NucleoSpin® 96 PCR Clean-up – 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® 96 PCR Clean-up kits using a centrifuge, additional equipment is necessary:

- A microtiterplate centrifuge which is able to accommodate the NucleoSpin® PCR Clean-up Binding Plate stacked on a Round- or Square-well Block and reaches accelerations of 5,600–6,000 x *g* (bucket height: 85 mm)
- Round-well Block (REF 740671), Round-well Block Low (REF 740482), and MN Square-well Block (REF740476)
- For transfer of the samples from the Round-well Block to the NucleoSpin® PCR Clean-up Binding Plate, we recommend use of an electronic eight-channel pipetting device with extra long tips capable of holding more than 500 µL. .

1 Adjust the volume of reaction mixture

Before starting the purification procedure, add Tris buffer (10 mM, pH 7.0) to adjust the PCR reaction mixture to a final volume of 100 µL.

Note: Removal of mineral oil is not necessary.

Transfer **100 µL sample** to each well of the Round-well Block (not provided in the kit).

2 Dispense binding buffer to the NucleoSpin® PCR Clean-up Binding Plate

Add **200 µL of Buffer NT** to **100 µL sample** in the wells of the Round-well Block and pipette up and down several times for mixing.

3 Transfer PCR samples to the NucleoSpin® PCR Clean-up Binding Plate

Place the NucleoSpin® PCR Clean-up Binding Plate on a MN Square-well Block (not provided in the kit) and transfer all of the sample/ Buffer NT mixture into the wells of the NucleoSpin® PCR Clean-up Binding Plate. Do not moisten the rims while dispensing samples. Moistened rims may cause cross contamination during centrifugation steps.

4 Bind DNA to silica membrane

Place MN Square-well Block (not provided in the kit) and NucleoSpin® PCR Clean-up Binding Plate onto the centrifuge carrier and place it into the rotor buckets. Centrifuge at **5,600 x *g*** for **2 min**.

Typically, samples will pass through the columns within ≤ 1 min.

5 Wash silica membrane

1st wash

Empty the Block and add **900 µL** of **Buffer NT3** to each well of the NucleoSpin® PCR Clean-up Binding Plate 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

Repeat wash step with **900 µL** of **Buffer NT3.** Centrifuge again at **5,600 x *g*** for **1–2 min** and discard the flow-through.

6 Dry NucleoSpin® PCR Clean-up Binding Plate

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.

7 Elute plasmid DNA

Place the NucleoSpin® PCR Clean-up Binding Plate on a Round-well Block Low (not provided in the kit).

Dispense **75–150 µL Elution Buffer NE** to each well of the NucleoSpin® PCR Clean-up Binding Plate. Dispense buffer directly onto the membrane. Incubate at room temperature for **1 min**. Centrifuge at **5,600–6,000 x *g*** for **2–3 min**.

Optional: Preheat Elution Buffer NE to 70 °C before dispensing. This will increase recovery for PCR products > 1000 bp.