Using NucleoSpin® 96 Plasmid with a Benchtop centrifuge

This protocol is designed for up to 192 (2x96) 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:

- MN Square-well Block (REF 740476) or Square-well Block (REF 740481)

Please note that there are only few centrifuges which can be used for handling of **NucleoSpin® 96 Plasmid** 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.

All centrifugation steps are performed at room temperature. It is useful to perform 2x96 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 a Square-well Block, please refer to the NucleoSpin® 96 user manual.

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

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Support protocol

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.

4. Place **NucleoSpin® Plasmid Filter Plate** (purple rings) on top of a new Square-well Block.

5. Transfer the lysates from step 3 to the wells of the NucleoSpin® Plasmid Filter Plate.

6. Load 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. Place **NucleoSpin® Plasmid Binding Plate** (transparent rings) on top of new 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 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. Empty the 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.

9. Empty the block. Add **900 µL** of **Buffer A4** to each well.
Centrifuge at **5,600 x *g*** for **4 min**.

Repeat this washing step once.

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Support protocol

10. Empty the 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.

11. 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.

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