Supplementary protocol

MACHEREY-NAGEL

Support protocol – Centrifuge processing NucleoSpin® 96 RNA Plant and Fungi kit (REF 740128.1 / .4) (Rev. 01, June 2026)

This protocol is only a supplement to the products general user manual. Please refer to the product 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 can be requested from our technical service (support@mn-net.com). Material safety data sheets (MSDS) can be downloaded from ***www.mn-net.com/MSDS****.*

- For hardware requirements, refer to section 2.3 of the manual: For centrifugation, a centrifuge suitable for deep-well plates is required. **This centrifuge must be able to accommodate the NucleoSpin® RNA Plant and Fungi Binding or Filter Plate stacked on a MN Square-well Block and Rack of Tube Strips (bucket height: 85 mm)** and reach accelerations of 5,600 – 6,000 x *g*. Regarding waste collection, suitable consumables (e.g., MN Square-well Blocks) are necessary and they are not included in the kit. For the most convenient handling, without the need of emptying and reusing the MN Square-well Blocks, we recommend using six MN Square-well Blocks, if two 96-well plates are processed at once (see ordering information). Alternatively, it is possible to empty the MN Square-well Blocks after every centrifugation step, reducing the amount of MN Square-well Blocks needed. Please use this product only with a balanced centrifuge.
- For use of the NucleoSpin® 96 RNA Plant and Fungi Core Kit (REF 740129.4), refer to section 2.4 of the manual regarding recommended accessories.

Before starting the preparation:

Check if Buffer PFW2 was prepared according to section 3 of the manual

1 Harvest sample

Harvest fresh material. Refer to section 5.1 for recommendations of sample amounts, depending on your sample disruption process (e.g. MN Bead Tubes or MN Beads Plates see section 2.8, paragraph Bead Plates). Frozen sample material is not recommended due to high risk of RNA degradation during sample thawing.

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2 Disruption and lysis of sample material

Add sample material to sample disruption container.

MN Bead Tubes Type G:

Add 550 µL Lysis Buffer PFL

Add 10 to 50 µL Buffer PFR. See table in section 5.1 for volume recommendations.

Disrupts sample material in the presence of buffer PFL and PFR (optional PFN)

MN Bead Plates Type G:

Add 100 to 250 µL Buffer PFL and 10 to 50 µL PFR.

Disrupts sample material in the presence of buffer PFL and PFR (optional PFN).

Note: For some sample types it is advisable to supplement the lysing reaction with Buffer PFN (see table in section 5 of the user manual)

3 Heat incubation (optional)

For samples disrupted in MN Bead Tubes Type G: Incubate the lyses sample for 5 min at 56 °C.

For samples disrupted in MN Bead Plates Type G: Heat incubation is not recommended.

Note: Do not perform this heat incubation for samples with high starch content, e.g. potato tubers or wheat kernels.

Sample dependent, heat incubation can increase lysis efficiency, reduces risk of filter clogging and can increase RNA yield. However, heat incubation may reduce RNA quality (reduced RIN values).

4 Preparation of lysates

Note: A centrifugation before filtration is recommended in order to clear the lids and to disinteragte foam.

5 For Bead Tubes Type G:

Remove the steel beads with a magnet! (see section 2.8, paragraph Bead Tubes)

Note: It is important to centrifuge without steel beads in order to avoid risk of tube rupture and for ease of subsequent lysate removal.

Centrifuge samples 1 min at 14,000 x *g* in order to clear the lids of the disruption container.

For Bead Plates Type G:

Centrifuge for 5 min at 2,000 x *g* (steel beads can stay in the plate during centrifugation). In case of sample lysis in < 550 µL, fill the lysate up to a total volume of 550 µL with lysis buffer PFL.

6 Filtration of lysates

Place the NucleoSpin® RNA Plant and Fungi Filter Plate (white rings) onto the MN Square-well block (not included in the kit).

Load 500 µL lysate onto the NucleoSpin® Plant and Fungi Filter Plate.

Check the weight of the stack to balance the centrifuge. Use only rotor buckets, which can accommodate the complete stack height (min. 85 mm).

Place the NucleoSpin® RNA Plant and Fungi Filter Plate stacked onto the MN Square-well block into the rotor buckets. Centrifuge at 5,600 – 6,000 x *g* for 5 min.

Note: Overlay crude lysate on the NucleoSpin® RNA Plant and Fungi Filter Plate slowly (50 µL/s) with 100 µL Buffer PFW2 in case of excessive foaming.

Typically, the lysates will have passed through the silica membrane within a few minutes. The centrifugation process can be extended to 20 min, if the lysates have not passed completely.

Discard NucleoSpin® Plant and Fungi Filter Plate.

7 Prepare binding conditions

Add 500 µL Buffer PFB to the flowthrough in the MN Square-well Block and mix by pipetting up and down at least 2 – 3 times or shaking.

Note: Please refer to Table in Section 5.1 for recommendations on Buffer PFB increase for certain samples.

Incubate for 5 min at room temperature.

8 Transfer crude lysates to NucleoSpin® RNA Plant and Fungi Binding Plate

Place the NucleoSpin® RNA Plant and Fungi Binding Plate (light green rings) onto a new MN Square-well Block.

Transfer up to 1 mL of the lysates to the NucleoSpin® RNA Plant and Fungi Binding Plate.

Do not moisten the rims of the individual wells while dispensing samples. After transfer, it is recommended to seal the openings of the NucleoSpin® RNA Plant and Fungi binding plates with gas-permeable foil (not included)

9 Bind RNA to silica membrane

Place the NucleoSpin® RNA Plant and Fungi Filter Plate stacked onto the MN Square-well block into the rotor buckets.

Check the weight of the stack to balance the centrifuge. Use only rotor buckets, which can accommodate the complete stack height (min. 85 mm).

Centrifuge at 5,600 – 6,000 x *g* for 5 min.

10 **Wash silica membrane**

1st wash

Add **500 µL Buffer PFW1** to each well of the NucleoSpin® RNA Plant and Fungi Binding Plate. It is recommended to seal the plate with a new gas-permeable foil.

Centrifuge at 5,600 – 6,000 x *g* for 2 min

2nd wash

Add **500 µL Buffer PFW2** to each well of the NucleoSpin® RNA Plant and Fungi Binding Plate. It is recommended to seal the plate with a new gas-permeable foil.

Centrifuge at 5,600 – 6,000 x *g* for 2 min.

3rd wash

Add **500 µL Buffer PFW2** to each well of the NucleoSpin® RNA Plant and Fungi Binding Plate. It is recommended to seal the plate with a new gas-permeable foil.

Centrifuge at 5,600 – 6,000 x *g* for 2 min

11 **Dry NucleoSpin® RNA Plant and Fungi Binding Plate**

Place the NucleoSpin® RNA Plant and Fungi Binding Plate onto a new MN-Square-well block.

Centrifuge at 5,600 – 6,000 x *g* for 5 – 15 min.

For critical ethanol-sensitive applications, it is recommended to prolong the centrifugation time up to 15 min or incubate at higher temperature. Remove the adhesive foil and place the stack into an incubator for 20 min at 37 °C to evaporate residual ethanol. Removal of ethanol by evaporation at 37 °C is more effective than additional, prolonged centrifugation.

12 **Elute RNA**

Place the NucleoSpin® RNA Plant and Fungi Binding Plate on an opened Rack of Tube Strips.

Dispense 100 µL RNase-free H_2O to each well of the NucleoSpin® RNA Plant and Fungi Binding Plate. Dispense directly onto the membrane. Incubate at room temperature for 1 min.

Centrifuge at 5,600 – 6,000 x g for 2 min.

Remove the plate from the Rack of Tube Strips.