EMORY UNIVERSITY | Emory Integrated Genomics Core

EIGC.015_Maxiprep_ZymoPURE II Plasmid Maxiprep Kit

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP Review

Standard Operating Procedure Approval Page:

Date Implemented: 29 May 2019

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

Product Description

The **ZymoPURE™ II Plasmid Maxiprep 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 20 minutes. The eluted plasmid DNA is EndoZero 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 precipitations, lengthy endotoxin removal incubations, and time-consuming centrifugation steps.

ZymoPURE™ technology uses a modified alkaline lysis method and features novel binding chemistry, which enables the highest yields and concentration of plasmid DNA (up to 3 µg/µl) directly from a spin-column. Coupling ZymoPURE with the innovative **EndoZero™ Spin-Columns**, to eliminate endotoxins, achieves EndoZero plasmid DNA (≤ 0.025 EU/µg of plasmid DNA), making it suitable for transfection, restriction endonuclease digestion, in vivo studies, bacterial transformation, PCR amplification, DNA sequencing, and other sensitive downstream applications.

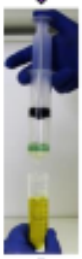
As an added convenience, the **ZymoPURE™ II Plasmid Maxiprep Kit** contains colored buffers that permit error-free visualization and identification of complete bacterial cell lysis and neutralization. Syringe filters are included for rapid clearing of the lysate and the unique spin-column design allows the binding step to be performed using a vacuum or table top centrifuge.

Procedure Overview:

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

The solution will turn dark purple and viscous following the addition of **ZymoPURE™ P2** (green) 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** 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-P Column** using a vacuum manifold.

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

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

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

Buffer Preparation:

- Add 88 ml of 95% ethanol to the 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!

Before Starting:

- Centrifuge up to 150 ml of bacterial culture at ≥ 3,400 x g for 10 minutes to pellet the cells. Discard supernatant.

Protocol:

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

1. Add 14 ml of ZymoPURE™ P1 (Red) to the bacterial cell pellet and resuspend completely by vortexing or pipetting.

2. Add 14 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 14 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 in one continuous motion until approximately 33-35 ml of cleared lysate is recovered. Save the cleared lysate!

6. Add 14 ml ZymoPURE™ Binding Buffer to the cleared lysate from step 5 and mix thoroughly by inverting the capped tube 10 times.

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

Vacuum Protocol: ***(Recommended)***

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.

7. Ensure the connections of the Zymo-Spin™ V-P Column Assembly are finger-tight and place onto a vacuum manifold. (If vacuum is not available, see page 6 for the centrifugation protocol.)

8. With the vacuum off, add the entire mixture from step 6 into the Zymo-Spin™ V-P Column Assembly, and then turn on the vacuum1 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-P Column Assembly.

10. 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.

11. 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.

12. 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.

13. Transfer the column into a clean 1.5 ml microcentrifuge tube and add 400 µl of ZymoPURE™ Elution Buffer directly to the column matrix. Wait 2 minutes, and then centrifuge at ≥ 10,000 x g for 1 minute in a microcentrifuge.

14. Optional: For EndoZero Plasmid DNA5 , remove the Luer Lock cap from the EndoZero™ Spin-Column and place the column in a clean 1.5 ml microcentrifuge tube. Add the entire eluate from step 13 into the EndoZero™ 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

Centrifugation Protocol: ***(Alternative)***

Perform steps 1-6 as indicated in the general protocol, see page 4.

7. Remove the 50 ml Reservoir from the top of the Zymo-Spin™ V-P Column Assembly. Ensure the connection between the 15 ml Conical Reservoir and Zymo- Spin™ V-P column is finger-tight and place the assembly into a 50 ml conical tube.

8. Add 14 ml of the mixture from step 6 into the 15 ml Conical Reservoir/ZymoSpin™ V-P column assembly, and then centrifuge the column at 500 x g for 2 minutes. Empty the 50 ml conical tube and repeat this step until the entire sample has passed through the column.

9. Add 5 ml of ZymoPURE™ Wash 1 to the Zymo-Spin™ V-P column assembly and centrifuge the column at 500 x g for 2 minutes.

10. Add 5 ml of ZymoPURE™ Wash 2 to the Zymo-Spin™ V-P column assembly and centrifuge the column for 2 minutes at 500 x g. Repeat the wash step.

11. Remove and discard the 15 ml Conical Reservoir and place the Zymo-Spin™ V-P Column into a Collection Tube. Centrifuge the column 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 microcentrifuge tube and add 400 µl of ZymoPURE™ Elution Buffer1,2,3 directly to the column matrix. Wait 2 minutes, and then centrifuge at ≥ 10,000 x g for 1 minute in a microcentrifuge.

13. Optional: For EndoZero Plasmid DNA4 , remove the Luer Lock cap from the EndoZero™ Spin-Column and place the column in a clean 1.5 ml tube. Add the entire eluate from step 12 into the EndoZero™ 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.