SPINeasy 96-Well Plasmid Miniprep Kit

Cat. No.: 116534096 (96 PREPS) or Cat. No.: 116534496 (4 x 96 PREPS)

Revision 1.0 Jan 2024

Scan QR code for more information from instruction manual

This protocol is designed for the purification of plasmid DNA from bacteria host.

Notes before starting:

- Briefly spin down the vial of **RNase A** and add the entire solution to **Resuspension Buffer SN1**. Mark the bottle and store at 2 – 8 ° C.
  Optional: Vortex the vial of Indicator Solution to mix well before use. Add the entire solution to Resuspension Buffer SN1.
- Add 18 mL (for 96 PREPS kit) or 36 mL (for 4 x 96 PREPS kit) of Isopropanol (> 99.5%) to each bottle of **Wash Buffer SN1** stock before use. Mark the bottle and store at room temperature.
- Add 80 mL (for 96 PREPS kit) or 160 mL (for 4 x 96 PREPS kit) of Absolute ethanol (96-100%) to each bottle of **Wash Buffer SN2** stock before use. Mark the bottle and store at room temperature.
- The centrifugation speed stated in the manual is a guideline. Use the maximum speed available if the recommended setting is not feasible.

Quick-Start Centrifugation Protocol

Refer to Instruction Manual for details on the germiculture of bacteria.

Bacteria Harvest

1. (A) Seal the 96-well culture plate with sealing film. Centrifuge for **10 min @ 3,000 *g*.**
   (B) Alternatively, pellet the culture in microcentrifuge tube by centrifugation for **3 min @ 10,000 *g*.**
2. Discard supernatant. Tap plate or tube on paper towel a few times to remove as much supernatant as possible.

Lysis

3. Resuspend each pellet in **250 µL Resuspension Buffer SN1**.
4. Add **250 µL Alkaline Lysis Buffer SN2** to the resuspended bacterial cells.
   (A) If using 96-well culture plate, seal plate with sealing film and mix well by inverting several times. Perform a short spin to bring liquid to the bottom of the plate.
   (B) If using microcentrifuge tube, mix well by inverting several times.
5. Incubate for **2 min at room temperature**. **Do not exceed 5 min of lysis time**.
   Note: *If Indicator Solution was added to the Resuspension Buffer SN1, the mixture will turn purple after the addition of Alkaline Lysis Buffer SN2.*

Neutralization

6. (A) If using 96-well culture plate, remove the seal of the plate, add **350 µL Neutralization Buffer SN3** per well, seal the plate again and mix well by inverting several times.
   (B) If using microcentrifuge tube, add **350 µL Neutralization Buffer SN3** per tube and mix well by inverting several times.
   Note: *If Indicator Solution was added to the Resuspension Buffer SN1, the lysate will turn from purple to yellow upon complete neutralization.*

Bind

7. Centrifuge to pellet the lysate debris.
   (A) If using 96-well culture plate, centrifuge for **10 min @ ≥ 3,000 *g***.
   (B) If using microcentrifuge tube, centrifuge for **5-10 min @ 15,000 *g***.
8. Place the **96 DNA Plate G** on a 96-well waste collection plate (not provided).
9. Transfer the **clear supernatant (~750 µL)** into each well of the 96 DNA Plate G. Centrifuge for **2 min @ 3,000 *g***. Discard the flow through and re-use the waste collection plate.

Wash

10. Add **500 µL Wash Buffer SN1** into each well of the 96 DNA Plate G. Centrifuge for **2 min @ 3,000 *g***. Discard the flow through and re-use the waste collection plate.
11. Add **750 µL Wash Buffer SN2** into each well of the 96 DNA Plate G. Centrifuge for **2 min @ 3,000 *g***. Discard the flow-through and re-use the waste collection plate.
12. Centrifuge for another **10 min @ 3,000 - 4000 *g*** to remove any residual wash buffer from the wells. Tap the plate on clean paper towel to dry the plate as much as possible.

Quick-Start Centrifugation Protocol - continued

Elute

13. Transfer the 96 DNA Plate G onto a clean 96-well collection plate (not provided).
14. Add **120 µL Elution Buffer SN** directly onto the membrane of each well of the plate. Incubate for **5 min @ room temperature**.
15. Centrifuge for **5 - 10 min @ 3,000 - 4,000 *g*** to collect the plasmid DNA in the 96-well collection plate.
    Recommended: *Maximize the plasmid DNA recovery by performing a second elution with 80 µL Elution Buffer SN and repeat step 15 to collect the second eluate.*

Quick-Start Vacuum Protocol

Bacteria harvest, lysis and neutralization steps are the same as the centrifugation protocol.

Bind

1. Centrifuge to pellet the lysate debris.
   (A) If using 96-well culture plate, centrifuge for **10 min @ ≥ 3,000 *g***.
   (B) If using microcentrifuge tube, centrifuge for **5-10 min @ 15,000 *g***.
2. Place the **96 DNA Plate G** on the vacuum manifold (with the waste tray).
3. Transfer the **clear supernatant (~750 µL)** into each well of the 96 DNA Plate G. Apply vacuum until all supernatant has passed through into the waste tray.

Wash

4. Add **500 µL Wash Buffer SN1** into each well of the 96 DNA Plate G. Apply vacuum until all buffer has passed through into the waste tray.
5. Add **750 µL Wash Buffer SN2** into each well of the 96 DNA Plate G. Apply vacuum until all buffer has passed through into the waste tray.
6. Apply vacuum for another **10 min** to remove any residual wash buffer from the wells. Tap the plate on clean paper towel to dry the plate as much as possible.

Elute

7. Remove the waste tray from the vacuum manifold. Set up the vacuum manifold with the 96 DNA Plate G placed on top of a clean 96-well collection plate (not provided).
8. Add **120 µL Elution Buffer SN** directly onto the membrane of each well of the plate. Incubate for **5 min @ room temperature**.
9. Apply vacuum (Keep the pressure at **300-400 mbar**) until all plasmid DNA is collected into the 96-well collection plate.
   Optional: *Alternatively, centrifuge for 5 - 10 min at 3,000 - 4,000 g to collect the plasmid DNA in the 96-well collection plate.*
   Recommended*: Maximize the plasmid DNA recovery by performing a second elution with 80 µL Elution Buffer SN and repeat step 9 to collect the second eluate.*

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Quick-Start Protocol

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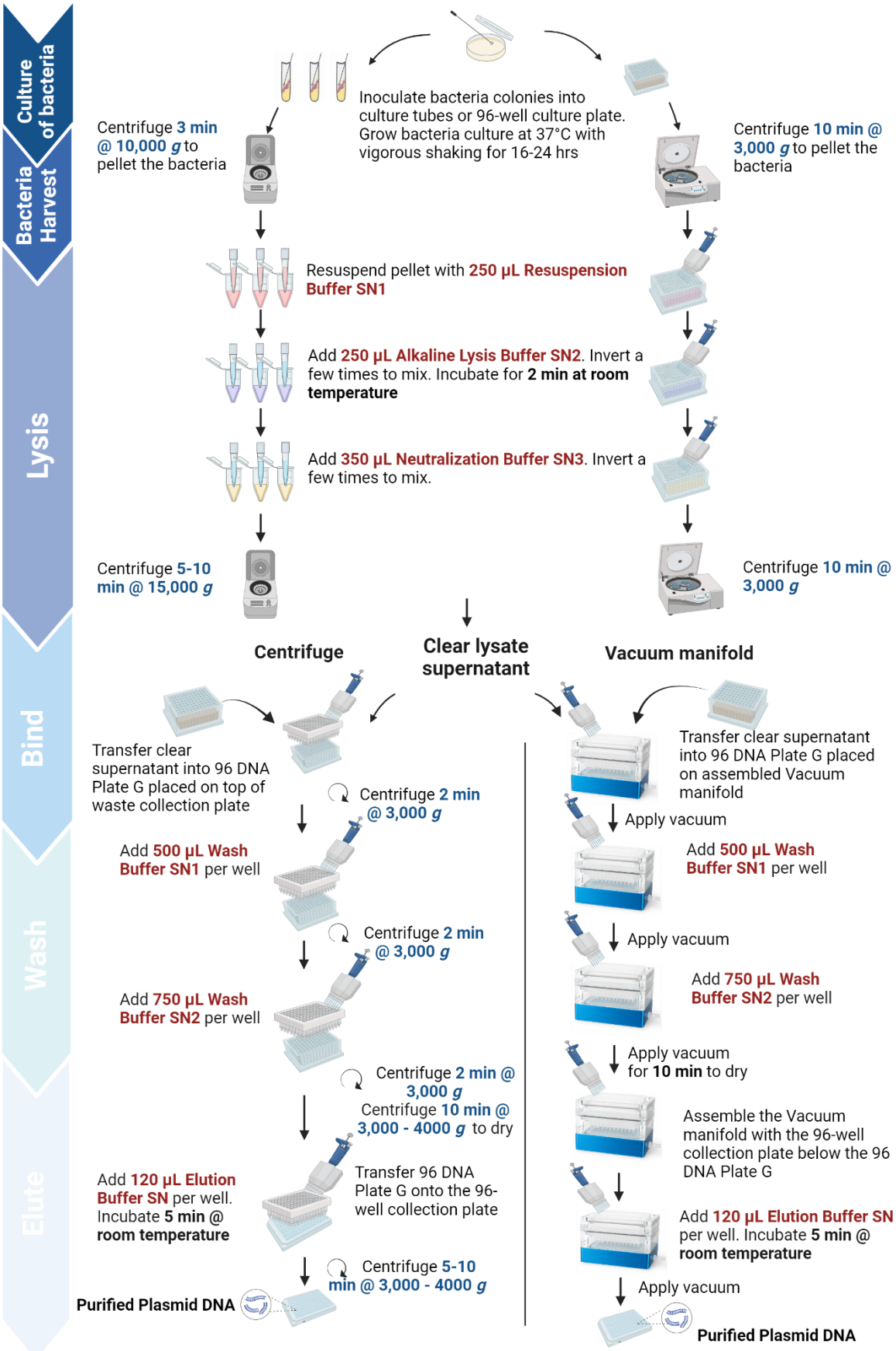

MP BIOMEDICALS
APAC: +65 6775 0008 | custserv.ap@mpbio.com
EUROPE: 00800 7777 9999 | custserv.eur@mpbio.com
AMERICAS: 800 854 0530 | custserv.na@mpbio.com
Learn more at www.mpbio.com