

# ZymoPURE™ Plasmid Midiprep

Catalog Nos. D4200 & D4201



## Quick Protocol

- ✓ Buffer Preparation: Add 38 ml of 95% ethanol to the **10 ml ZymoPURE™ Wash 2 (Concentrate)** (D4200), or add 88 ml of 95% ethanol to the **23 ml ZymoPURE™ Wash 2 (Concentrate)** (D4201) before use.
- ✓ The **ZymoPURE™ P2** and **ZymoPURE™ Binding Buffer** may have precipitated. If this occurs, dissolve the precipitate by incubating the bottles at 30-37 °C for 10-20 minutes and mix by inversion. Do not microwave!
- ✓ Before Starting: Centrifuge up to 50 ml of bacterial culture at  $\geq 3,400 \times g$  for 10 minutes to pellet the cells in a 50 ml conical tube. Discard supernatant.

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

1. Add 8 ml of **ZymoPURE™ P1 (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.
2. Add 8 ml of **ZymoPURE™ P2 (Green)** and immediately mix by gently inverting the tube 6 times. Do not vortex! Let sit at room temperature for 2-3 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! *The sample will turn yellow when the neutralization is complete and a yellowish precipitate will form.*
4. Ensure the plug is attached to the Luer Lock at the bottom of the **ZymoPURE™ Syringe Filter**. Place the syringe filter upright in a tube rack and load the lysate into the ZymoPURE™ Syringe Filter and wait 5-8 minutes for the precipitate to float to the top.
5. Remove the Luer Lock plug from the bottom of the syringe and place it into a clean 50 ml conical tube. Place the plunger in the syringe and push the solution through the ZymoPURE™ Syringe Filter to clear the debris. Save the cleared lysate!
6. Add 8 ml **ZymoPURE™ Binding Buffer** to the cleared lysate from step 5 and mix thoroughly by inverting the capped tube 8 times.

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ZYMO RESEARCH

*The Beauty of Science is to Make Things Simple*

## Vacuum Protocol

*The vacuum pump should be a single or double-staged unit capable of producing up to 400 mm Hg pressure.*

7. Ensure the connections of the **Zymo-Spin™ III-P Column Assembly** are finger-tight and place onto a vacuum manifold.
8. Add the entire mixture from step 6 into the Zymo-Spin™ III-P Column Assembly, and then turn on the vacuum until all of the liquid has passed completely through the column.
9. Unscrew the purple Luer Lock cap from the top of the **Zymo-Spin™ III-P Column** and discard the **Reservoirs**.

## Centrifugation Protocol

*A swinging bucket rotor is required for centrifugation.*

7. Remove the **50 ml Reservoir** from the top of the **Zymo-Spin™ III-P Column Assembly**. Ensure the connection between the **15 ml Conical Reservoir** and **Zymo-Spin™ III-P column** is finger-tight and place the assembly into a 50 ml conical tube.
8. Add 10 ml of the mixture from step 6 into the **15 ml Conical Reservoir/Zymo-Spin™ III-P Column** assembly, and centrifuge at 500 x g for 2 minutes.
9. Empty the 50 ml conical tube and repeat step 9 until the entire sample has passed through the column.

**Note:** Steps 10-11 can also be completed using a microcentrifuge instead of the vacuum manifold (see full instruction manual).

10. With the vacuum off, add 800 µl of **ZymoPURE™ Wash 1** to the Zymo-Spin™ III-P Column. Turn on the vacuum until all of the liquid has passed completely through the column.
11. With the vacuum off, add 800 µl of **ZymoPURE™ Wash 2** to the Zymo-Spin™ III-P Column. Turn on the vacuum until all of the liquid has passed completely through the column. Repeat this wash step.
12. Place the Zymo-Spin™ III-P Column in a **Collection Tube** and transfer to a microcentrifuge. Centrifuge at  $\geq 10,000 \times g$  for 1 minute in order to remove any residual wash buffer.
13. Transfer the Zymo-Spin™ III-P Column into a clean 1.5 ml tube and add 200 µl of **ZymoPURE™ Elution Buffer** directly to the column matrix. Incubate at room temperature for 2 minutes, and then centrifuge at  $\geq 10,000 \times g$  for 1 minute in a microcentrifuge.