# SPINeasy™ Plasmid Midiprep Kit

Cat. No.: 116539025 (25 PREPS) / 116539000 (2 PREPS)

## Quick-Start Protocol

Revision Nov 2023

Scan QR code for more information from instruction manual

### Notes before starting:

- Briefly spin down **the vial of RNase A** and add the **entire solution** to **Resuspension Buffer SD1**. Mark the bottle and store **Resuspension Buffer SD1** with **RNase A** at 2-8 °C.
- Add **30 mL** (**3 mL** for sample kit) isopropanol to **Wash Buffer DW1** and mark the bottle.
- Add **100 mL** (**10 mL** for sample kit) absolute ethanol to **Wash Buffer DW2** and mark the bottle.
- Prepare two labelled 15 mL centrifuge tubes per prep: one for lysate binding preparation and another for elution of plasmid DNA.

**Column preparation**

1. Pipette **450 µL Equilibration Buffer** into **Column SD with collection tube**. Incubate for **1 min** at room temperature and centrifuge for **30 sec @ 14,000 *g***. Discard flow through and reuse collection tube.
2. Keep the columns aside for later use.

**Culture**

3. Grow **25–50 mL** transformed bacteria culture by incubation at 37 °C overnight (approximately **16 hours**) with vigorous shaking at **180–250 rpm**.
4. Harvest the cells by centrifugation for **20 mins @ 3,000 *g***. Discard the supernatant.

**Lyse**

5. Resuspend bacterial cell pellet in **2 mL Resuspension Buffer SD1**.
6. Add **2 mL Alkaline Lysis Buffer SD2** and mix well by gently inverting the tube several times. Do not vortex or pipette vigorously. Proceed to the next step **directly** or within **5 mins**.
7. Add **3 mL Neutralization Buffer SD3** and mix well by gently inverting **several times**. Do not vortex or pipette vigorously.
8. Centrifuge for **10 mins @ 15,000 *g***.

**Bind**

9. Transfer lysate supernatant to a 15 mL centrifuge tube. Add **2 mL** ethanol to the lysate supernatant and mix by inverting the tube.
10. Load **3.5 mL** mixture onto a Column SD with collection tube.
11. Centrifuge for **2 min @ 5,000 *g*** or until all the fluid has passed through the column. Discard flow through and reuse collection tube.
12. Repeat Steps 10 and 11 until all lysate has been loaded.

**Wash**

13. Add **3.5 mL Wash Buffer DW1** to the column.
14. Centrifuge for **2 min @ 5,000 *g*** or until all the fluid has passed through the column. Discard flow through and reuse collection tube.
15. Add **3.5 mL Wash Buffer DW2** to the column.
16. Centrifuge for **2 min @ 5,000 *g*** or until all the fluid has passed through the column. Discard flow through and reuse collection tube.
17. Centrifuge for **an additional 10 mins @ 5,000 *g*** to dry column.
18. Air dry membrane by allowing the column to stand at room temperature for **3 mins**.

**Elute**

19. Remove collection tube and place column into a clean 15 mL centrifuge tube.
20. Add **500 µL Elution Buffer SD** to the center of the membrane. Incubate at room temperature for **3 mins**.
21. Centrifuge for **5 mins @ 5,000 *g*** to elute plasmid DNA. Transfer the eluted plasmid DNA into a clean 1.5 mL centrifuge tube for easier storage.

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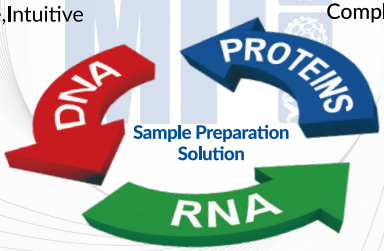



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