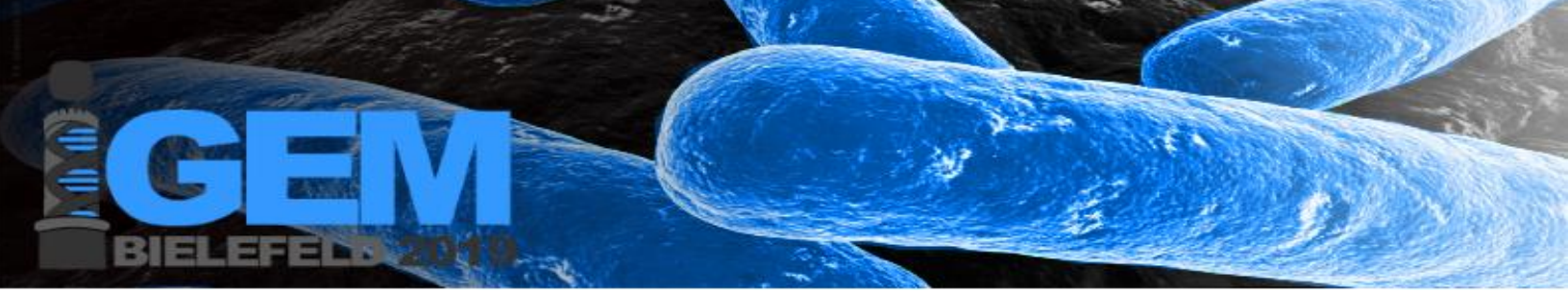

ZymoPURE™ Plasmid Miniprep Kit

Catalog Nos. D4208, D4209, D4210, D4211 & D4212

Centrifugation Protocol

Before Starting: Incubate **ZymoPURE™ P3** on ice for 30 minutes before use.

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 3 minutes.

4. Add 250 µl of **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.

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 the supernatant from step 6 into a clean 1.5 ml microcentrifuge tube

8. Add 275 µl of **ZymoPURE™ Binding Buffer** to the cleared lysate from step 77 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 column/Collection Tube** assembly at room temperature for 2 minutes and then centrifuge at *5,000 x g* for 1 minute. Discard the flow through.

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.
    Repeat this wash step with 200 µl of ZymoPURE™ Wash 2.

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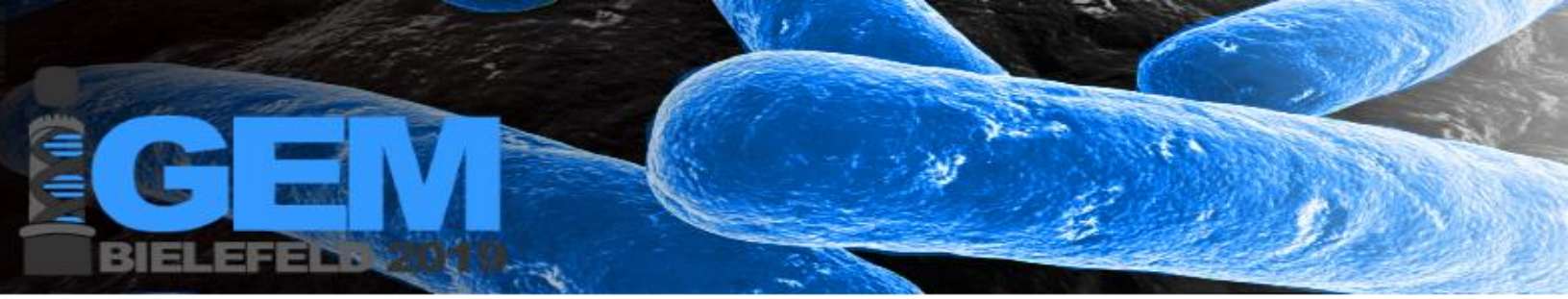

12. Add 800 µl of **ZymoPURE™ Wash 2** to the Zymo-Spin™ II-P column and centrifuge at *5,000 x g* for 1 min. Discard the flow through. Repeat this wash step with 200 µl of ZymoPURE™ Wash 2.

13. Place the Zymo-Spin™ II-P Column in a Collection Tube and transfer to a microcentrifuge. Centrifuge at ≥ *10,000 x g* for 1 minute in order to remove any residual wash buffer.

14. 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.

From: iGEM Bielefeld-CeBiTec

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