NucleoSpin® 96 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****.*

This protocol is designed for up to 192 (2 x 96) parallel plasmid DNA preparations from 1.3–5 mL overnight culture.

For use of the NucleoSpin® 96 Plasmid kit in a centrifuge, additional equipment is required:

Protocol step	Suitable consumables, not supplied	REF	Remark
Wash step, collection of cleared lysate	MN Square-well Block Square-well Block	740476 740481	
Elution	Rack of Tube Strips Round-well Block Low Round-well Block Square-well Block	740477 740487 740475 740481	*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® 96 Plasmid Binding Plate stacked on a round or Square-well Block and reaches accelerations of 5,600–6,000 x *g* (bucket height: 85 mm)

All centrifugation steps are performed at room temperature. It is useful to perform 2 x 96 preparations at one time since in all cases the rotor must be balanced.

Add the provided RNase A to Buffer A1, mix, and store at 4 °C. Prepare Buffer A4 according to the user manual.

For information about cultivation of bacteria in the 96-well Culture Plate, please refer to the NucleoSpin® 96 Plasmid user manual.

A repeating pipette and a multichannel pipette facilitate liquid handling during the procedure.

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 Plate

Place NucleoSpin® Plasmid Filter Plate (purple rings) on top of a new (MN) Square-well Block. Transfer the lysates (from step 4) to the wells of the NucleoSpin® Plasmid Filter Plate. Do not moisten the rims while dispensing samples. Moistened rims may cause cross contamination during centrifugation steps.

6 Clear crude lysates by centrifugation

Load (MN) Square-well Block with NucleoSpin® Plasmid Filter Plate onto the carrier then place in the rotor bucket. Centrifuge at **5,600 x *g*** for **4 min**.

7 Bind DNA to silica membrane

Place NucleoSpin® Plasmid Binding Plate (transparent rings) on top of new (MN) Square-well Block. Mark the plate for later identification. Transfer the flow-through from step 6 to the wells of the NucleoSpin® Plasmid Binding Plate. Load (MN) Square-well Block and NucleoSpin® Plasmid Binding Plate onto the carrier then place in the rotor bucket. 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. Centrifuge at **5,600 x *g*** for **4 min**.

This step is necessary to remove trace nuclease activity when using endA strains such as the JM series, HB 101 and its derivatives, or any wild-type strains that have high levels of nuclease activity or high carbohydrate content.

2nd wash

Discard the flow-through from the (MN) Square-well Block. Add **900 µL** of **Buffer A4** to each well. Centrifuge at **5,600 x *g*** for **4 min**.

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 Plate

Discard the flow-through from the (MN) Square-well Block. Centrifuge at **5,600 x *g*** for **10–15 min** in order to dry the membrane. Alternatively incubate NucleoSpin® Plasmid Binding Plate for 10 min at 70 °C in a suitable incubator.

10 Elute plasmid DNA

Place NucleoSpin® Plasmid Binding Plate on new deep-well block (e.g., (MN) Square-well Block). Dispense **50–75 µL Buffer AE** to each well of the plate. Incubate for 1–3 min at RT. Centrifuge at **5,600 x *g*** for **4 min** to collect DNA.

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