Support protocol

Use of NucleoSpin® 8 Plasmid with a Benchtop centrifuge (Rev. 01)

For complete information regarding the NucleoSpin® 8 Plasmid kits e.g. kit contents, product description including basic principles and kit specifications, storage conditions, troubleshooting, and ordering information see NucleoSpin® 8 Plasmid manual.

For the handling of NucleosSpin® 8 Plasmid kits using a centrifuge additional equipment is necessary (see protocol).
- For centrifugation a microtiterplate centrifuge which is able to accommodate the NucleoSpin® 8 Plasmid Binding Strips stacked on a round or Square-well Block and reaches accelerations of 5,600 – 6,000 x *g* is required (bucket height: 85 mm).

- For processing the NucleoSpin® 8 Plasmid Strips the Starter Set C (REF 740684), containing Column Holders C, Dummy Strips, MN Square-well Blocks, Tube Strips is also required. For detailed information refer to the Starter Set C manual.

- For using the NucleoSpin® 8 Plasmid with a centrifuge the Round-well Block (REF 740671) and the MN Square-well Block (REF740476) is required.

- For transfer of the sample from the Round-well Block to the NucleoSpin® 8 Plasmid Binding Strips 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 1250 µL tips (Matrix # 8251).

Procedure

1. 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.

2. Add **250 µL** of **Buffer A2** to each sample, seal the block with adhering foil and mix by moderate shaking or inverting the block 4-6 times to mix. The solution becomes viscous and slightly clear when mixed sufficiently.

3. Remove the tape from the block. Add **350 µL** of **Buffer A3** to each sample and seal the block with a new tape. Gently invert the block 4-6 times. Mixing can also be performed before transferring the lysate to the filter plate with a single aspirate/dispense cycle of 850 µL.

The solutions should become cloudy.

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4. 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.

5. 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**.

6. 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 5 to the wells of the NucleoSpin® 8 Plasmid Binding Strips. Centrifuge at **5,600 x *g*** for **4 min**.

7. Empty the Block and 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.

8. Empty the block and 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.

Repeat this washing step once.

9. 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. Place the Column Holder C with the NucleoSpin® 8 Plasmid Binding Strips on top of the rack with MN Tube Strips.
Insert MN 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 MN Tube Strips supplied in the Starter Set C.

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

11. 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**.

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