NucleoSpin® 8 Plasmid – centrifuge processing (Rev. 01)

This protocol is only a supplement to the kit's general user manual. Please refer to the kit manual for more detailed information regarding safety instructions, product-specific disclaimers, and especially preparations needed before starting the procedure. The latest version of the user manual is available at ***www.mn-net.com/usermanuals*** *or can be requested from our technical service (tech-bio@mn-net.com). Material safety data sheets (MSDS) can be downloaded from* ***www.mn-net.com/MSDS****.*

For the handling of NucleosSpin® 8 Plasmid kits using a centrifuge, additional equipment is necessary:

- MN Square-well Block (REF 740476), Rack of Tube Strips (REF 740477)

  Note: Do not use the (supplied) Elution Plate for elution. This plate may crack when centrifuged > 1,500 rpm.

- A microtiterplate centrifuge which is able to accommodate the NucleoSpin® 8 Plasmid Binding Strips stacked on a MN Square-well Block or Rack of Tube Strips and reaches accelerations of 5,600–6,000 x *g* (bucket height: 85 mm)

- Starter Set C (REF 740684), containing Column Holders C, Dummy Strips, MN Square-well Blocks, and Rack of Tube Strips (for detailed information, please refer to the Starter Set C manual.

1 Harvest bacterial cells in the Culture Plate

Centrifuge the bacteria cultures (1.5–5 mL LB or up to 2.5 mL 2 x YT or TB) for **10 min** at **1,000 x *g***. Discard supernatant.

2 Resuspend bacterial cells

Resuspend pelleted bacterial cells in **250 µL** of **Buffer A1** by pipetting up and down or placing the plate on a suitable microplate shaker. Mark the block for later identification. Ensure that RNase A has been added to Buffer A1.

No cell clumps should be visible after resuspension of the pellets.

3 Lyse bacterial cells

Add **250 µL** of **Buffer A2** to each sample and mix by moderate shaking. The solution becomes viscous and slightly clear when mixed sufficiently.

4 Neutralize

Add **350 µL** of **Buffer A3** to each sample and mix before transferring the lysate to the filter plate with a single aspirate / dispense cycle of 850 µL.

The solutions should become cloudy.

5 Transfer crude lysates onto the NucleoSpin® Plasmid Filter Strips

Insert the NucleoSpin® 8 Plasmid Filter Strips (purple rings) into the Column Holder C. Place Column Holder C on a MN Square-well Block (not supplied with the kit) and transfer all of the lysate into the wells of the NucleoSpin® 8 Plasmid Filter Strips. Do not moisten the rims while dispensing samples. Moistened rims may cause cross contamination during centrifugation steps.

6 Clear crude lysates by centrifugation

Place the MN Square-well Block and the Column Holder C with the NucleoSpin® 8 Plasmid Filter Strips onto the centrifuge carrier and place it into the rotor buckets. Centrifuge at **5,600 x *g*** for **4 min**.

7 Bind DNA to silica membrane

Insert NucleoSpin® 8 Plasmid Binding Strips (transparent rings) into the Column Holder C. Place Column Holder C on a MN Square-Well Block and transfer the flow-through from step 6 to the wells of the NucleoSpin® 8 Plasmid Binding Strips. Centrifuge at **5,600 x *g*** for **4 min**.

8 Wash silica membrane

1st wash

Discard the flow-through from the MN Square-well Block. Add **600 µL** of **Buffer AW** to each well of the NucleoSpin® 8 Plasmid Binding Strips and centrifuge again at **5,600 x *g*** for **1–2 min**. After centrifugation, discard flow-through collected in the MN Square-well Block.

2nd wash

Discard the flow-through from the MN Square-well Block. Add **900 µL** of **Buffer A4** to each well of the NucleoSpin® 8 Plasmid Binding Strips and centrifuge again at **5,600 x *g*** for **1–2 min**. After centrifugation, discard flow-through collected in the MN Square-well Block.

3rd wash

Repeat wash step with **900 µL** of **Buffer A4.** Centrifuge again at **5,600 x *g*** for **1–2 min**.

9 Dry NucleoSpin® Plasmid Binding Strips

Discard the flow-through from the MN Square-well Block. Centrifuge for **5–10 min** at **5,600 x *g*** in order to remove residual washing buffer from the silica membrane and for drying the membrane.

10 Elute plasmid DNA

Place the Column Holder C with the NucleoSpin® 8 Plasmid Binding Strips on top of the Rack of Tube Strips.

Insert Tube Strips supplied with the NucleoSpin® 8 Plasmid kit only into the rows underneath the NucleoSpin® 8 Plasmid Binding Strips. The remaining rows have to be filled with the tube strips supplied in the Starter Set C.

Note: The Rack of Tube Strips has to be filled with tube strips completely for centrifugation.

Dispense **50–75 µL Elution Buffer AE** to each well of the NucleoSpin® 8 Plasmid Binding Strips. Dispense buffer directly onto the membrane. Incubate at room temperature for **1–3 min**. Centrifuge at **5,600–6,000 x *g*** for **2–3 min**.

Note: Do not use (supplied) Elution Plate for elution. This plate may crack when centrifuged > 1,500 rpm.