

Support protocol

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 NucleoSpin[®] 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 Impact² 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.

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(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 μ L 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 μ L 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.