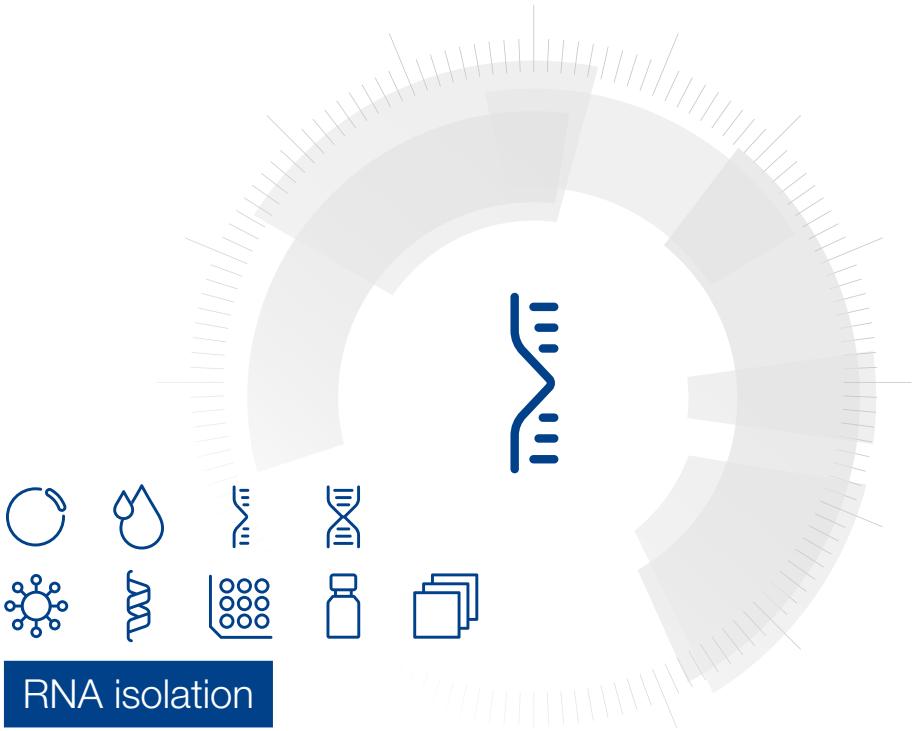
Bioanalysis

MACHEREY-NAGEL

User manual



RNA isolation

- NucleoSpin® 96 RNA Plant and Fungi
- NucleoSpin® 96 RNA Plant and Fungi Core Kit

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Table of contents

1 Components

1.1 Kit contents

	NucleoSpin® 96 RNA Plant and Fungi	
REF	**1 × 96 preps 740128.1**	**4 × 96 preps 740128.4**
Lysis Buffer PFL	125 mL	250 mL
Reduction Buffer PFR	20 mL	20 mL
Binding Buffer PFB	200 mL	2 × 200 mL
Wash Buffer PFW1	125 mL	250 mL
Wash Buffer PFW2 (Concentrate)[1]	25 mL	100 mL
RNase-free H_2O	60 mL	125 mL
NucleoSpin® RNA Plant and Fungi Filter Plate (white rings)	1	4
NucleoSpin® RNA Plant and Fungi Binding Plate (light green rings)	1	4
MN Wash Plates (including six Paper Sheets)	1	4
Square-well Block	1	4
Rack of Tube Strips (including Caps Strips)	1	4
Leaflet	1	1

[1] For preparation of working solutions and storage conditions see 3.

Kit contents continued

	NucleoSpin® 96 RNA Core Kit
REF	**4 × 96 preps 740129.4**
Lysis Buffer PFL	250 mL
Reduction Buffer PFR	20 mL
Binding Buffer PFB	2 × 200 mL
Wash Buffer PFW1	250 mL
Wash Buffer PFW2 (Concentrate)	100 mL
RNase-free H_2O	125 mL
NucleoSpin® RNA Plant and Fungi Filter Plate (white rings)	4
NucleoSpin® RNA Plant and Fungi Binding Plate (light green rings)	4
Leaflet	1

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol (for preparation of working solutions PFW2; see section 3)
- Neutralization Buffer PFN for processing acidic samples (see section 6.2 for ordering information)

Consumables

- Disposable pipette tips
- MN Bead Tubes Type G or MN Bead Plate Type G (optional, see section 6.2 for ordering information)

Equipment

- Pipettes
- Centrifuge for microcentrifuge tubes in case of use of MN Bead Tubes Type G
- Centrifuge suitable for Deep-well Plates in case of use of MN Bead PlatesType G (see section 2.3)
- Equipment for sample disruption and homogenization (see section 2.7)
- Personal protection equipment (lab coat, gloves, goggles)

For more detailed information regarding special hardware required for centrifuge, vacuum or positive pressure processing, please see section 2.4. For ordering information, please see section 6.2.

For recommended accessories for use of the flexible **NucleoSpin® 96 RNA Plant and Fungi Core Kit** (reduced kit composition; REF 740129.4), please see section 2.4.

1.3 About this user manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous or updated revisions.

2 Product description

2.1 The basic principle

The **NucleoSpin® 96 RNA Plant and Fungi** kit is designed for the isolation of RNA from diverse plant and fungal material, including samples rich in starch, sugar, secondary metabolites and other compounds that might interfere with common RNA isolation procedures.

First, plant material is mechanically disrupted (e.g. by MN Bead Tubes or Bead Plates, grinding in liquid nitrogen, or any other suitable disruption method) in lysis buffer containing large amounts of chaotropic ions. This lysis buffer immediately inactivates RNases, which are present in virtually all biological materials. After removal of plant debris with the NucleoSpin® 96 Plant and Fungi Filter plate, a binding solution is added which creates appropriate binding conditions which favor adsorption of RNA to the silica membrane. Washing steps with two different buffers remove salts, metabolites and macromolecular cellular components. Pure RNA is finally eluted under low ionic strength conditions with RNase-free water.

The RNA preparation using **NucleoSpin® RNA Plant and Fungi** can be performed at room temperature. The eluate, however, should be treated with care because RNA is very sensitive to trace contaminations of RNase, often found on general lab ware, fingerprints, and dust. To ensure RNA stability, keep RNA frozen at -20 °C for short-term or at -70 to -80 °C for long-term storage.

2.2 Kit specifications

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® 96 RNA Plant and Fungi
Technology	Silica membrane technology
Format	96-well plates
Processing	Manual or automated, vacuum, positive pressure or centrifugation
Sample material	Up to 500 mg plant or fungal material (wet weight)
Typical yield	10 – 60 µg depending on sample material and quality
Fragment size	≥ 200 nt
A_{260}/A_{280}	1.9 – 2.1
A_{260}/A_{230}	~ 2
Typical RIN (RNA integrity number)	7 – 9
Elution volume	100 µL (70 – 150 µL)
Preparation time	50 min/plate (without lysis)
Theoretical binding capacity	200 µg
Use	For research use only

2.3 Required hardware

NucleoSpin® 96 RNA Plant and Fungi can be processed under vacuum, positive pressure or centrifugation. Certain hardware for processing is required.

Centrifugation of MN Bead Tubes or MN Bead Plates

Please check section 1.2

Centrifugation

For centrifugation, a centrifuge suitable for deep-well plates is required. This centrifuge must be able to accomodate the NucleoSpin® RNA Plant and Fungi Binding or Filter Plate stacked on a MN Square-well Block and Rack of Tube Strips and reach accelerations of 5,600 – 6,000 x *g* (bucket height: 85 mm).

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.

Vacuum processing

The **NucleoSpin® 96 RNA** Plant and Fungi kit can be used with the NucleoVac 96 Vacuum Manifold (see ordering information). When using **NucleoSpin® 96 RNA Plant and Fungi kit** with less than 96 samples, Self adhering PE Foil (see ordering information) should be used in order to close and protect non-used wells of the NucleoSpin® RNA Plant and Fungi Binding and Filter Plate and thus guarantee proper vacuum.

Establish a reliable vacuum source for the NucleoVac 96 Vacuum Manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of -0.2 to -0.4 bar (reduction of atmospheric pressure). The use of the NucleoVac Vacuum Regulator (see ordering information) is recommended. Alternatively, adjust the vacuum so that during the purification the sample flows through the column with a rate of 1 – 2 drops per second. Depending on the amount of sample being used, the vacuum times may need to be increased for complete filtration. Additionally, a suitable centrifuge for sample preparation steps may be required.

Positive pressure processing

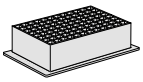
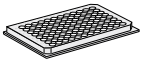
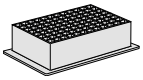
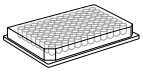
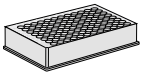
Please contact Technical service regarding information for positive pressure processing.

2.4 Recommended accessories for use of the NucleoSpin® 96 RNA Plant and Fungi Core Kit

The **NucleoSpin® 96 RNA Plant and Fungi Core Kit** provides buffers and NucleoSpin® RNA Plant and Fungi Binding and Filter Plates. Accessory plates (e.g., lysis plates, elution plates) are not provided with the core kit. The reduced kit composition along with a variety of separately available accessories, allow optimal adjustment of the kit to individual user needs. The user can select additional consumables according to his / her requirements for highest flexibility.

For use of **NucleoSpin® 96 RNA Plant and Fungi Core Kit**, follow the standard protocols (see section 5.1 and 5.2).

Recommended accessories for use of the **NucleoSpin® 96 RNA Plant and Fungi Core Kit** (4 × 96 preps) are available from MACHEREY-NAGEL (see ordering information, section 6.2).

Protocol step	Suitable consumables, not supplied with the core kits	Remarks
Lyse samples	4 x Rack of Tubes Strips with Cap Strips per 4 × 96 preps or  4 x MN Bead Plate Type G 384 x MN Bead Tubes Type G	
Filtration of samples	4 x Square-well Block 	Collection of flow through after lysate filtration
Bind RNA to the membrane	4 x MN Wash Plate per 4 × 96 preps 	MN Wash Plate minimizes the risk of cross contamination (vacuum processing only).
	2 x MN Square-well Block 	For waste collection during centrifugation (reusable)
Elute RNA	4 x Rack of Tubes Strips with Cap Strips per 4 × 96 preps or  4 x Round-well Block with Cap Strips per 4 × 96 preps or 	
	4 x Elution Plate U-bottom or 	For vacuum processing only
	4 x Round-well Block Low, U-bottom 	For processing under centrifugation

2.5 Automated processing and automation support

NucleoSpin® 96 RNA Plant and Fungi can be used fully automated on many common laboratory workstations. Please contact MN for the availability of scripts and general considerations about adapting **NucleoSpin® 96 RNA Plant and Fungi** on a certain workstation.

Visit MN online at ***www.mn-net.com*** or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions, and selection of the protocol. Several application notes of the **NucleoSpin® 96 RNA Plant and Fungi** kit on various liquid handling instruments can also be found at ***www.mn-net.com*** at Bioanalysis / Automation.

Full processing under vacuum enables complete automation without the need of centrifugation steps for drying or elution.

The risk of cross-contamination is reduced by optimized vacuum settings during all steps and by the improved shape of the outlets of the NucleoSpin® RNA Plant and Fungi Binding Plate.

During the washing steps the bottom of the plate is protected by the MN Wash Plates. The NucleoSpin® RNA Plant and Fungi Binding Plates can be dried under vacuum. Thorough cleaning of the vacuum chamber is recommended after each run to prevent DNA-containing aerosols from forming.

MN extraction kits are designed for streamlined automation, offering compatibility with a range of leading open robotic systems. Whether you're using magnetic rod systems or liquid handlers like Hamilton, Tecan, Eppendorf, or other platforms, our kits ensure efficient and reliable extraction processes. Reach out to us for comprehensive support and tailored automation solutions, making your extraction experience seamless and hassle-free.

Questions about MACHEREY-NAGEL's scripting support or automation service?

Please contact us for personal assistance:

Phone: +49 2421 969-0
Email: support@mn-net.com
For more information, visit our website: ***www.mn-net.com/automation***

2.6 Handling, preparation, and storage of starting material

RNA is not protected against digestion by plant RNase until the sample material is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents. Therefore it is important that samples are processed as fresh as possible or flash frozen in liquid N_2 immediately and stored at -70 °C. If frozen samples are used as sample material, it is very important that the sample will only thaw during the mechanical disruption in the presence of lysis buffer. Otherwise the RNA quality will be immediately impaired.

Plant material lysed in Lysis buffer PFL can be stored at -20 °C for at least 2 weeks.

Wear gloves at all times during the preparation. Change gloves frequently.

2.7 Lysis, disruption and clarification of sample material

For most plant sample material a mechanical disruption is a necessity. Several disruption options are possible.

MN Bead Tubes

MN Bead Tubes Type G (see section 6.2 for ordering information) 5 mm steel balls are recommended in combination with a swing-mill (e.g., MM200, MM300, MM400 (Retsch®) or other bead beating devices (e.g. Precellys, Bertin Technologies; FastPrep System, MP-Bio; Speed Mill, Analytik Jena). Respect the leaflet of the MN Bead Tubes for handling and safety aspects.

MN Bead Tubes Type D (3 mm steel balls, REF 740814) and MN Bead Tubes Type A (0.6 – 0.8 mm ceramic balls, certified DNA and nuclease free, REF 740786) might be sufficient for disruption of soft plant material and tissues / cells with weak cell walls.

After disruption, a centrifugation step is typically required to clear the lysate and eliminate foam:

Bead removal: Place a magnet on top of the Bead Tube lid and briefly invert the tube to fix the bead to the lid. Screw open the tube, remove the magnet in order to dispose the beads and reclose the tube. Centrifuge for 1 min at 14.000x g in order to clear the lysate.

Subsequently, the cleared lysate can be applied to the NucleoSpin® 96 RNA Plant and Fungi Filter plate to remove residual debris from the lysate. Depending on the sample material, amount and effectiveness of removal of cleared lysate from the bead tube, the lysate may be processed (addition of binding buffer, transfer onto the RNA binding plate) without usage of the NucleoSpin® 96 RNA Plant and Fungi Filter plate. Only cleared lysates shall be used for addition of binding buffer and transfer onto the NucleoSpin® RNA Plant and Fungi binding plate. Therefore, filtration of the lysate is generally recommended!

The use of MN Bead Tube Holder (REF 740469) in combination with MN Bead Tubes is not recommended for NucleoSpin® 96 RNA Plant and Fungi due to insufficient disruption of most plant material.

For suitable sample amounts for processing with MN Bead Tubes Type G refer to section 5.

MN Bead Plate

Reduces buffer volume (100 – 250 µL) during disruption can help to disintegrate plant material, because liquid reduces considerably the disruption efficiency. After disruption, add lysis buffer up to a total volume of 550 µL.

For disruption of plant material MN 96 Bead Plates Type G (REF 740855) are highly recommended. Generally, disruption of plant material often generates foam, which hinders further processing and bears the risc of cross contamination. MN Bead Plate Type G contains antifoam to avoid / reduce foam formation and thus simplifies further processing. After sample disuption clear the lids of the cap strips by centrifugation of the bead plate for 5 min at 2,000 x *g*. This is highly recommended in order to avoid cross contamination.

Subsequently, the cleared lysate can be applied to the NucleoSpin® 96 RNA Plant and Fungi Filter plate to remove residual debris from the lysate.

The use of MN Bead Plate Type D is not recommended for plant samples due to excessive foam formation and reduced disruption effectiveness compared to MN Bead Plate Type G.

Mortar, pestle and liquid nitrogen

This common sample disruption method can be used for most sample types. It typically gives excellent RNA quality; however, RNA yield can be lower compared to the extraction with bead tubes or extraction bags (see below).

MN Bead material

MACHEREY-NAGEL offers a range of beads in bulk (nuclease and DNA-free). Especially MN Beads Type D and G Bulk (3 mm and 5 mm diameter steel balls) offer the possibility to create your own bead tube or plate according to your needs. Please contact our technical service for further advice.

Disruption devices

With disruption devices (e.g. ULTRA-TURRAX®, IKA ; Omni Tissue Master, Omni; Polytron, Kinematica) it is possible to sufficiently homogenize plant material. Required processing time and risk of cross contamination should be considered before use.

Dissociators

Dissociator devices (e.g. gentleMACS, Miltenyi Biotec; ULTRA TURRAX® Tube Drive, IKA) can effectively dissrupt plant material but typically require liquid volumes exceeding the recommended lysis buffer volume for one preparation with NucleoSpin® 96 RNA Plant and Fungi and are thus rather recommended for low throughput applications with higher lysate volumes.

Syringe and needle

Not recommended. For most plant materials it will not be possible to disrupt them using syringe and needle. Disruption effectiveness of syringe and needle is typically restricted to cultured mammalian cells.

Disposable micro pestles for disruption within a centrifugal tube

Not recommended due to inconsistent effectiveness, impracticability, and high processing time.

2.8 Elution procedures of pure RNA

Due to dead volume of the silica membrane by using vacuum, please notice that the difference between the dispensed elution buffer and the recovered elution buffer containing RNA is approximately 20 µL. For RNA elution, a volume of 50 – 150 µL nuclease free water is recommended. Higher RNA concentrations are obtained when using a dispense volume of 50 µL, however, higher elution efficiencies are obtained when using dispense volumes of > 100 µL.

Recovered elution volume = Dispensed elution volume minus 20 µL

3 Storage conditions and preparation of working solutions

Attention: Buffers PFL and PFW1 contain chaotropic salt. Wear gloves and goggles!

CAUTION: Lysis Buffer contains guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

All kit components should be stored at 15 – 25 °C and are stable until: see package label. Storage at lower temperatures may cause precipitation of salts.

Before starting any NucleoSpin® RNA Plant and Fungi protocol prepare the following:

- **Wash Buffer PFW2**: Add the indicated volume of 96 – 100 % ethanol (see table below) to Wash Buffer PFW2 concentrate. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer PFW2 can be stored at 15 – 25 °C for at least one year.

	NucleoSpin® 96 RNA Plant and Fungi / Core Kit		
REF	**1× 96 preps 740128.1**	**4 × 96 preps 740128.4**	**4 × 96 preps 740129.4**
Wash Buffer PFW2 (Concentrate)	1 × 25 mL Add 100 mL ethanol	1 × 100 mL Add 400 mL ethanol	1 × 100 mL Add 400 mL ethanol

4 Safety instructions

When working with the **NucleoSpin® 96 RNA Plant and Fungi** and **NucleoSpin® 96 RNA Plant and Fungi Core** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***).

Caution: Guanidine hydrochloride in Buffer PFL can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® 96 RNA Plant and Fungi** and **NucleoSpin® 96 RNA Plant and Fugi Core** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocols

5.1 General recommendations

Please refer to the following for choosing the optimal protocol, sample amount and buffer volumes using MN Bead Tubes Type G or MN Bead Plates Type G for disruption. Other disruption techniques might require reduced sample input.

Table 2: Recommendations for different sample types

	Sample amount per preparation	Buffer PFR	Buffer PFB	Recommended protocol
Samples rich in secondary metabolites				
Grape vine leaf	100 mg	50 µL	500 µL	5.1
Noble fir	50 mg	20 µL	500 µL	5.1
Spruce needle	50 mg	20 µL	500 µL	5.1
Ginger rhizome	500 mg	50 µL	500 µL	5.1
Fruit tissue				
Kiwi	500 mg	20 µL	750 µL	5.2
Citrus fruit	500 mg	20 µL	750 µL	5.2
Apple	500 mg	10 µL	750 µL	5.2
Grape berry	500 mg	50 µL	750 µL	5.1
Blueberry	500 mg	20 µL	500 µL	5.2
Tomato	500 mg	20 µL	750 µL	5.1
Leaves				
Tobacco	100 mg	50 µL	500 µL	5.1
Wheat	100 mg	20 µL	500 µL	5.1
Maize	100 mg	20 µL	500 µL	5.1
Arabidopsis	100 mg	20 µL	500 µL	5.1
Samples with high starch content				
Maize kernel	100 mg	50 µL	500 µL	5.1
Wheat kernel	90 mg	20 µL	500 µL	5.1
Potato tuber	50 mg	50 µL	500 µL	5.1

Table 2: Recommendations for different sample types				
	Sample amount per preparation	Buffer PFR	Buffer PFB	Recommended protocol
Other seeds				
Arabidopsis seeds	100 seeds	20 µL	750 µL	5.1
Alfalfa seed	50 mg	20 µL	750 µL	5.1
Cotton seed	1 seed (~100 mg)	20 µL	750 µL	5.1
Roots				
Alfalfa root	300 mg	10 µL	500 µL	5.1
Pea root	180 – 280 mg	20 µL	500 µL	5.1
Sugar beet (root)	500 mg	10 µL	500 µL	5.1
Other sample types				
Sugar cane (stem)	500 mg	20 µL	500 µL	5.1
Fungal hyphae	50 mg	20 µL	750 µL	5.2
Fungal fruiting body	50 – 100 mg	10 µL	500 µL	5.1
Moss	100 mg	10 µL	500 µL	5.1

For sample types, specifically for RNA isolation from acidic samples (e.g. fruits) and other samples, it is advisable to supplement the lysing reaction with Buffer PFN.

Table 3: Recommended volume of Buffer PFN (not included)	
Sample type (fruit tissue)	Buffer PFN per preparation
Kiwi	50 µL
Lemon	50 µL
Apple	15 µL
Orange	15 µL
Blueberry	50 µL
Fungal hyphae	0 – 50 µL

5.2 NucleoSpin® 96 RNA Plant and Fungi@ vacuum processing

- For hardware requirements, refer to section 2.4.
- For detailed information regarding the vacuum manifold setup, see page 19.
- For detailed information about each step, see page 20.
- For use of the NucleoSpin® 96 RNA Plant and Fungi Core Kit (REF 740129.4), refer to section 2.5 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer PFW2 was prepared according to section 3.

Protocol at a-glance

1	**Harvest**	**Provide plant or fungi sample material in a disruption tube**
2	**Lyse plant material**	**550 µL PFL + 10 – 50 µL PFR and mechanically homogenize**
	MN Bead Tube Type G:	
	Optional: Heat incubation	**56 °C, 5 min**
	Clear lids and break foam	**Remove steel beads with a magnet** **14,000 x *g* 1 min**
	MN Bead Plate Type G	
	Clear lids	**2,000 x *g*, 5 min** **Fill up to 550 µL with PFL**
3	**Filter lysate**	**Assemble filtration setup** **Load samples** **-0.2 to -0.4 bar[1], 1 min**
4	**Adjust binding conditions** Mix by pipetting up and down at least 2 – 3 times or shaking	**550 µL PFB** **Assemble binding setp**
5	**Transfer** clarified lysates to NucleoSpin® RNA Plant and Fungi Binding Plate	**Load 500 µL of clarified sample**
6	**Bind** RNA to silica membrane of the NucleoSpin® RNA Plant and Fungi Binding Plate	**-0.2 to -0.4 bar[1], 1 min**

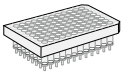
[1] Reduction of atmospheric pressure

7	**Wash** silica membrane	**500 µL PFW1** **500 µL PFW2** **500 µL PFW2** **– 0.2 to -0.4 bar[1], 1 min each step** **Remove MN Wash Plate**
8	**Dry** NucleoSpin® RNA Plant and Fungi Binding Plate by applying vacuum *Optional: Dry the outlets of the NucleoSpin® RNA Plant and Fungi Binding Plate by placing it on a Paper Sheet before applying vacuum*	**Maximum vacuum (-0.6 bar[1]) 10 min**
9	**Elute** RNA	**100 µL RNase-free H_2O** **Incubate 1 min** **-0.6 bar[1],1 min**

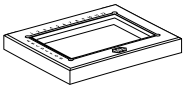
[1] Reduction of atmospheric pressure

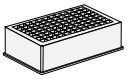
Setup of vacuum manifold:

Filtration setup

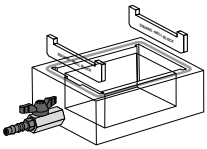


NucleoSpin® Stool Filter Plate



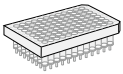


Square-well Block

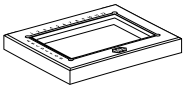


Manifold base with spacers 'SQUARE-WELL BLOCK' inserted

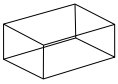
Binding setup



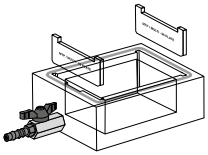
NucleoSpin® Stool Binding Plate



MN Wash Plate

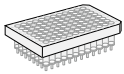


Waste Container

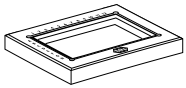


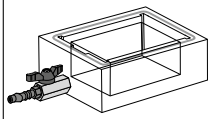
Manifold base with spacers 'MTP/MULTI-96 PLATE' inserted

Drying setup



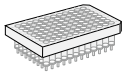
NucleoSpin® Stool Binding Plate



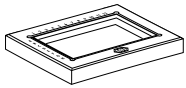


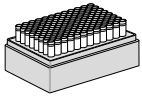
Manifold base without spacers

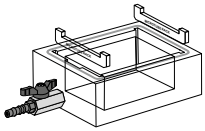
Elution setup



NucleoSpin® Stool Binding Plate







Manifold base with spacers 'MICROTUBE RACK' inserted

Detailed protocol

- For hardware requirements, refer to section 2.3.
- For detailed information regarding the vacuum manifold setup, see page 19.
- For use of the NucleoSpin® 96 RNA Plant and Fungi Core Kit (REF 740129.4), refer to section 2.4 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer PFW2 was prepared according to section 3.

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 are not recommended due to high risk of RNA degradation during sample thawing.

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)

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 risc of filter clogging an can increase RNA yield. However, head incubation may reduce RNA quality (reduced RIN values).

[1] Reduction of atmospheric pressure

4 Preparation of lysates for filtration

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

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.

5 Prepare NucleoVac 96 Vacuum Manifold

Insert spacers 'SQUARE-WELL BLOCK', notched side up, into the grooves located on the short sides of the manifold. Insert the Square-well Block on top of the spacers in the manifold base.

Place the vacuum manifold's lid onto the manifold.

Place the NucleoSpin® 96 RNA Plant and Fungi Filter Plate (white rings) into the manifold's lid.

Note: Seal unused wells with a self-adhering foil (see section 2.3)

6 Filtration of lysates

Load **500 µL lysate** onto the NucleoSpin® 96 Plant and Fungi Filter Plate.

Apply vacuum until all lysates have passed through the wells (-0.2 to -0.4 bar; approximately 1 min, but at least until all lysates passed through the filter). Release the vacuum.

Note: Overlay crude lysate on the NucleoSpin® 96 RNA Plant and Fungi Filter Plate slowly (50 µL/s) with 100 µL Buffer PFW2 in case of excessive foaming.

Discard NucleoSpin® 96 Plant and Fungi Filter Plate.

7 Prepare binding

Add **500 µL Buffer PFB** to the flowthrough in the 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 Prepare NucleoVac 96 Vacuum Manifold

Insert spacers 'MTP/MULTI-96 PLATE', notched side up, into the grooves located on the short sides of the manifold. Insert the waste container into the center of the manifold. Place the MN Wash Plate on top of the spacers in the manifold base.

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

Place a NucleoSpin® RNA Plant and Fungi Binding Plate into vacuum manifold's lid.

Load up to 1 mL of the binding mixture from step 7 onto the binding plate.

10 Bind RNA to silica membrane

Apply vacuum until all lysates have passed through the wells (-0.2 to -0.4 bar[1]; approximatel 1 min). Increase binding time until all lysates have passed through the silica. Release the vacuum.

11 Wash silica membrane

1st wash

Add **500 µL Buffer PFW1** to each well of the NucleoSpin® RNA Plant and Fungi Binding Plate. Apply vacuum (-0.2 to -0.4 bar[1]; 1 min) until all buffer has passed through the wells. Release the vacuum.

12 2nd wash

Add **500 µL Buffer PFW2** to each well of the NucleoSpin® RNA Plant and Fungi Binding Plate. Apply vacuum (-0.2 to -0.4 bar[1]; 1 min) until all buffer has passed through the wells. Release the vacuum.

3rd wash

Add **500 µL Buffer PFW2** to each well of the NucleoSpin® RNA Plant and Fungi Binding Plate. Apply vacuum (-0.2 to 0.4 bar[1]; 1 min) until all buffer has passed through the wells. Release the vacuum.

13 Remove MN Wash Plate and reassemble the vacuum manifold

After the final wash step, close the valve, release the vacuum and remove the NucleoSpin® RNA Plant Fungi Binding Plate from the vacuum manifold. Put it on a clean paper sheet (supplied with the MN Wash Plate) to remove residual EtOH-containing wash buffer. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

14 Dry NucleoSpin® RNA Binding Plate

Insert a clean Waste Container into the chamber (no spacer required).

Insert the NucleoSpin® RNA Binding Plate into the manifold lid and close the manifold. Build up the vacuum with the valve closed. Once the **maximum vacuum (-0.6 bar[1])** is achieved, open the valve and apply vacuum for at least **10 min** to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer PFW2 inhibits enzymatic reactions and has to be removed completely before eluting RNA.

Finally, release the vacuum and recover the NucleoSpin® RNA Plant and Fungi Binding Plate.

[1] Reduction of atmospheric pressure

15 Elute RNA

Assemble elution setup: Insert spacers "MICROTUBE RACK" into the vacuum manifold base. Place the Rack of Tube Strips onto the spacers. Close the manifold with the manifold lid and place the NucleoSpin® RNA Plant and Fungi Binding Plate back on top of the lid (see Elution Setup on page 19)

Pipette **100 µL RNase-free H_2O** directly to the bottom of each well (100 µL are recommended, 50 – 150 µL are possible, see section 2.8). Incubate for **1 min** at **room temperature**. Build up the vacuum with the valve closed. Once the **maximum vacuum (-0.6 bar[1])** is achieved, open the valve and apply vacuum for 1 min.

[1] Reduction of atmospheric pressure

5.3 Removal of DNA

In case samples with high initial DNA content are analyzed by downstream applications highly sensitive towards DNA contamination, an additional DNA digest might be required. Protocols for DNase treatments are given below. The rDNase Set (see ordering information) is required for this procedure.

Protocol A: DNA digestion in solution

1 Digest DNA (Reaction setup)

Add **6 µL Reaction Buffer for rDNase** and **0.6 µL rDNase** to **60 µL eluted RNA**.

(Alternatively premix 100 µL Reaction Buffer for rDNase and 10 µL rDNase and add 1/10 volume to one volume of RNA eluate). Gently swirl the tube in order to mix the solution. Spin down gently (approx. 1 s at 1,000 x *g*) to collect every droplet of the solution at the bottom of the tube.

2 Incubate sample

Incubate for **10 min** at **37 °C**.

Repurify RNA

Repurify RNA with a suitable RNA cleanup procedure, for example by use of the NucleoSpin® RNA Clean-up, NucleoSpin® RNA Clean-up XS kits (see ordering information), or by ethanol precipitation.

Ethanol precipitation, exemplary

Add **0.1 volume** of **3 M sodium acetate, pH 5.2** and **2.5 volumes** of **96 – 100 % ethanol** to **one volume of sample**. Mix thoroughly.

Incubate **several minutes** to **several hours** at **-20 °C** or **4 °C**.

Note: Choose long incubation times if the sample contains low RNA concentration.

Short incubation times are sufficient if the sample contains high RNA concentration.

Centrifuge for **10 min** at **maximum speed**.

Wash RNA pellet with 70 % ethanol.

Dry RNA pellet and resuspend RNA in RNase-free H_2O.

Protocol B: On-column DNA digestion

1 Reconstitution of rDNase

Add 4 mL Reaction Buffer for rDNase into a rDNase Vial Size F and dissolve the DNase.

2 On-column digestion into purification procedure

Follow the purification procedure according to section 5.2 until the column has been washed with 500 µL Buffer PFW1 (in step 11).

Apply **95 µL rDNase reaction mixture** directly onto the center of the silica membrane of the column.

Incubate at **room temperature** for **15 min**.

Continue the procedure 5.1, step 5, by adding 500 µL Buffer PFW2 onto the column.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded / no RNA obtained	*RNase contamination* • Create an RNase-free environment on the worktable. Clean trough reservoirs with appropriate solutions. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Do not fill back unused buffer from the trough reservoir into the bottle. • Use sterile tips with filter. *Insufficient sample quality* • Control sample harvest, storage, and lysis. Make sure that samples are harvested, stored and lysed adequately in order to preserve RNA integrity. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid nitrogen. Samples should always be kept at -70 °C. Never allow tissues to thaw before addition of Lysis Buffer. Perform disruption of samples in liquid nitrogen.
Poor RNA quality or yield	*Reagents not applied or prepared properly* • Store kit components at room temperature. Storage at low temperatures may cause salt precipitation. • Keep bottles tightly closed in order to prevent evaporation or contamination. • Prepare Buffer PFW2 by adding ethanol according to the description. *Insufficient sample disruption* • Choose a different disruption method. If one disruption method gives unsatisfactory results, try an alternative disruption method. Alternatively, use less sample material. *Fruit tissue sample not cleared prior to heat incubation* • Clear fruit tissue sample lysates and perform the heat incubation with the clear supernatant only. *Sample with high starch content was heat incubated* • Samples such as potato tubers, maize kernels, wheat kernels and similar should not be incubated at elevated temperatures during the RNA purification procedure • However, banana fruit tissue of ripe fruits should be heat incubated in order to obtain high RNA yield. • Sample disrupted in MN Bead Plate was heat incubated. If heat incubation is desired, do not exeed approx. 30 sec. in a water bath.

Problem	Possible cause and suggestions
Poor RNA quality or yield *(continued)*	*Elution* • Be sure that all of the water gets into contact with the silica membrane. No water drops should stick to the walls of the columns. The membrane has to be wetted completely. *Clogging of the NucleoSpin® RNA Plant and Fungi Binding Plate* • If using too much sample or if tissue lysate has not been successfully cleared, clogging of the NucleoSpin® RNA Plant and Fungi Binding Plate may appear. Reduce sample amount and raise time for vacuum filtration steps to prevent this. If clogging happens during the run, take the remaining lysate off the NucleoSpin® RNA Plant and Fungi Binding Plate, discard it, and proceed with the next step.
Suboptimal performance of RNA in downstream experiments	*Carry over of ethanol* • Be sure to remove all of ethanolic Buffer PFW2 after the final wash step. Dry the NucleoSpin® RNA Plant and Fungi Binding Plate for at least 10 min with maximum vacuum.
Insufficient vacuum pressure	*Vacuum pressure is not sufficient* • Check if the vacuum manifold lid fits tightly on the manifold base while vacuum is applied.
Insufficient buffer volumes	*Buffer volumes are not enough* • Buffers are delivered in sufficient, but limited amounts. Calculate the required buffer volumes and pour an additional amount of 10 % into the reservoirs if using a robotic platform. Do not fill back unused buffer from reservoir into the bottle to avoid contaminations. Ask technical service for extended buffer volumes.
Cross-contamination	*Splattering of eluate* • Reduce the vacuum strength during the elution step. Alternatively a Round-well Block or Rack of Tube Strips (see ordering information) can be used for collecting the eluate if a higher vacuum strength is required during the elution. • Be sure that no liquid drops out of the tips while moving the tips above the binding plate.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 96 RNA Plant and Fungi	740128.1 740128.4	1 × 96 preps 4 × 96 preps
NucleoSpin® 96 RNA Plant and Fungi Core Kit	740129.4	4 × 96 preps
Lysis Buffer PFL	740122.30	30 mL
Reduction Buffer PFR	740123.5	5 mL
Neutralization Buffer PFN	740121.5	5 mL
Wash Buffer PFW1	740119.30	30 mL
MN Bead Tubes Type D	740814.50	50
MN Bead Tubes Type G	740817.50	50
MN Bead Plates Type G	740855.1 740855.4	1 4
MN Beads Type D (bulk)	740814.B.1000	500 g
MN Beads Type G (bulk)	740817.B.250	500 g
TCEP	740395.107	107 g
MN Square-well Block	740476 740476.24	4 24
Square-well Block (8 x Square-well Block required for 4 × 96 samples)	740481 740481.24	4 24
Round-well Block Low, U-bottom (set consists of 1 Round-well Block Low, U-bottom and Self adhering PE Foil)	740487 740487.24	4 sets 24 sets
Round-well Block with Cap Strips (set consists of 1 Round-well Block 12 Cap Strips)	740475 740475.24	4 sets 24 sets
Rack of Tube Strips with Cap Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Elution Plate U-bottom (set consists of Elution Plate U-bottom and Self adhering PE Foil)	740486.24	24 sets
Cap Strips	740478 740478.24	48 288
MN Wash Plate	740479 740479.24	4 24

Product	REF	Pack of
Self adhering PE Foil	740676	50
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
MN Frame	740680	1

Visit ***www.mn-net.com*** for more detailed product information.

6.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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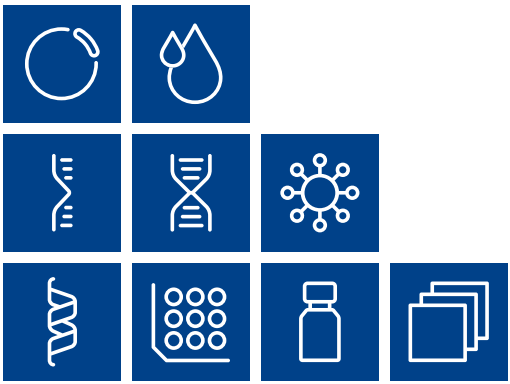
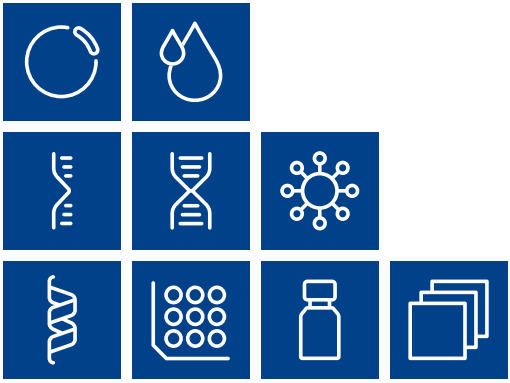


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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