ZYMO RESEARCH

DNA Purification Made Simple™

ZymoPURE™ II Plasmid Midiprep Kit

Rapid purification of endotoxin-free plasmid DNA from up to 50 ml of overnight *E. coli* culture.

Highlights

- Perform plasmid midipreps in only 16 minutes using a simple spin-column protocol.
- Purify up to 1.2 mg of highly concentrated plasmid DNA directly from a spin-column.
- Eluted plasmid DNA is Endotoxin-free and Transfection-Ready.

Catalog Numbers:
D4200, D4201

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

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

Table of Contents

Product Contents

ZymoPURE™ II Plasmid Midiprep Kit	D4200 (25 prep)	D4201 (50 prep)	Storage Temperature
ZymoPURE™ P1[1] (Red)	210 ml	410 ml	4°C
ZymoPURE™ P2[2,3] (Blue)	210 ml	410 ml	Room Temp.
ZymoPURE™ P3 (Yellow)	210 ml	410 ml	Room Temp.
ZymoPURE™ Binding Buffer[3]	210 ml	410 ml	Room Temp.
ZymoPURE™ Wash 1	55 ml (3x)	55 ml (5x)	Room Temp.
ZymoPURE™ Wash 2 (concentrate)[4]	28 ml (2x)	28 ml (4x)	Room Temp.
ZymoPURE™ Elution Buffer	6 ml	12 ml	Room Temp.
Zymo-Spin™ V-PS Column Assemblies[5]	25 pcs	50 pcs	Room Temp.
ZymoPURE™ Syringe Filter-X	25 pcs	50 pcs	Room Temp.
ZymoPURE™ Syringe Plungers	25 pcs	50 pcs	Room Temp.
EndoZero™ II Spin-Columns	25 pcs	50 pcs	Room Temp.
Collection Tubes	25 pcs	50 pcs	Room Temp.
Instruction Manual	1 pc	1 pc	-

[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 °C for 10-20 minutes and mix by inversion. Do not microwave!
[4] ZymoPURE™ Wash 2 included with D4200S is supplied ready-to-use and does not require the addition of ethanol prior to use. ZymoPURE™ Wash 2 included with D4200 and D4201 are supplied as a concentrate and require the addition of ethanol prior to use. See Buffer Preparation (page 5) for instructions.
[5] The Zymo-Spin™ V-PS, 15 ml Reservoir-X and 50 ml Reservoir are pre-assembled as a single unit.

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 using the Standard Protocol. Suitable for transfecting stable, primary, and sensitive cell lines.

      ≤ 0.025 EU/µg of plasmid DNA with optional EndoZero™ II Spin-Column. Suitable for in *vivo* studies.
- **Plasmid DNA Yield** – Up to 1.2 mg per preparation. Actual yield is dependent on the plasmid copy number, culture growth conditions, and strain of *E. coli* utilized. Typical yields from 50 ml of overnight culture grown in LB are 200 – 400 µg for high copy number plasmids and 25 – 50 µg for low copy number plasmids.
- **Plasmid DNA Size** – Up to ~200 kb
- **Recovery Volume** – ≥ 150 µl of ZymoPURE™ Elution Buffer or DNase-free water
- **Processing Time** – ≤ 18 min
- **Required Equipment** – Microcentrifuge and vacuum/vacuum manifold (recommended) or swinging-bucket centrifuge.

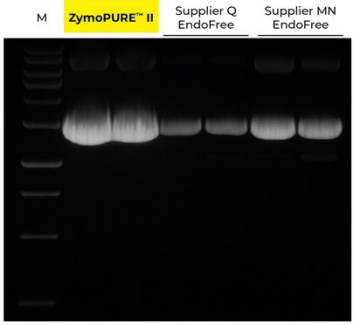
Product Description

The **ZymoPURE™ II Plasmid Midiprep Kit** features a simple spin-column based method for the purification of up to 1.2 mg of transfection grade plasmid DNA in less than 18 minutes. The eluted plasmid DNA is endotoxin-free 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 precipitation, lengthy endotoxin removal incubations, and time-consuming centrifugation steps.

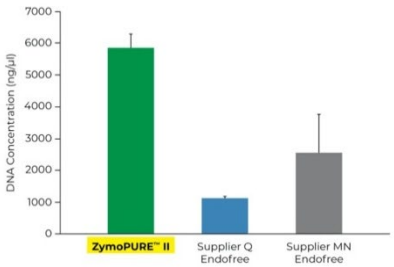
ZymoPURE™ technology uses a modified alkaline lysis method and features our patented binding chemistry and EZ-Flow™ spin-column design, which enables the highest DNA binding capacity and rapid loading of the lysate and wash buffer, resulting in the fastest purification of highly concentrated (up to 6 mg/ml) plasmid DNA directly from a spin-column. Coupling ZymoPURE with the innovative **EndoZero™ II Spin-Columns**, to eliminate residual endotoxins, achieves endotoxin-free plasmid DNA (≤ 0.025 EU/µg of plasmid DNA), making it suitable for transfection, recombinant virus production, lentivirus production, genome editing, *in vivo* studies, sequencing, restriction endonuclease digestion, *in vitro* transcription/translation, PCR, transformation, and other sensitive 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 also included for rapid clearing of the lysate and the unique spin-column design allows the binding step to be performed using a vacuum or centrifuge.

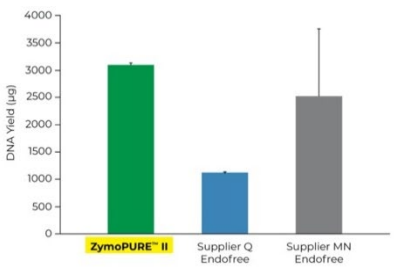
Rapid Purification of Highly Concentrated Endotoxin-Free Plasmid DNA



Up to 6x More Concentrated Plasmid



Up to 3x More Plasmid DNA Yield



Yield and concentration for plasmid DNA isolated using the ZymoPURE™ II Maxiprep kit compared to two endotoxin-free kits from Supplier Q and Supplier MN. 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) µl of eluted plasmid DNA was visualized post agarose gel electrophoresis. M, ZR 1 kb DNA Marker (Zymo Research).

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™ Syringe Filter-X** 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™ V-PS Column** using a vacuum manifold.

The 50 ml Reservoir is removed and the **Zymo-Spin™ V-PS Column** is washed using a vacuum manifold.

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

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

Protocol

Buffer Preparation:

- ✓ Add 107 ml of 95% ethanol to the **28 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!

Before Starting:

- ✓ Centrifuge up to 50 ml of bacteria culture grown in LB at ≥ 3,400 *x g* for 10 minutes to pellet the cells (wet pellet weight of 0.25 – 0.45 g). Discard supernatant. Please refer to the Growing Overnight Culture section in the appendix for optimal culture conditions.

Plasmid DNA Purification

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

1. Add 8 ml of cold **ZymoPURE™ P1 (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.

2. Add 8 ml of **ZymoPURE™ P2 (Blue)** and immediately mix by gently inverting the tube 6 times. Do not vortex! Let sit at room temperature for 2-3 minutes[1]. *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. Do not vortex! Invert the tube 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.*

4. Ensure the plug is attached to the Luer Lock at the bottom of the **ZymoPURE™ Syringe Filter-X.** Place the syringe filter upright in a 50 ml or microcentrifuge tube rack and load the lysate in the ZymoPURE™ Syringe Filter-X[2]. 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-X in one continuous motion until approximately 20 ml of cleared lysate is recovered. Save the cleared lysate! It is critical that approximately 20 ml of lysate is recovered from the syringe filter for the next step. Please refer to page 10 in the appendix regarding the adjustment of the volume of ZymoPURE™ Binding Buffer used in step 6 if the clarified lysate volume is below approximately 20 ml.

6. Add 8 ml of **ZymoPURE™ Binding Buffer** to the cleared lysate from step 5 and mix thoroughly by inverting the tube 8 times[3].

   To continue processing the lysate using the recommended vacuum protocol, proceed to the next page. If a vacuum is not available, proceed to page 7 for an alternative centrifugation method.

[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 **ZymoPURE™ Syringe Filter-X**.
[3] The sample can become hazy/slightly cloudy after this step if a lot of plasmid DNA is present in the lysate.

Vacuum Protocol:

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 manifold[1].

7. Ensure the connections of the **Zymo-Spin™ V-PS Column Assembly** are finger-tight and place onto a vacuum manifold. (If vacuum is not available, see page 7 for the centrifugation protocol.)

8. With the vacuum off, add the entire mixture from step 6 into the Zymo-Spin™ V-PS Column Assembly, and then turn on the vacuum[1] until all of the liquid has passed completely through the column.

9. Remove and discard the **50 ml Reservoir** from the top of the Zymo-Spin™ V-PS Column Assembly.

10. With the vacuum off, add 5 ml of **ZymoPURE™ Wash 1** to the Zymo-Spin™ V-PS Column Assembly. Turn on the vacuum until all of the liquid has passed completely through the column[2].

11. With the vacuum off, add 5 ml of **ZymoPURE™ Wash 2** to the Zymo-Spin™ V-PS Column Assembly. Turn on the vacuum until all of the liquid has passed completely through the column.

12. With the vacuum off, add 5 ml of **ZymoPURE™ Wash 2** to the Zymo-Spin™ V-PS Column Assembly. Turn on the vacuum and keep it on for an additional two minutes after the liquid has passed completely through the column.

13. Remove and discard the **15 ml Reservoir-X** and place the **Zymo-Spin™ V-PS Column** in a **Collection Tube**. Centrifuge at ≥ 16,000 x *g* for 1 minute, in a microcentrifuge, to remove any residual wash buffer.

14. Transfer the column into a clean 1.5 ml microcentrifuge tube and add 200 µl of **ZymoPURE™ Elution Buffer**[3,4,5] directly to the center of the column matrix. Wait 2 minutes, and then centrifuge at ≥ 16,000 x *g* for 1 minute in a microcentrifuge.

15. *Optional:* For removal of residual endotoxins[6,7], place the **EndoZero™ II Spin-Column** in a clean 1.5 ml microcentrifuge tube. Add the entire eluate from step 14 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 ≤ -20°C.

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[1] The lysate and wash buffers might take longer to pass through the column when less than 400 mm Hg is used.
[2] The matrix bed inside the column will potentially become a Pinkish/Purple color after this step depending on the amount of plasmid DNA that was loaded onto the column. This is normal and not an issue as long as the binding capacity of the spin-column 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. For high plasmid DNA loads, more plasmid DNA can generally be recovered from the column by performing a second elution.
[5] For low-copy number plasmids or if higher concentration is desired, the plasmid DNA can be eluted in as little as 150 µl.
[6] This optional step will reduce endotoxin levels from ≤ 1 EU/µg of plasmid DNA to ≤ 0.025 EU/µg of plasmid DNA.
[7] Due to the **EndoZero™ II Spin-Column** chemistry, some plasmid DNA will be lost during this step. The percent of plasmid DNA loss will be dependent on the amount of plasmid DNA that is going through the spin-column. The plasmid DNA loss is generally not significant for moderate and high-copy number plasmids. However, it can be significant for low copy number plasmids or lower yielding preps.

Centrifugation Protocol:

Perform steps 1-6 as indicated in the general protocol on page 5 and continue with the protocol below using a swinging-bucket centrifuge.

7. Remove the **50 ml Reservoir** from the top of the **Zymo-Spin™ V-PS Column Assembly.** Ensure the connection between the **15 ml Reservoir-X** and **Zymo-Spin™ V-PS 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 Reservoir-X/Zymo-Spin™ V-PS Column Assembly**, and centrifuge at 500 *x g* for 2 minutes. Empty the 50 ml conical tube and repeat this step until the entire mixture has passed through the column.

9. Add 5 ml of **ZymoPURE™ Wash 1** to the Zymo-Spin™ V-PS Column Assembly and centrifuge the column at 500 x *g* for 2 minutes[1]. Discard the flow through.

10. Add 5 ml of **ZymoPURE™ Wash 2** to the Zymo-Spin™ V-PS Column Assembly and centrifuge at 500 x *g* for 2 minutes.

11. Add 5 ml of **ZymoPURE™ Wash 2** to the Zymo-Spin™ V-PS Column Assembly and centrifuge at 500 x *g* for 2 minutes.

12. Remove and discard the 15 ml Reservoir-X from the **Zymo-Spin™ V-PS Column.** Place the Zymo-Spin™ V-PS Column in a **Collection Tube** and centrifuge at ≥ 16,000 x *g* for 1 minute, in a microcentrifuge, to remove any residual wash buffer.

13. Transfer the Zymo-Spin™ V-PS Column into a clean 1.5 ml tube and add 200 µl of **ZymoPURE™ Elution Buffer**[2,3,4] directly to the center of the column matrix. Incubate at room temperature for 2 minutes, and then centrifuge at ≥ 16,000 x *g* for 1 minute in a microcentrifuge.

14. *Optional:* For removal of residual endotoxins[5,6], 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 ≤ -20°C.

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[1] The matrix bed inside the column will potentially become a Pinkish/Purple color after this step depending on the amount of plasmid DNA that was loaded onto the column. This is normal and not an issue as long as the binding capacity of the spin-column is not exceeded.

[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 **ZymoPURE™ Elution Buffer** to 50 ºC and/or increasing the incubation period up to 10 minutes prior to centrifugation. For high plasmid DNA loads, more plasmid DNA can generally be recovered from the column by performing a second elution.

[4] For low copy number plasmids or if higher concentration is desired, the plasmid DNA can be eluted in as little as 150 µl.

[5] This optional step will reduce endotoxin levels from ≤ 1 EU/µg of plasmid DNA to ≤ 0.025 EU/µg of plasmid DNA.

[6] Due to the **EndoZero™ II Spin-Column** chemistry, some plasmid DNA will be lost during this step. The percent of plasmid DNA loss will be dependent on the amount of plasmid DNA that is going through the spin-column. The plasmid DNA loss is generally not significant for moderate and high-copy number plasmids. However, it can be significant for low-copy number plasmids or lower yielding preps.

Appendices

Low-Copy Number Plasmid Protocol

When working with low-copy number plasmid DNA, it is possible to increase plasmid DNA yield by processing up to 100 ml of overnight culture grown in LB using the protocol below.

Before Starting:

✓ Centrifuge up to 100 ml of bacterial culture grown in LB at ≥ 3,400 *x g* for 10 minutes to pellet the cells (wet pellet weight of 0.50 – 0.90 g). Discard supernatant.

Plasmid DNA Purification

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

1. Add 8 ml of cold **ZymoPURE™ P1 (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.

2. Add 8 ml of **ZymoPURE™ P2 (Blue)** and immediately mix by gently inverting the tube 8-10 times. Do not vortex! Let sit at room temperature for 5 minutes. *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. Do not vortex! Invert the tube 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.*

4. Centrifuge the neutralized lysate for 10 minutes at ≥ 3,400 *x g* and load the recovered supernatant in the ZymoPURE™ Syringe Filter-X.

5. Remove the Luer Lock plug from the bottom of the **ZymoPURE™ Syringe Filter-X** 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-X in one continuous motion until approximately 20 ml of cleared lysate is recovered. Save the cleared lysate! It is critical that approximately 20 ml of lysate is recovered from the syringe filter for the next step. Please refer to page 10 in the appendix regarding the adjustment of the volume of ZymoPURE™ Binding Buffer used in step 6 if the clarified lysate volume is below approximately 20 ml.

6. Add 8 ml of **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 page 6. If a vacuum is not available, proceed to page 7 for an alternative centrifugation method.

Gram-Positive Bacteria Protocol

It is possible to isolate plasmid DNA from Gram-Positive species with the ZymoPURE™ II Midiprep 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.

Plasmid DNA Purification

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

1. Add 8 ml of cold **ZymoPURE™ P1 (Red)** containing lysozyme[1] at a final concentration of 1 mg/ml to the bacterial cell pellet and resuspend completely by vortexing or pipetting. Incubate the resuspended cell pellet at 37°C for 15-60 minutes[2].

2. Add 8 ml of **ZymoPURE™ P2 (Blue)** and immediately mix by gently inverting the tube 6 times. Do not vortex! Let sit at room temperature for 2-3 minutes[3]. *Cells are completely lysed when the solution appears clear, purple, and viscous.*

3. Add 8 ml of **ZymoPURE™ P3 (Yellow)** and mix thoroughly by inversion. Do not vortex! Invert the tube 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.*

4. Ensure the plug is attached to the Luer Lock at the bottom of the **ZymoPURE™ Syringe Filter-X.** Place the syringe filter upright in a 50 ml or microcentrifuge tube rack and load the lysate in the ZymoPURE™ Syringe Filter-X[4]. 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-X in one continuous motion until approximately 20 ml of cleared lysate is recovered. Save the cleared lysate! It is critical that approximately 20 ml of lysate is recovered from the syringe filter for the next step. Please refer to page 10 in the appendix regarding the adjustment of the volume of ZymoPURE™ Binding Buffer used in step 6 if the clarified lysate volume is below approximately 20 ml.

6. Add 8 ml of **ZymoPURE™ Binding Buffer** to the cleared lysate from step 5 and mix thoroughly by inverting the tube 8 times.

   To continue processing the lysate using the recommended vacuum protocol, proceed to page 6. If a vacuum is not available, proceed to page 7 for an alternative centrifugation method.

[1] Lytic enzymes other than lysozyme will require optimization and validation in the **ZymoPURE™ P1** buffer prior to use.
[2] 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.
[3] Do not allow the lysis reaction to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA.
[4] 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 **ZymoPURE™ Syringe Filter-X.**

Adjusting Volume of Binding Buffer

The ratio of lysate to binding buffer is critical for optimal plasmid DNA binding to the spin-column. Therefore, it is important that approximately 20 ml of cleared lysate is recovered from the ZymoPURE™ Syringe Filter-X during step 5 of the protocol. If the clarified lysate volume is below approximately 20 ml, please adjust the volume of ZymoPURE™ Binding Buffer used in step 6 of the protocol. This can be accomplished by multiplying the volume of recovered lysate by 0.4. Please see the example and table below for reference.

Example: For 16 ml of cleared lysate, you will add 6.4 ml of ZymoPURE™ Binding Buffer to the cleared lysate instead of 8 ml in step 6 of the protocol (16 ml x 0.4 = 6.4 ml).

Optimal Volume of ZymoPURE™ Binding Buffer for Various Volumes of Lysate

Approximate Neutralized Lysate Volume	Volume of ZymoPURE Binding Buffer to Add
20 ml	8.0 ml
19 ml	7.6 ml
18 ml	7.2 ml
17 ml	6.8 ml
16 ml	6.4 ml
15 ml	6.0 ml

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.

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 and the ZymoPURE™ Syringe Filter-X 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 *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 tube 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 30-37ºC 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	**Less than approximately 20 ml of neutralized lysate was used for the binding step.** The ratio of binding buffer to lysate is critical for optimal plasmid DNA binding to the spin-column. Plasmid DNA yield will be reduced if less than approximately 20 ml is recovered from the syringe filter. Please refer to page 10 in the appendix if less then approximately 20 ml of lysate is recovered from the syringe filter. **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 300 ml and use the Low-Copy Number Plasmid Protocol on page 8.
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.

Problem	Possible Causes and Suggested Solutions
RNA in Eluate	**ZymoPURE™ P1:** Ensure that ZymoPURE™ P1 has been stored at 4°C. RNase A can be purchased separately if necessary. **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 spin-column. 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 Spin-Column:** 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 Spin-Column:** 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 Spin-Column:** The lysate recovered from the ZymoPURE™ Syringe Filter-X should be free of debris. Prior to adding the ZymoPURE™ Binding Buffer to the lysate, centrifuge the lysate for 10 minutes at ≥ 3,400 *x g* and collect the supernatant if a lot of visible debris is present in the lysate recovered from the ZymoPURE™ Syringe Filter-X.

Ordering Information

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

Individual Kit Components	Catalog No.	Amount
ZymoPURE™ P1 (Red)	D4200-1-150 D4200-1-210 D4200-1-410	150 ml 210 ml 410 ml
ZymoPURE™ P2 (Blue)	D4200-2-150 D4200-2-210 D4200-2-410	150 ml 210 ml 410 ml
ZymoPURE™ P3 (Yellow)	D4200-3-150 D4200-3-210 D4200-3-410	150 ml 210 ml 410 ml
ZymoPURE™ Binding Buffer	D4200-4-150 D4200-4-210 D4200-4-410	150 ml 210 ml 410 ml
ZymoPURE™ Wash 1	D4200-5-55	55 ml
ZymoPURE™ Wash 2 (Concentrate)	D4200-6-28	28 ml
ZymoPURE™ Elution Buffer	D4200-7-6 D4200-7-12 D4200-7-30	6 ml 12 ml 30 ml
Zymo-Spin™ V-PS Column Assembly w/ 15 ml Reservoir-X and 50 ml Reservoir	C1083-5	5
15 ml Reservoir-X	C1084-25	25
50 ml Reservoir	C1032-25	25
ZymoPURE™ Syringe Filter-X	C1092-5	5
ZymoPURE™ Syringe Plunger	C1037-5	5
EndoZero™ II Spin-Columns	C1060-25	25
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000

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 D4309 D4210 D4211 D4212
ZymoPURE™ 96 Plasmid Miniprep Kit	2 x 96 Preps. 4 x 96 Preps.	D4214 D4215

✓ **18 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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Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

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.

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.

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