wash step, or performing the wash steps out of order can result in recovering an eluate with a pinkish/purple color. **Exceeded Binding Capacity of Zymo-Spin P-96 Plate:** Using more than the recommended culture volume or using enriched culture media when preparing high-copy number plasmids might result in incomplete washing of the column and recovering an eluate with a pinkish/purple color. Reduce the volume of culture being processed or switch to LB media.
Column Clogs	**Exceeded Binding Capacity of Zymo-Spin P-96 Plate:** Using more than the recommended culture volume or using enriched culture media when preparing high-copy number plasmids can reduce column flow and potentially completely clog the spin-column. Reduce the volume of culture being processed or switch to LB media. **Lysate Debris is loaded onto the Zymo-Spin P-96 Plate:** The lysate recovered from the ZymoPURE™ Filter Plate should be free of debris. If a lot of visible debris is present in the lysate recovered from the ZymoPURE Filter Plate, centrifuge the lysate for 10 minutes at ≥ 2,000 x g and collect the supernatant prior to adding the ZymoPURE™ Binding Buffer to the lysate.

Ordering Information

Product Description	Catalog No.	Size
ZymoPURE™ 96 Plasmid Miniprep Kit	D4214 D4215	2 x 96 4 x 96

Individual Kit Components	Catalog No.	Amount
ZymoPURE™ P1 (Red)	D4200-1-30 D4200-1-100 D4200-1-150 D4200-1-210	30 ml 100ml 150ml 210 ml
ZymoPURE™ P2 (Blue)	D4200-2-30 D4200-2-100 D4200-2-150 D4200-2-210	30 ml 100ml 150 ml 210 ml
ZymoPURE™ P3 (Yellow)	D4200-2-30 D4200-2-100 D4200-3-150 D4200-3-210	30 ml 100ml 150 ml 210 ml
ZymoPURE™ Binding Buffer	D4200-2-30 D4200-2-110 D4200-4-150 D4200-4-210	30 ml 110ml 150 ml 210 ml
ZymoPURE™ Wash 1	D4200-5-80	80 ml
ZymoPURE™ Wash 2 (Concentrate)	D4200-6-23	23 ml
ZymoPURE™ Elution Buffer	D4200-7-12 D4200-7-30	12 ml 30 ml
ZymoPURE™ Filter Plate	C2022	2
Zymo-Spin P-96 Plate	C2023	2
Wash Plate	C2024	2
96-Well 2.0 ml Deep Well Plate	C2015	2
Air Permeable Sealing Cover	C2011-2 C2011-4 C2011-8	2 4 8
96-Well Plate Cover Foil	C2007-2 C2007-4 C2007-6	2 4 6

Complete Your Cloning Workflow

✓ **Transfection-grade plasmid DNA from a miniprep**

ZymoPURE™ Plasmid Miniprep	Size	Catalog No.
ZymoPURE™ Plasmid Miniprep Kit	10 Preps. 50 Preps. 100 Preps. 400 Preps. 800 Preps.	D4208T D4209 D4210 D4211 D4212

✓ **20 Minute Endotoxin-Free Midi & Maxipreps**

ZymoPURE™ II Plasmid Prep Kits	Size	Catalog No.
ZymoPURE™ II Plasmid Midiprep Kit	25 Preps. 50 Preps.	D4200 D4201
ZymoPURE™ II Plasmid Maxiprep Kit	10 Preps. 20 Preps.	D4202 D4203
ZymoPURE™ II Plasmid Gigaprep Kit	5 Preps.	D4204

✓ **Simple 20 second High Efficiency Transformations**

Mix & Go! Competent Cells	Size	Catalog No.
DH5α	10 x 100 µl aliquots 96 x 50 µl aliquots 96 x 50 µl aliquots PCR Plate	T3007 T3009 T3010
JM109	10 x 100 µl aliquots 96 x 50 µl aliquots	T3003 T3005
Zymo10B	10 x 100 µl aliquots 96 x 50 µl aliquots	T3019 T3020
HB101	10 x 100 µl aliquots 96 x 50 µl aliquots	T3011 T3013
TG1	10 x 100 µl aliquots	T3017

✓ **Recover ultra-pure highly concentrated DNA from PCR & other sources**

DNA Clean & Concentrator™	Size	Catalog No.
DNA Clean & Concentrator™-5	50 Preps. 200 Preps.	D4003 D4004
ZR-96 DNA Clean-Up Kit™	2 x 96 Preps. 4 x 96 Preps.	D4017 D4018

✓ **Rapid extraction of ultra-pure DNA from agarose gels**

Zymoclean Gel DNA Recovery™	Size	Catalog No.
Zymoclean™ Gel DNA Recovery Kit	50 Preps. 200 Preps.	D4001 D4002

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