Innovation to support science....

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZymoPURE - Express™ Plasmid Midiprep Kit

Catalog No. **D4213** (Patent Pending)

Highlights

- Innovative ZymoPURE - Express™ pellet-free procedure bypasses the standard cell-pelleting and resuspension steps. No centrifugation required!!
- The fastest, easiest, most reliable method for purification of up to 1.2 mg of ultra-pure endotoxin-free plasmid DNA.
- Plasmid DNA is eluted directly from a microcentrifuge column and is well-suited for transfection and other sensitive applications

Contents

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

Product Contents:

ZymoPURE - Express™ Plasmid Midiprep (Kit Size)	D4213 (25 preps.)	Storage Temperature
ZP Express™ Lysis Buffer[1]	210 ml	Room Temp.
ZP Express™ Neutralization Buffer	260 ml	4 – 8 °C
ZP Express™ Binding Buffer	260 ml	Room Temp.
ZymoPURE™ Wash 1	3 x 55 ml	Room Temp.
ZymoPURE™ Wash 2 (Concentrate)	3 x 23 ml	Room Temp.
ZymoPURE™ Elution Buffer	12 ml	Room Temp.
RNase A	30 mg	4 - 8 °C (after mixing)
Zymo-Spin™ V-P Column Assemblies[2]	25	Room Temp.
ZymoPURE™ Syringe Filters	25	Room Temp.
ZymoPURE™ Syringe Plungers	25	Room Temp.
Collection Tubes	25	Room Temp.
Instruction Manual	1	-

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

[1] Caution: ZP Express™ Lysis Buffer contains NaOH. Please use proper safety precautions.

[2] The Zymo-Spin™ V-P, 15 ml Conical Reservoir and 50 ml Reservoir are pre-assembled as a single unit.

Specifications:

- **DNA Purity:** Eluted DNA is ultrapure, endotoxin-free, and well suited for transfection, transformation, sequencing, restriction endonuclease digestion, *in vitro* transcription, and other sensitive applications.
    - Typical $Abs_{260/280} \geq 1.8$ and $Abs_{260/230} \geq 2.0$
- **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)*
- **Plasmid DNA Size:** Up to 25 kb
- **Recommended Sample Volume:** 25 ml *E. Coli* Culture grown in LB Medium
- **Recovery Volume:** ≥ 200 µl of ZymoPURE™ Elution Buffer or DNase free water
- **Required Equipment:** Microcentrifuge and vacuum/vacuum manifold
- **Processing Time:** 15 min

Notes:

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Product Description

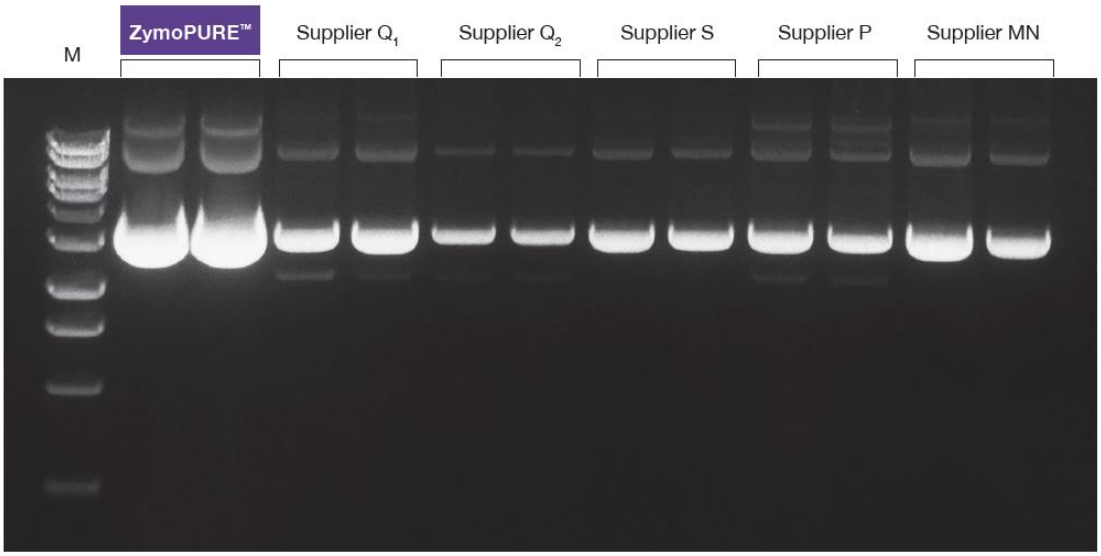
The **ZymoPURE - Express™ Plasmid Midiprep Kit** utilizes a patented alkaline lysis method for purifying up to 1.2 mg of high-quality endotoxin-free plasmid DNA directly from the culture without pelleting and resuspension steps. Just add the **ZP Express™ Lysis Buffer** *directly to your bacterial culture*, neutralize, filter debris, and purify using the patented Zymo-Spin™ V-P microcentrifuge column.

The **ZymoPURE - Express™ Plasmid Midiprep Kit** is the fastest and easiest method for isolating plasmid DNA from *E. coli*. Innovative binding chemistry (patent pending) enables highly concentrated (up to 1.5 µg/µl) DNA to be eluted directly from microcentrifuge 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, restriction endonuclease digestion, bacterial transformation, PCR amplification, DNA sequencing, and other sensitive downstream applications.

As an added convenience, the **ZymoPURE - Express™ Plasmid Midiprep Kit** contains patented colored buffers that permit error-free visualization and identification of complete bacterial cell lysis and neutralization and syringe filters are included for rapid lysate clearing.

No large centrifuge!
No cell pelleting!
No gravity filtration!
No ethanol precipitation!

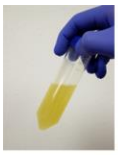
From culture to endotoxin-free plasmid DNA in less than 15 minutes!



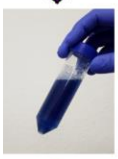
Plasmid DNA yield and concentration from the ZymoPURE – ExpressTM Maxiprep kit compared to other major suppliers. Plasmid DNA (pGL3) was isolated from 25 ml of JM109 E. coli culture grown overnight following the manufacturer's suggested protocol in duplicate. The eluted plasmid DNA was visualized post agarose gel electrophoresis. M, ZR 1 kb DNA Marker (Zymo Research).

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

Procedure Overview:



1) Aliquot 25 ml of bacterial culture.

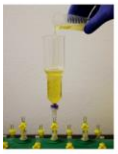


2) The solution will turn dark blue following the direct addition of **ZP Express™ Lysis Buffer**.

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

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

5) **ZP Express™ Binding Buffer** is added to the cleared lysate and mixed thoroughly



6) The mixture is loaded into the **Zymo-Spin™ V-P Column** using a vacuum manifold.

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

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

Buffer Preparation:

- ✓ Resuspend **RNase A** with 1 ml of water and mix thoroughly by pipetting or vortexing. Add entire mixture into **ZP Express™ Neutralization Buffer** before use.
- ✓ Add 88 ml of 95% ethanol to the 23 ml **ZymoPURE™ 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 ºC for 10-20 minutes and mix by inversion. Do not microwave!

Protocol:

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

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.

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

1. Add 8 ml of **ZP Express™ Lysis Buffer** directly to 25 mL of bacterial culture in LB Medium[1, 2] and immediately mix by gently inverting the tube 6 times[3]. Do not vortex! Let sit at room temperature for 3 minutes[4]. *Cells are completely lysed when the solution appears clear, viscous, and blue.*
2. Add 10 ml of **ZP Express™ Neutralization Buffer** and mix gently but thoroughly by inverting the tube 8-12 times. Do not vortex! *The sample will turn yellow when the neutralization is complete and a yellow precipitate will form.*
3. Ensure the plug is attached to the Luer Lock at the bottom of the **ZymoPURE™ Syringe Filter.** Place the syringe filter upright in a tube rack and load the lysate into the ZymoPURE™ Syringe Filter[5] and wait 5-8 minutes for the precipitate to float to the top.
4. Remove the Luer Lock plug from the bottom of the syringe. Place the syringe filter into a clean 50 ml conical tube. Place the plunger in the syringe and slowly push the solution through the ZymoPURE™ Syringe Filter until 35-36 ml of lysate is recovered[6]. Save the cleared lysate!
5. Add 10 ml **ZP Express™ Binding Buffer** to the cleared lysate from step 4 and mix thoroughly by inverting the capped tube 8 times.

Notes:

[1] To process up to 50 ml of culture OR culture grown in medium other than LB, please refer to appendix for an alternate protocol.

[2] For best results, allow culture to cool to room temperature (15-30°C) before starting protocol.

[3] To minimize the amount of denatured plasmid, add **ZP Express™ Lysis Buffer** to culture rapidly and invert the sample immediately.

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

[5] 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**.

[6] For best results, push plunger through **ZymoPURE™ Syringe Filter** in one motion, without releasing downward pressure.

6. Ensure the connections of the **Zymo-Spin™ V-P Column Assembly** are finger-tight and place onto a vacuum manifold.

7. Add the entire mixture from step 5 into the Zymo-Spin™ V-P Column Assembly, and then turn on the vacuum 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™ V-P Column Assembly.

9. With the vacuum off, add 5 ml of **ZymoPURE™ Wash 1** to the **15 ml Conical Reservoir.** Turn on the vacuum until all of the liquid has passed completely through the column.

10. With the vacuum off, add 5 ml of **ZymoPURE™ Wash 2** to the 15 ml Conical Reservoir. Turn on the vacuum until all of the liquid has passed completely through the column. Repeat this wash step.

11. Remove and discard the 15 ml Conical Reservoir and place the **Zymo-Spin™ V-P Column** in a **Collection Tube**. Centrifuge at ≥ 10,000 x *g* for 1 minute, in a microcentrifuge, to remove any residual wash buffer.

12. Transfer the column into a clean 1.5 ml tube and add ≥ 200 µl of **ZymoPURE™ Elution Buffer**[1, 2] directly to the column matrix. Incubate at room temperature for 2 minutes, and then centrifuge at ≥ 10,000 x *g* for 1 minute in a microcentrifuge. Store the eluted plasmid DNA at ≤ -20°C.

Notes:

[1] The **ZymoPURE™ Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.

[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 5 minutes prior to centrifugation.

Appendix:

To process > 25 ml of culture:

1. Centrifuge up to 50 ml of bacterial culture in LB Medium (or 25 ml of bacterial culture grown in medium other than LB) at ≥ 3,400 x g for 10 minutes to pellet the cells in a 50 ml conical tube. Discard the supernatant.

2. Add 25 mL of Buffer P1 (sold separately[3]) to the bacterial cell pellet and resuspend completely by vortexing or pipetting.

3. Begin with the protocol on page 4.

[3] **ZR Plasmid Miniprep – Classic**
Buffer P1 (Red)
(Catalog Number: D4027-1)

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	
Culture growth conditions	• **Poor aeration of culture.** The optimal culture volume to air volume ratio is 1:5 or less. For best aeration, use baffled culture flasks, or a vented or gas-permeable seal on the culture vessel. • **The culture was overgrown, undergrown or contaminated.** Use a fresh culture for optimal performance. (An A_{600} of 0.2-0.35 is the optimal optical density of a tenfold dilution of the culture.) • **Antibiotics were omitted from the growth medium** • **Culture was not grown in LB Medium.** Only LB Medium is recommended for the standard protocol. Other culture media are not recommended for direct lysis but can be processed by following the procedure within the appendix. The composition of LB Medium per liter is as follows: 10 g Tryptone, 5 g Yeast Extract, and 10 g NaCl.
Cell density is too high	• **Too much culture used.** Lysis and neutralization will be incomplete and the ZymoPURE™ Syringe Filter may clog during filtration. More culture does not always equal more plasmid. Incomplete lysis and neutralization are two of the most common causes of failed plasmid preps and both are caused by too much culture being used. For optimal performance, no more than 1.5 x 10^{11} bacterial cells should be used (A_{600} of 1.0 = 8 x 10^8 cells/ml) • **Incomplete lysis:** After addition of ZP Express™ Lysis Buffer, the solution should change to a clear blue color, 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 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 Express™ Neutralization Buffer.
Temperature of culture	• **Culture needs to be at room temperature.** Allow culture to cool down to room temperature (15-30°C) before processing. Failure to perform this step will lead to incomplete neutralization and a clogging of the syringe filter.
Wash buffer	• **Ensure that ethanol has been added** to the ZymoPURE™ Wash 2. • **Ensure that the bottle cap is screwed on tightly** after each use to prevent evaporation of the ethanol.
DNA elution	• **Incomplete elution:** For large size plasmids (> 10 kb), add ZymoPURE™ Elution Buffer and incubate the column for 5-10 minutes before centrifugation. Also, pre-warm the ZymoPURE™ Elution Buffer to 50 ºC prior to elution.
Inefficient recovery of cleared lysate	• **Less than 35 ml of cleared lysate recovered from syringe filter.** At least 35 ml of cleared lysate should be recovered during filtration step in order to have appropriate binding conditions. Failure to obtain this amount of cleared lysate will result in significantly diminished yield. • **Syringe Filter Clogged.** If the culture used is above room temperature, there is a higher likelihood of the syringe filter clogging and/or breaking during filtration. To avoid this, allow samples to cool after being removed from an incubator.
ZP Express Lysis Buffer and/or ZP Express Binding Buffer precipitated	• **Both buffers 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.

Low DNA Quality

DNA does not perform well	• **Incomplete neutralization** Incomplete neutralization generates poor quality supernatant. Ensure that neutralization is complete by inverting the sample 9-12 times after the addition of ZP Express™ Neutralization Buffer. • **Ethanol contamination in eluate.** Centrifuge the Zymo-Spin™ V-P column matrix to dryness as indicated in the protocol prior to adding the ZymoPURE™ Elution Buffer.
Genomic DNA in eluate	• **Improper handling** (Sample was vortexed or handled too roughly). Excessive genomic DNA contamination is usually caused by excessive mechanical shearing during the lysis and neutralization steps. Also, prolonged lysis or incomplete mixing of lysis or neutralization buffers may contribute to genomic DNA contamination in your sample. • **Too much cleared lysate recovered.** Genomic DNA resides within the flocculent of the lysate. In order to avoid excessive amounts of genomic DNA within the eluate, recover between 35-37 mL of cleared lysate from the syringe filter.
Denatured Plasmid DNA in eluate	• **Incomplete Mixing** Denatured plasmid can result from samples not being mixed properly. To avoid this, add ZP Express™ Lysis Buffer to the culture rapidly to promote mixing and be sure to invert each sample **immediately**. Prolonged incubation before mixing leads to increased amounts of denatured plasmid. • **Extended Incubation** Prolonging lysis beyond 3 minutes can lead to increased levels of denatured plasmid in your sample.
Flocculent in cleared lysate	• **Improper handling** For best results with the ZymoPURE Syringe Filter, push the plunger through the syringe slowly but firmly. Plunging the lysate through the syringe too forcefully can result in debris passing through the filter into the cleared lysate. The cleared lysate should not have any flocculent present.

Ordering Information

Product Description	Kit Size	Catalog No.
ZymoPURE - Express™ Plasmid Midiprep Kit	25 preps.	D4213

For Individual Sale	Amount	Catalog No.
ZP Express™ Lysis Buffer	210 ml	D4213-1-210
ZP Express™ Neutralization Buffer	260 ml	D4213-2-260
RNase A	30 mg	E1008-30
ZP Express™ Binding Buffer	260 ml	D4213-3-260
ZymoPURE™ Wash 1	20 ml 55 ml 410 ml	D4200-5-20 D4200-5-55 D4200-5-410
ZymoPURE™ Wash 2 (Concentrate)	10 ml 23 ml	D4200-6-10 D4200-6-23
ZymoPURE™ Elution Buffer	6 ml 12 ml 30 ml	D4200-7-6 D4200-7-12 D4200-7-30
Zymo-Spin™ V-P Column Assembly w/ 15 ml Conical and 50 ml Reservoir	5	C1040-5
15 ml Conical Reservoir	25	C1031-25
50 ml Reservoir	25	C1032-25
ZymoPURE™ Syringe Filter and Plunger Set	5	C1036-5
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000

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