ZymoPURE™ Plasmid Miniprep Kit from Zymo Research

- 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.
- Add 250 µL of **ZymoPURE™ P1 (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.
- 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.
- 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.
- Incubate the neutralized lysate on ice for 5 minutes.
- Centrifuge the neutralized lysate for 5 minutes at 16,000 x *g*.
- Transfer 600 µl of supernatant from the step above 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.
- Add 275 µl of **ZymoPURE™ Binding Buffer** to the cleared lysate and mix thoroughly by inverting the capped tube 8 times.
- Place a **Zymo-Spin™ II-P Column** in a Collection Tube and transfer the entire mixture into the **Zymo-Spin™ II-P Column**.
- 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 through.
- 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.

- 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.
- 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.
- Centrifuge the **Zymo-Spin™ II-P Column** at ≥ 10,000 x *g* for 1 minute in order to remove any residual wash buffer
- Transfer the **Zymo-Spin™ II-P Column** into a clean 1.5 mL tube and add 25 µL of **ZymoPURE™ Elution Buffer** 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.

From: Zymo Research