



## PLASMID MINIPREP (ZYMO, D4209)

### MATERIALS

- ZymoPure P1
- ZymoPURE P2
- ZymoPURE P3
- ZymoPURE Binding Buffer
- ZymoPURE Wash 1
- ZymoPURE Wash 2
- ZymoPURE Elution Buffer
- Zymo-Spin II-P Columns
- Collection Tubes

### EQUIPMENT

- Microcentrifuge

### PROTOCOL

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

1. Centrifuge 0.5-5 ml of bacterial culture in a clear 1.5 ml tube at full speed for 15- 20 seconds in a microcentrifuge. Discard supernatant.
2. Add 250 µl of ZymoPURE™ P1 (Red) to the bacterial cell pellet and resuspend completely by vortexing or pipetting.
3. Add 250 µl of ZymoPURE™ P2 (Green) and immediately mix by gently inverting the tube 6-8 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.
4. Add 250 µl of ice cold ZymoPURE™ P3 (Yellow) and mix thoroughly by inversion. Do not vortex! Invert the tube an additional 3-4 times after the sample turns completely yellow. The sample will turn yellow when the neutralization is complete and a yellowish precipitate will form.
5. Incubate the neutralized lysate on ice for 5 minutes.
6. Centrifuge the neutralized lysate for 5 minutes at 16,000 x g.
7. Transfer 600 µl of supernatant from step 6 into a clean 1.5 ml microcentrifuge tube. Be careful not to disturb the yellow pellet and avoid transferring any cellular debris to the new tube.
8. Add 275 µl of ZymoPURE™ Binding Buffer to the cleared lysate from step 7 and mix thoroughly by inverting the capped tube 8 times.
9. Place a Zymo-Spin™ II-P Column in a Collection Tube and transfer the entire mixture from step 8 into the Zymo-Spin™ II-P Column.

10. Incubate the Zymo-Spin™ II-P/Collection Tube assembly at room temperature for 2 minutes and then centrifuge at 5,000 x g for 1 min. Discard the flow through1 .
11. Add 800 µl of ZymoPURE™ Wash 1 to the Zymo-Spin™ II-P Column and centrifuge at 5,000 x g for 1 min. Discard the flow through.
12. Add 800 µl of ZymoPURE™ Wash 2 to the Zymo-Spin™ II-P Column and centrifuge at 5,000 x g for 1 min. Discard the flow through.
13. Add 200 µl of ZymoPURE™ Wash 2 to the Zymo-Spin™ II-P Column and centrifuge at 5,000 x g for 1 min. Discard the flow through.
14. Centrifuge the Zymo-Spin™ II-P Column at  $\geq 10,000 \times g$  for 1 minute in order to remove any residual wash buffer.
15. Transfer the Zymo-Spin™ II-P Column into a clean 1.5 ml tube and add 25 µl of ZymoPURE™ Elution Buffer2,3 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. Store the eluted plasmid DNA at  $\leq -20^{\circ}\text{C}$ .

References:

[https://files.zymoresearch.com/protocols/\\_d4208t\\_d4209\\_d4210\\_d4211\\_d4212\\_zymopure\\_plasmid\\_miniprep.pdf](https://files.zymoresearch.com/protocols/_d4208t_d4209_d4210_d4211_d4212_zymopure_plasmid_miniprep.pdf)