Using NucleoSpin® 96 PCR Clean-up with a Benchtop centrifuge (Rev. 02)

For complete information regarding the NucleoSpin® 96 PCR Clean-up kits e.g. kit contents, product description including basic principles and kit specifications, storage conditions, troubleshooting, and ordering information see NucleosSpin® 96 PCR Clean-up manual.

For use of the **NucleoSpin® 96 PCR Clean-up** kit in a centrifuge additional equipment is required:

- MN Square-well Block (REF 740476) or Square-well Block (REF 740481)
- Round-well Block Low (REF 740482)
  Note: The Elution Plate, U-bottom supplied with the kit is for vacuum elution only.

Please note that there are only few centrifuges which can be used for handling of **NucleoSpin® 96 PCR Clean-up** kits. The centrifuge should be able to pick up a swing out rotor which is capable of accommodating the NucleoSpin® Plasmid Binding Plate/ Square-well Block sandwich (bucket height: 85 mm) and reaches accelerations of 5,600 – 6,000 x g.

For transfer of the sample from the Round-well Block to the NucleoSpin® PCR Binding Plate we recommend usage of an electronic eight-channel pipetting device with extra long tips capable of holding more than 500 µl. A good choice is the Matrix Impact^2 multichannel pipettor with 102-mm-long 1,250 µl tips (Matrix # 8251).

Procedure

1. Perform PCR reaction according to the standard protocols.
For PCR reaction volumes below 100 µL: Before starting the purification procedure, add Tris buffer (10 mM, pH 7.0) to adjust the reaction mixture to a final volume of 100 µL. The amount of added mineral oil has not to be considered.

Note: Removal of mineral oil is not necessary.

2. Transfer **100 µL sample** to each well of the (MN) Square-well Block (not supplied with the kit).

3. Add **200 µL of Binding Buffer NT** into the wells of the (MN) Square-well Block and pipette up and down several times for mixing.

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany

Germany and international:
Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

Switzerland:
MACHEREY-NAGEL AG
Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

France:
MACHEREY-NAGEL EURL
Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

USA:
MACHEREY-NAGEL Inc.
Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com

Support protocol

(Alternatively, add to 1 vol of PCR reaction and 2 vol of Buffer NT into the PCR plate. Mix by pipetting up and down.)

4. 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.

5. Place the (MN) Square-well Block (not provided with 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.

6. Add **900 µL of 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.

Repeat this washing step once.

7. 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.

8. Place the **NucleoSpin® PCR Clean-up Binding Plate** on a Round-well Block, low (not provided with the kit).

9. Dispense **75-150 µL Elution Buffer NE** or water 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: Prewarm Elution Buffer to 70°C before dispensing. This will increase recovery for PCR products > 1000 bp.