

# Produktinformation



Forschungsprodukte & Biochemikalien
Zellkultur & Verbrauchsmaterial
Diagnostik & molekulare Diagnostik
Laborgeräte & Service

Weitere Information auf den folgenden Seiten! See the following pages for more information!



Lieferung & Zahlungsart siehe unsere Liefer- und Versandbedingungen

### Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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### ZymoPURE - Express<sup>™</sup> Plasmid Midiprep Kit

Pellet-free method for purifying transfection-grade plasmid DNA from 25 ml of overnight E. coli culture.

#### **Highlights**

- Direct lysis omits conventional cell-pelleting protocol and resuspension steps.
- · Purify up to 1.2 mg of highly concentrated plasmid DNA directly from a spin-column.
- Prepare transfection-grade plasmid DNA in only 15 minutes.

Catalog Numbers: D4213



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







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### **Product Contents**

ZymoPURE - Express <sup>™</sup> Plasmid Midiprep Kit	<b>D4213</b> (25 prep)	Storage Temperature
ZP Express <sup>™</sup> Lysis Buffer <sup>1,2</sup>	210 ml	Room Temp.
ZP Express <sup>™</sup> Neutralization Buffer	260 ml	4-8° C
ZP Express <sup>™</sup> Binding Buffer <sup>2</sup>	260 ml	Room Temp.
ZymoPURE <sup>™</sup> Wash 1	55 ml (3x)	Room Temp.
ZymoPURE <sup>™</sup> Wash 2 (concentrate) <sup>3</sup>	28 ml (2x)	Room Temp.
ZymoPURE <sup>™</sup> Elution Buffer	12 ml	Room Temp.
RNase A	30 mg	4-8° C (after mixing)
Zymo-Spin <sup>™</sup> V-PS Column Assemblies⁴	25 pcs	Room Temp.
ZymoPURE <sup>™</sup> Syringe Filter-X	25 pcs	Room Temp.
ZymoPURE <sup>™</sup> Syringe Plungers	25 pcs	Room Temp.
Collection Tubes	25 pcs	Room Temp.
Instruction Manual	1	-

<sup>&</sup>lt;sup>1</sup> Caution: ZP Express<sup>™</sup> Lysis Buffer contains NaOH. Please use proper safety precautions. <sup>2</sup> The ZP Express<sup>™</sup> Lysis Buffer and ZP Express<sup>™</sup> 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! <sup>3</sup> ZymoPURE<sup>™</sup> Wash 2 included with D4213 is supplied as a concentrate and requires the addition

of ethanol prior to use. See Buffer Preparation (page 5) for instructions. <sup>4</sup> The Zymo-Spin<sup>™</sup> V-PS, 15 ml Reservoir-X and 50 ml Reservoir are pre-assembled as a single unit.

### **Specifications**

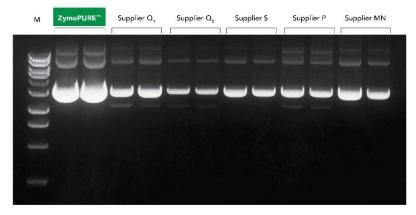
- DNA Purity Eluted DNA is ultra-pure, transfection-grade, and well suited for transfection, transformation, lentivirus production, adenovirus production, AAV production, CRISPR, genome editing, sequencing, restriction endonuclease digestion, *in vitro* transcription/translation, PCR, and other sensitive applications.
  - Typical Abs260/280  $\geq$  1.8 and Abs260/230  $\geq$  2.0
  - ⊙ Endotoxin levels: ≤ 1 EU/µg of plasmid DNA using the Standard Protocol. Suitable for transfecting stable, primary, and sensitive cell lines.
- 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 25 ml of overnight culture grown in LB are 100 – 200 µg for high copy number plasmids and 12 – 25 µg for low copy number plasmids.
- Plasmid DNA Size Up to 200 kb
- Recovery Volume ≥ 150 µl of ZymoPURE<sup>™</sup> Elution Buffer or DNasefree water
- Processing Time 15 min
- **Required Equipment** Microcentrifuge and vacuum/vacuum manifold (recommended) or swinging-bucket centrifuge.

### **Product Description**

The **ZymoPURE – Express**<sup>™</sup> **Plasmid Midiprep kit** utilizes a patented alkaline lysis method for purifying up to 1.2 mg of transfection-grade plasmid DNA directly from *E. coli* culture without conventional cell-pelleting and resuspension steps. The **ZP Express**<sup>™</sup> **Lysis Buffer** is simply added directly to the culture and the plasmid DNA is purified following neutralization and removal of debris.

ZymoPURE<sup>™</sup> technology features our patented binding chemistry and EZ-Flow<sup>™</sup> 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. The wash regimen has been optimized to ensure the plasmid DNA is free of endotoxins, salt, and protein. The result is plasmid DNA ideal for transfection, transformation, lentivirus production, adenovirus production, AAV production, CRISPR, genome editing, sequencing, restriction endonuclease digestion, *in vitro* transcription/translation, PCR, and other sensitive applications.

As an added convenience, the **ZymoPURE** - **Express**<sup>™</sup> **Plasmid Midiprep Kit** contains patented colored buffers that permit error-free visualization and identification of complete bacterial cell lysis. 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.



#### **Superior Plasmid DNA Yield**

Plasmid DNA Yield and concentration from the ZymoPURE- Express<sup>™</sup> Midiprep kit compared to other major suppliers. Plasmid DNA (pGL3®) was isolated from 25 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**



Aliquot 25 ml of overnight bacterial culture.



The solution will turn dark blue and viscous following the addition of **ZP Express™ Lysis Buffer** (blue) indicating bacterial lysis is complete.



The solution will turn yellow and a precipitate will form after adding **ZP Express™ Neutralization Buffer** (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.



**ZP Express<sup>™</sup> 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<sup>™</sup> V-PS Column** is washed using a vacuum manifold.



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

### Protocol

#### **Buffer Preparation:**

- ✓ Resuspend RNase A with 1 ml of water and mix thoroughly by pipetting or vortexing. Add entire mixture into ZP Express<sup>™</sup> Neutralization Buffer before use.
- ✓ Add 107 ml of 95 100<sup>3</sup>% ethanol to the 28 ml ZymoPURE<sup>™</sup> Wash 2 (Concentrate) before use.
- ✓ The ZP Express™ Lysis Buffer and ZP Express™ 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!

#### **Plasmid DNA Purification**

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

A vessel with a minimum volume of 50 ml is required to prepare the bacterial lysate.

- 1. Add 8 ml of **ZP Express<sup>™</sup> Lysis Buffer** <u>directly</u> to 25 ml of bacterial culture<sup>1</sup> in LB Medium<sup>2,3</sup> and <u>immediately mix</u> by gently inverting the tube 6 times<sup>4</sup>. Do not vortex! Let sit at room temperature for 3 minutes<sup>3</sup>. *Cells are completely lysed when the solution appears clear, viscous, and blue.*
- 2. Add 10 ml of **ZP Express<sup>™</sup> Neutralization Buffer** and mix gently but thoroughly by inverting the tube 8-12 times. <u>Do not vortex!</u> The sample will turn yellow when the neutralization is complete, and a yellow precipitate will form.
- Ensure the plug is attached to the Luer Lock at the bottom of the ZymoPURE<sup>™</sup> Syringe Filter-X. Place the syringe filter upright in a 50 ml or microcentrifuge tube rack and load the lysate in the ZymoPURE<sup>™</sup> Syringe Filter-X<sup>5</sup>. Wait 5-8 minutes for the precipitate to float to the top.
- 4. 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<sup>™</sup> Syringe Filter-X in one continuous motion until approximately 35-36 ml of cleared lysate is recovered. <u>Save the cleared lysate!</u> The volume of lysate recovered from the syringe filter is critical for optimal plasmid DNA binding to the spin-column. Please ensure that approximately 35-36 ml of cleared lysate is recovered from the syringe filter and used in step 5 of the protocol.
- 5. Add 10 ml of **ZP Express<sup>™</sup> Binding Buffer** to the cleared lysate from step 4 and mix thoroughly by inverting the capped tube 8 times<sup>6</sup>.

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.

<sup>&</sup>lt;sup>1</sup>Please refer to the Growing Overnight Culture section in the appendix for optimal culture conditions.

<sup>&</sup>lt;sup>2</sup>To process up to 50 ml of culture OR culture grown in medium other than LB, please refer to appendix for an alternate protocol.

<sup>&</sup>lt;sup>3</sup>For best results, allow culture to cool to room temperature (15-30° C) before starting protocol.

<sup>&</sup>lt;sup>4</sup>To minimize the amount of denatured plasmid, add **ZP Express<sup>TM</sup> Lysis Buffer** to the culture rapidly and invert the sample immediately. Do not allow the lysis reaction to proceed for more than 3 minutes.

<sup>&</sup>lt;sup>5</sup>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 into the **ZymoPURE<sup>TM</sup> Syringe Filter-X**.

<sup>&</sup>lt;sup>6</sup> 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<sup>1</sup>.

- 6. Ensure the connections of the **Zymo-Spin**<sup>™</sup> **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.)
- With the vacuum off, add the entire mixture from step 5 into the Zymo-Spin<sup>™</sup> V-PS Column Assembly, and then turn on the vacuum<sup>1</sup> until all of the liquid has passed completely through the column.
- 8. Remove and discard the **50 ml Reservoir** from the top of the Zymo-Spin<sup>™</sup> V-PS Column Assembly.
- 9. <u>With the vacuum off</u>, add 5 ml of **ZymoPURE™ Wash 1** to the Zymo-Spin<sup>™</sup> V-PS Column. Turn on the vacuum until all of the liquid has passed completely through the column.
- 10. <u>With the vacuum off</u>, add 5 ml of **ZymoPURE<sup>™</sup> Wash 2** to the Zymo-Spin<sup>™</sup> V-PS Column. Turn on the vacuum until all of the liquid has passed completely through the column.
- 11. <u>With the vacuum off</u>, add 5 ml of **ZymoPURE<sup>™</sup> Wash 2** to the Zymo-Spin<sup>™</sup> 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.
- 12. Remove and discard the **15 ml Reservoir-X** and place the **Zymo-Spin<sup>™</sup> 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.
- 13. Transfer the column into a clean 1.5 ml microcentrifuge tube and add 200 µl of ZymoPURE<sup>™</sup> Elution Buffer<sup>2,3,4</sup> 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. Store the eluted plasmid DNA at ≤ -20°C.

<sup>&</sup>lt;sup>1</sup>The lysate and wash buffers might take longer to pass through the column when less than 400 mm Hg is used.

<sup>&</sup>lt;sup>2</sup>The **ŻymoPURE™ 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.

<sup>&</sup>lt;sup>3</sup> The DNA yield can be increased by pre-warming the **ZymoPURE**<sup>™</sup> **Elution Buffer** to 50 °C 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.

<sup>&</sup>lt;sup>4</sup> For low-copy number plasmids or if higher concentration is desired, the plasmid DNA can be eluted in as little as 150 µl.

#### **Centrifugation Protocol:**

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

- Remove the 50 ml Reservoir from the top of the Zymo-Spin<sup>™</sup> V-PS Column Assembly. Ensure the connection between the 15 ml Reservoir-X and Zymo-Spin<sup>™</sup> V-PS Column is finger-tight and place the assembly into a 50 ml conical tube.
- Add 10 ml of the mixture from step 5 into the 15 ml Reservoir-X/Zymo-Spin<sup>™</sup> 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.
- 8. Add 5 ml of **ZymoPURE<sup>™</sup> Wash 1** to the Zymo-Spin<sup>™</sup> V-PS Column Assembly and centrifuge the column at 500 x *g* for 2 minutes. Discard the flow through.
- 9. Add 5 ml of **ZymoPURE<sup>™</sup> Wash 2** to the Zymo-Spin<sup>™</sup> V-PS Column Assembly and centrifuge at 500 x *g* for 2 minutes.
- 10. Add 5 ml of **ZymoPURE**<sup>™</sup> **Wash 2** to the Zymo-Spin<sup>™</sup> V-PS Column Assembly and centrifuge at 500 x *g* for 2 minutes.
- Remove and discard the 15 ml Reservoir-X from the Zymo-Spin<sup>™</sup> V-PS Column. Place the Zymo-Spin<sup>™</sup> 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.
- 12. Transfer the Zymo-Spin<sup>™</sup> V-PS Column into a clean 1.5 ml tube and add 200 µl of ZymoPURE<sup>™</sup> Elution Buffer<sup>1,2,3</sup> directly to the column matrix. Incubate at room temperature for 2 minutes, and then centrifuge at ≥ 16,000 x g for 1 minute in a microcentrifuge. Store the eluted plasmid DNA at ≤ -20°C.

<sup>&</sup>lt;sup>1</sup> The **ZymoPURE<sup>™</sup> 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.

<sup>&</sup>lt;sup>2</sup>The DNA yield can be increased by pre-warming the **ZymoPURE<sup>™</sup> Elution Buffer** to 50 °C 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.

<sup>&</sup>lt;sup>3</sup>For low-copy number plasmids or if higher concentration is desired, the plasmid DNA can be eluted in as little as 150 μl.

### Appendices

#### Processing more than 25 ml of culture

It is possible to process up to 50 ml of overnight culture grown in LB with the ZymoPURE - Express<sup>™</sup> Plasmid Midiprep Kit. However, the 50 ml of culture must be pelleted and resuspended in 25 ml of Buffer P1 (sold separately<sup>1</sup>).

- 1. Centrifuge up to 50 ml of bacterial culture in LB medium (or 25 ml of bacterial culture grown in medium other than LB)<sup>2</sup> at  $\geq$  3,400 *x g* for 10 minutes to pellet the cells in a 50 ml conical tube. Discard supernatant.
- 2. Add 25 ml of **Buffer P1**<sup>1</sup> to the bacterial cell pellet and resuspend completely by vortexing or pipetting.
- Add 8 ml of ZP Express<sup>™</sup> Lysis Buffer <u>directly</u> to the resuspended cells and <u>immediately mix</u> by gently inverting the tube 6 times<sup>3</sup>. Do not vortex! Let sit at room temperature for 3 minutes<sup>3</sup>. Cells are completely lysed when the solution appears clear, viscous, and blue.
- 4. Add 10 ml of ZP Express<sup>™</sup> Neutralization Buffer and mix gently but thoroughly by inverting the tube 8-12 times. <u>Do not vortex!</u> The sample will turn yellow when the neutralization is complete, and a yellow precipitate will form.
- 5. Ensure the plug is attached to the Luer Lock at the bottom of the ZymoPURE<sup>™</sup> Syringe Filter-X. Place the syringe filter upright in a 50 ml or microcentrifuge tube rack and load the lysate in the ZymoPURE<sup>™</sup> Syringe Filter-X<sup>4</sup>. Wait 5-8 minutes for the precipitate to float to the top.
- 6. 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<sup>™</sup> Syringe Filter-X in one continuous motion until approximately 35-36 ml of cleared lysate is recovered. <u>Save the cleared lysate!</u> The volume of lysate recovered from the syringe filter is critical for optimal plasmid DNA binding to the spin-column. Please ensure that approximately 35-36 ml of cleared lysate is recovered from the syringe filter and used in step 5 of the protocol.
- 7. Add 10 ml **ZP Express<sup>™</sup> Binding Buffer** to the cleared lysate from step 4 and mix thoroughly by inverting the capped tube 8 times<sup>5</sup>.

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.

<sup>&</sup>lt;sup>1</sup> **Buffer P1** can be purchased through Zymo Research – Catalog # D4027-1-500.

<sup>&</sup>lt;sup>2</sup>Please refer to the Growing Overnight Culture section in the appendix for optimal culture conditions.

<sup>&</sup>lt;sup>3</sup>To minimize the amount of denatured plasmid, add **ZP Express<sup>TM</sup> Lysis Buffer** to the culture rapidly and invert the sample immediately. Do not allow the lysis reaction to proceed for more than 3 minutes.

<sup>&</sup>lt;sup>4</sup> 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 into the **ZymoPURE™ Syringe Filter-X.** 

<sup>&</sup>lt;sup>5</sup> The sample can become hazy/slightly cloudy after this step if a lot of plasmid DNA is present in the lysate.

#### **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
	<b>Poor aeration of culture.</b> 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 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 <sub>600</sub> of 0.2-0.35 is the optimal optical density of a tenfold dilution of the culture. Please refer to the Growing Overnight Culture section in the appendix for optimal culture conditions.
	<b>Too much culture used.</b> Lysis and neutralization will be incomplete and the ZymoPURE <sup>™</sup> Syringe Filter-X may clog during filtration. <u>More culture does not always equal more plasmid.</u> 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.
Low DNA Yield	<b>Incomplete lysis:</b> After addition of ZP Express <sup>™</sup> Lysis Buffer, the solution should change to a clear blue color, indicating complete lysis. Different <i>E. coli</i> strains often require different growth conditions and may vary in their susceptibility to alkaline lysis.
	<b>Incomplete neutralization:</b> The solution should not be viscous following neutralization and the orange 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 ZP Express <sup>™</sup> Neutralization Buffer.
	<b>Temperature of Culture:</b> Allow culture to cool down to room temperature (15-30°C) before processing. Failure to perform this step will lead to incomplete neutralization and clogging of the syringe filter.
	ZP Express <sup>™</sup> Lysis buffer and/or Binding Buffer 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.

Problem	Possible Causes and Suggested Solutions
	Less than approximately 35-36 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 35-36 ml is recovered from the syringe filter.
	<b>ZymoPURE Wash 2:</b> Ensure that the correct volume of ethanol was added to the ZymoPURE <sup>™</sup> Wash 2 prior to use. Also, ensure that the bottle cap is screwed on tightly after each use to prevent evaporation of the ethanol.
Low DNA Yield	<b>Incorrect column washing:</b> 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.
	<b>Incomplete elution:</b> For large size plasmids (> 10 kb), add ZymoPURE <sup>™</sup> Elution Buffer and incubate the column for 5-10 minutes before centrifugation. Also, pre-warm the ZymoPURE <sup>™</sup> Elution Buffer to 50 °C prior to elution.
	<b>Low copy-number plasmid:</b> Increase the overnight culture processing volume up to 50 ml and use the Protocol on page 8.
	<b>Culture was not grown in LB Medium.</b> Only LB Medium is recommended for use with the direct lysis protocol. Other culture media are not recommended for direct lysis but can be used with the pellet-based procedure on page 8 in the Appendix. The composition of LB Medium per liter is as follows: 10 g Tryptone, 5 g Yeast Extract, and 10 g NaCl.
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 ZP Express <sup>™</sup> Neutralization Buffer.
	<b>Insufficient centrifugation:</b> 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.

Problem	Possible Causes and Suggested Solutions
Genomic DNA in eluate	<b>Improper handling:</b> 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.
	<b>Overgrown culture.</b> Overgrown or old cultures may contain more genomic DNA contamination than fresh cultures.
	<b>ZP Express<sup>™</sup> Neutralization Buffer:</b> Ensure that ZP Express <sup>™</sup> Neutralization Buffer has been stored at 4°C. RNase A can be purchased separately if necessary.
RNA in Eluate	<b>Too much culture used.</b> 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 ZP Express <sup>™</sup> Binding Buffer with the lysate will result in degraded RNA also binding to the spin-column. Ensure the correct volume of ZP Express <sup>™</sup> Binding Buffer is used.
	<b>Exceeded Binding Capacity of Spin-Column:</b> 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.
Column Clogs	Lysate Debris is loaded onto the Spin-Column: The lysate recovered from the ZymoPURE <sup>™</sup> Syringe Filter-X should be free of debris. Prior to adding the ZP Express <sup>™</sup> Binding Buffer to the lysate, centrifuge the lysate for 10 minutes at $\geq$ 3,400 <i>x g</i> and collect the supernatant if a lot of visible debris is present in the lysate recovered from the ZymoPURE <sup>™</sup> Syringe Filter-X.

## **Ordering Information**

Product Description	Catalog No.	Size
ZymoPURE - Express <sup>™</sup> Plasmid Midiprep Kit	D4213	25 Preps.
Individual Kit Components	Catalog No.	Amount
ZP Express <sup>™</sup> Lysis Buffer	D4213-1-210	210 ml
ZP Express <sup>™</sup> Neutralization Buffer	D4213-2-260	260 ml
RNase A	E1008-30	30 mg
ZP Express <sup>™</sup> Binding Buffer	D4213-3-260	260 ml
ZymoPURE <sup>™</sup> Wash 1	D4200-5-55	55 ml
ZymoPURE <sup>™</sup> Wash 2 (Concentrate)	D4200-6-28	28 ml
ZymoPURE <sup>™</sup> 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 <sup>™</sup> Syringe Filter-X	C1092-5	5
ZymoPURE <sup>™</sup> Syringe Plunger	C1037-5	5
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000

### **Complete Your Cloning Workflow**

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

ZymoPURE <sup>™</sup> 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 <sup>™</sup> 96 Plasmid Miniprep Kit	2 x 96 Preps. 4 x 96 Preps.	D4214 D4215

#### ✓ 18 Minute Endotoxin-Free Midi & Maxipreps

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

#### ✓ Simple 20 second High Efficiency Transformations

Mix & Go! Competent Cells	Size	Catalog No.
DH5a	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 <sup>™</sup> -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 <sup>™</sup> 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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