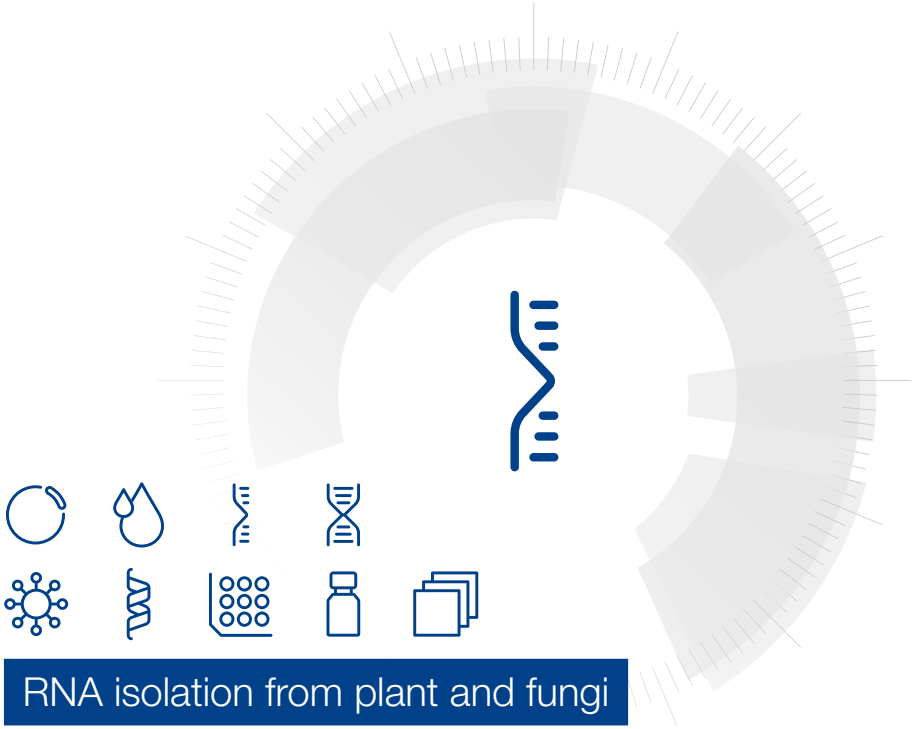
Bioanalysis

MACHEREY-NAGEL

User manual



RNA isolation from plant and fungi

■ NucleoSpin® RNA Plant and Fungi

December 2021 / Rev. 04

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RNA isolation from plant and fungi

Protocol at a glance (Rev. 04)

		NucleoSpin® RNA Plant and Fungi
1 Homogenize and lyse sample	 	500 µL PFL 10–50 µL PFR Mix 56 °C, 5 min 14,000 x *g*, 1 min
2 Filtrate lysate	 	Load lysate 14,000 x *g*, 1 min
3 Adjust RNA binding conditions		500 µL PFB Mix RT, 5 min
4 Bind RNA	 	Load 650 µL sample 14,000 x *g*, 30 s Load residual sample 14,000 x *g*, 30 s
5 Wash silica membrane	 	1st wash 500 µL PFW1 2nd wash 500 µL PFW2 3rd wash 500 µL PFW2 14,000 x *g*, 1 min after each washing step
6 Elute RNA	 	50 µL RNase-free H_2O RT, 1 min 14,000 x *g*, 1 min

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1 Components

1.1 Kit contents

	NucleoSpin® RNA Plant and Fungi		
REF	**10 preps 740120.10**	**50 preps 740120.50**	**250 preps 740120.250**
Lysis Buffer PFL	8 mL	30 mL	150 mL
Reduction Buffer PFR	5 mL	5 mL	20 mL
Binding Buffer PFB	10 mL	45 mL	200 mL
Wash Buffer PFW1	8 mL	30 mL	150 mL
Wash Buffer PFW2 (concentrate)*	6 mL	25 mL	3 x 25 mL
RNase-free H_2O	13 mL	13 mL	60 mL
NucleoSpin® RNA Plant and Fungi Filter	10	50	250
NucleoSpin® RNA Plant and Fungi Columns (light blue rings – plus Collection Tube)	10	50	250
Collection Tubes (2 mL)	30	150	750
Collection Tubes (1.5 mL)	10	50	250
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* For preparation of working solutions and storage, see section 3.

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

Reagents

- 96–100 % ethanol (for preparation of Buffer PFW2)
- Neutralization Buffer PFN for processing acidic samples (see section 6.3 for ordering information)

Consumables

- Disposable pipette tips
- NucleoSpin® Bead Tubes Type G (optional, see section 6.3 for ordering information)

Equipment

- Manual pipettes
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization (see section 2.4)
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoSpin® RNA Plant and Fungi** kit before using this product. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available online at ***www.mn-net.com***.

Please contact Technical Service regarding information about any changes to the current user manual compared with previous revisions.

2 Product description

2.1 The basic principle

The **NucleoSpin® 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 NucleoSpin® Bead Tubes, 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® Plant and Fungi Filter, 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 °C for long-term storage.

2.2 Kit specifications

- **NucleoSpin® RNA Plant and Fungi** is recommended for the isolation of RNA from diverse plant tissues and organs as well as filamentous fungi. The kit is not suitable for the isolation of small RNA (< 200 nt).
- Typically, 50–500 mg sample input is recommended per preparation. Please refer to Table 2 (page 11, f) for detailed recommendations.
- **NucleoSpin® RNA Plant and Fungi Filters** for removal of tissue debris are included in the kit.
- The kit allows the isolation of up to 70 µg RNA, suitable for downstream applications such as qRT-PCR, cDNA synthesis, Northern blotting and others.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® RNA Plant and Fungi
Format	Mini spin column
Sample material	< 500 mg plant / fungal material
Fragment size	> 200 nt
Typical yield	20–70 µg
A_{260}/A_{280}	1.9–2.1
A_{260}/A_{230}	~ 2
Typical RIN (RNA Integrity Number)	7–9
Elution volume	50 µL
Preparation time	25 min/6 preps
Binding capacity	200 µg

2.3 Handling, preparation, and storage of starting materials

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.

2.4 Lysis and disruption of sample material

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

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).

Bead tubes

NucleoSpin® Bead Tubes Type G (see section 6.3 for ordering information) are recommended in combination with a swing-mill (e.g., MM200, MM300, MM400

(Retsch®) for most plant materials. Bead Tubes typically give highest yield, avoid any cross-contamination, and enable time efficient sample disruption.

The MN Bead Tube Holder should not be used for disruption of plant material with NucleoSpin® Bead Tubes Type G because it is usually insufficient.

2.5 Elution procedures

It is possible to adapt the elution method and elution volume in order to achieve optimal RNA concentrations for the respective downstream application. In addition to the standard method described in the individual protocols (recovery rate about 70–90 %), modifications are possible.

- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. About 90–100 % of bound nucleic acid will be eluted.
- **High yield and high concentration:** Elute with the standard elution volume and apply the eluate once more onto the column for re-elution.

Eluted RNA should immediately be kept on ice for optimal stability. For short-term storage freeze at -20 °C, for long-term storage freeze at -70 °C.

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 for at least one year. Storage at lower temperatures may cause precipitation of salts.

During storage, especially at low temperatures, a precipitate may form in Buffer PFN. Such precipitates can be easily dissolved by incubating the bottle at 40 °C before use.

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. 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® RNA Plant and Fungi			
REF	**10 preps** **740120.10**	**50 preps** **740120.50**	**250 preps** **740120.250**
Wash Buffer PFW2 (concentrate)	6 mL Add 24 mL ethanol	25 mL Add 100 mL ethanol	3 x 25 mL Add 100 mL ethanol to each bottle

4 Safety instructions

When working with the **NucleoSpin® RNA Plant and Fungi** 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 ***http://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® RNA Plant and Fungi** 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 to 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

Please refer to Table 2 for choosing the optimal protocol, sample amount and buffer volumes.

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	500µL	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 (~100mg)	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

5.1 RNA isolation from plant and fungal material

Before starting the preparation:

- Check if Wash Buffer PFW2 was prepared according to section 3.
- During storage, especially at low temperatures, a precipitate may form in Buffer PFN. Such precipitates can be easily dissolved by incubating the bottle at 40 °C before use.

1 Homogenize sample

Option A: Mortar, pestle, and liquid nitrogen

500 µL PFL
10–50 µL PFR

Mix

Add **500 µL Buffer PFL** into a 1.5 or 2 mL microcentrifuge tube (not provided).

Add **10–50 µL Buffer PFR** to the tube. See table 2 for optimal volume of Buffer PFR.

Precool mortar and pestle with liquid nitrogen or at -70 °C in a freezer.

Add the sample into the mortar containing liquid nitrogen. For optimal sample input, follow the recommendations given in Table 2.

Grind sample

Grind sample under liquid nitrogen until a fine powder is obtained.

Transfer sample

56 °C, 5 min

Transfer sample to the Buffer PFL / PFR mixture and mix immediately. The plant material shall only thaw within the lysis buffer.

Incubate lysis tube for **5 min** at **56 °C**.

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

14,000 x *g*, 1 min

Centrifuge for **1 min** at **14,000 x *g*** in order to sediment cell debris.

Note: If the cell debris pellet is not sufficiently solid, centrifuge for a longer time (e.g., 3 min) and / or at 20,000 x g.

Continue with the clear supernatant.

Option B: Bead Tubes (not provided)

500 µL PFL
10–50 µL PFR

Add **500 µL Buffer PFL** into **NucleoSpin® Bead Tubes Type G**.

Add **10–50 µL Buffer PFR** to the tube. See table 2 for optimal volume of Buffer PFR.

Transfer sample

Transfer sample to the NucleoSpin® Bead Tube Type G. For optimal sample input, follow the recommendations given in table 2.

Place the Bead Tube into a swing-mill and **agitate twice** for **30 s** at 30 Hz with intermediate position change (please refer to the manufacturers' instructions for proper use of the instrument).

Agitate
2 x 30 s

Incubate NucleoSpin® Bead Tube Type G for **5 min** at **56 °C**.

56 °C, 5 min

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

Remove steel balls from the Bead Tube.

!

Attention: Removal of steel balls is necessary in order to avoid tube damage during subsequent centrifugation.

14,000 x *g*,
1 min

Centrifuge for **1 min** at **14,000 x *g*** in order to sediment cell debris.

Note: If the cell debris pellet is not sufficiently solid, centrifuge for a longer time (e.g. 3 min) and/or at 20,000 x g.

Continue with the clear supernatant.

2 Filtrate Lysate

Insert a **NucleoSpin® RNA Plant and Fungi Filter Column** (green ring) into a Collection Tube (2 mL, provided).

Load lysate

Note: Alternatively use a 2 mL microcentrifuge tube with lid (not provided). This facilitates mixing by vortexing in step 3.

Load the clear **lysate** from step 1 onto the column.

14,000 x *g*, 30 s

Centrifuge for **1 min** at **14,000 x *g***.

Note: In some cases a small pellet will form. This pellet does not have to be removed and can be processed together with the supernatant.

Note: If the sample does not pass the column completely, centrifuge at 20,000 x g for additional 3 min.

3 Adjust RNA binding conditions

Add **500 µL Buffer PFB** to the flowthrough and mix by pipetting.

500 µL PFB

Note: Please refer to Table 2 for recommendations on Buffer PFB increase for certain sample types.

RT, 5 min

Incubate for **5 min** at **room temperature**.

4 Bind RNA

For each preparation take one **NucleoSpin® RNA Plant and Fungi Column** (light blue ring) preassembled with a Collection Tube.

Load 650 µL sample

Load 650 µL of the sample onto the NucleoSpin® RNA Plant and Fungi column.

14,000 x *g*, 30 s

Centrifuge for **30 s** at **14,000 x *g***.

Discard the flowthrough and reuse the collection tube.

Load the **residual sample** volume (approx. 200 µL) onto the column.

Load residual sample

Centrifuge for **30 s** at **14,000 x *g***.

Discard collection tube with flowthrough and insert the column into a fresh Collection Tube (2 mL, provided).

14,000 x *g*, 30 s

5 Wash and dry silica membrane

1st wash

500 µL PFW1

Add **500 µL Buffer PFW1** onto the column.

Centrifuge for **1 min** at **14,000 x *g***.

14,000 x *g*, 1 min

Discard collection tube with flowthrough and insert column into a fresh Collection Tube (2 mL, provided).

2nd wash

500 µL PFW2

Add **500 µL Buffer PFW2** onto the column.

Centrifuge for **1 min** at **14,000 x *g***.

14,000 x *g*, 1 min

Discard flowthrough and reuse collection tube.

3rd wash

500 µL PFW2

Add **500 µL Buffer PFW2** onto the column.

Centrifuge for **1 min** at **14,000 x *g***.

14,000 x *g*, 1 min

Discard flowthrough and discard collection tube unless the following additional wash step is included.

<u>Optional:</u> For some samples an additional wash step is recommended. These samples cause a discoloring of the silica or the eluate after the 3rd washing step. Such samples are e.g., conifer needles, blueberry fruits, and grape leaves.

Add 500 µL Wash Buffer PFW2 onto the column.

*Centrifuge for **1 min** at **14,000 x g**.*

Discard collection tube with flowthrough.

6 Elute RNA

50 µL RNase-free H_2O

RT, 1 min

Insert column into a fresh Collection Tube (1.5 mL, provided).

Add **50 µL RNase-free H_2O** onto the column.

Incubate for approximately **1 min** at **room temperature**.

14,000 x *g*, 1 min

Centrifuge for **1 min** at **14,000 x *g***.

If higher RNA concentrations are desired, elution can be done with 40 µL. Overall yield, however, will decrease when using smaller volumes.

For further alternative elution procedures see section 2.5.

5.2 RNA isolation from acidic samples (e.g., fruits) and other samples

Before starting the preparation:

- Check if Wash Buffer PFW2 was prepared according to section 3.
- Check if Neutralization Buffer PFN is available (see section 6.3 for ordering information).

Table 3: Recommended volume of Buffer PFN

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

1 Homogenize sample

Option A: Mortar, pestle, and liquid nitrogen

Add **500 µL Buffer PFL** into a 1.5 or 2 mL microcentrifuge tube (not provided).

500 µL PFL
10–50 µL PFR
10–50 µL PFN

Add **10–50 µL Buffer PFR** to the tube. See Table 2 for optimal volume of Buffer PFR.

Add 1**0–50 µL Buffer PFN** to the tube. See Table 3 below for recommended volume of Buffer PFN.

Mix

Precool mortar and pestle with liquid nitrogen or at -70 °C.

Add **500 mg sample** to the mortar containing liquid nitrogen.

Grind sample in liquid nitrogen until a fine powder is obtained.

Grind sample

Transfer sample to the microcentrifuge tube containing the buffer mixture and mix immediately. The plant material shall only thaw within the lysis buffer.

Transfer sample

Centrifuge for **1 min** at **14,000 x *g*** in order to sediment cell debris.

14,000 x *g*, 1 min

<u>Note:</u> If the cell debris pellet is not sufficiently solid, centrifuge for a longer time (e.g., 3 min) and/or at 20,000 x g.

Transfer the clear **supernatant** to a fresh tube (not provided).

Transfer supernatant

<u>Note:</u> For acidic samples it is important to remove cell debris prior to heat incubation.

Incubate lysis tube for **5 min** at **56 °C**.

56 °C, 5 min

Option B: NucleoSpin® Bead Tubes Type G (not provided)

Add **500 µL Buffer PFL** into **NucleoSpin® Bead Tube Type G**.

500 µL PFL
10–50 µL PFR
10–50 µL PFN

Add **10–50 µL Buffer PFR** to the tube. See Table 2 for optimal volume of Buffer PFR.

Add **10–50 µL Buffer PFN** to the tube. For an appropriate amount see the Table 3.

Transfer sample

Transfer **500 mg sample material** into the NucleoSpin® Bead Tube Type G.

Place the Bead Tube into a swing-mill and **agitate twice** for **30 s** at 30 Hz with intermediate position change (please refer to the manufacturers' instructions for proper use of the machine).

Agitate 2 x 30 s

Remove steel balls from the NucleoSpin® Bead Tube Type G.

! *Attention: Removal of steel balls is necessary in order to avoid tube damage during subsequent centrifugation.*

Centrifuge for **1 min** at **14,000 x *g*** in order to sediment cell debris.

14,000 x *g*, 1 min

<u>Note:</u> If the cell debris pellet is not sufficiently solid, centrifuge for a longer time (e.g. 3 min) and/or at 20,000 x g

Transfer supernatant

Transfer the clear **supernatant** into a fresh tube (not provided).

<u>Note:</u> For acidic samples it is important to remove the cell debris before heat incubation.

Incubate sample for **5 min** at **56 °C**.

56 °C, 5 min

Continue with protocol 5.1, step 2: "Filtrate lysate"

6 Appendix

6.1 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.

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**.

3 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

Reconstitution of rDNase

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

On-column digestion into purification procedure

Follow the purification procedure according to section 5.1 until the column has been washed with 500 µL Buffer PFW1 (in step 5).

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.

6.2 Troubleshooting

Problem	Possible cause and suggestion
Clogged NucleoSpin® RNA Plant and Fungi Filter	*Too much sample material* • Use less sample material and / or centrifuge for 3 min at 20,000 x *g*.
Poor RNA quality or yield	*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.
Poor RNA purity and or colored silica membrane / eluate	*Washing steps not sufficient* • Perform an additional wash step with Buffer PFW1.

Problem	Possible cause and suggestion
	RNase contamination
	• Create an RNase-free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250 °C before use.
	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	*Insufficient sample disruption*
	• Choose a different disruption method. If one disruption method gives unsatisfactory results, try an alternative disruption method.
	Reagents not applied or restored properly
	• Prepare Buffer PFW2 by adding ethanol according to the description.
	• Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added.
	Kit storage
	• Store kit components at room temperature. Storage at low temperature my cause salt precipitation.
	• Keep bottles tightly closed in order to prevent evaporation or contamination

Problem	Possible cause and suggestion
Poor RNA quality or yield *(continued)*	*Ionic strength and pH influence A_{260} absorption as well as ratio A_{260}/A_{280}* • For adsorption measurement, use 5 mM Tris pH 8.5 as diluent. Please see also: - Manchester, K L. 1995. Value of A_{260} / A_{280} ratios for measurement of purity of nucleic acids. Biotechniques 19, 208–209. - Wilfinger, W W, Mackey, K and Chomczyski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 22, 474–481.
Low A_{260}/A_{230} ratio	*Carry-over of contaminants* • Carefully load the lysate to the NucleoSpin® RNA Plant and Fungi Column and try to avoid a contamination of the upper part of the column and the column lid. • Make sure that a sufficient amount / concentration of RNA is used for quantification so that the A_{230} value is significantly higher than the background level. • Measurement of low amount / concentration of RNA will cause unstable A_{260}/A_{230} ratio values.
Contamination of RNA with genomic DNA	*Too much cell material used* • Reduce quantity of sample material used. *DNA detection system too sensitive* • The amount of DNA contamination is reduced by the NucleoSpin® RNA Plant and Fungi Filter Column. However, dependent on the sample type and amount, it can not be guaranteed that the purified RNA is 100 % free of DNA. Therefore, in very sensitive applications, it might still be possible to detect DNA. The probability of DNA detection with PCR increases with: - the number of DNA copies per preparation: single copy target < plastidial / mitochondrial target < plasmid transfected into cells - decreasing of PCR amplicon size. • Use larger PCR targets (e.g., > 500 bp) or intron spanning primers if possible. • Use one of the support protocol, section 6.1, for subsequent DNA digestion in solution or on-column.

Problem	Possible cause and suggestion
Suboptimal performance of RNA in downstream experiments	*Carry-over of ethanol or salt* • Do not let the flowthrough touch the column outlet after the wash steps. Be sure to centrifuge at the corresponding speed for the respective time in order to remove last wash buffer completely. • Check if wash buffer has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by wash buffer. *Store isolated RNA properly* • Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at -20 °C, for long term storage freeze at 70 °C.
Damaged Bead Tubes Type G	*Beads not removed from Bead Tube* • Remove steel balls from the Bead Tube by placing a magnet on top of the lid. Invert the tube once. Open the tube and remove steel balls attached to the lid.

6.3 Ordering Information

Product	REF	Preps/Pack of
NucleoSpin® RNA Plant and Fungi	740120.10/.50	10/50
Lysis Buffer PFL	740122.30	30 mL
Reduction Buffer PFR	740123.5	5 mL
Neutralization Buffer PFN	740121.5	5 mL
Wash Buffer PFW2 (concentrate)	740124.12	12 mL
NucleoSpin® Bead Tubes Type G	740817.50	50
NucleoSpin® RNA Clean-up	740948.10/.50/.250	10/50/250
NucleoSpin® RNA Clean-up XS	740903.10/.50/.250	10/50/250
NucleoSpin® RNA/Protein	740933.10/.50/.250	10/50/250
NucleoSpin® TriPrep	740966.10/.50/.250	10/50/250
NucleoSpin® miRNA	740974.10/.50/.250	10/50/250
NucleoZOL	740404.200	200 mL
NucleoSpin® RNA Set for NucleoZOL	740406.10/.50	10/50
rDNase Set	740963	1
Collection Tubes (2 mL)	740600	1000

6.4 Product use restriction / warranty

NucleoSpin® RNA Plant and Fungi kit components were developed, designed and sold for research purposes only. They are suitable for in vitro uses only. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the NucleoSpin® RNA Plant and Fungi kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You

may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

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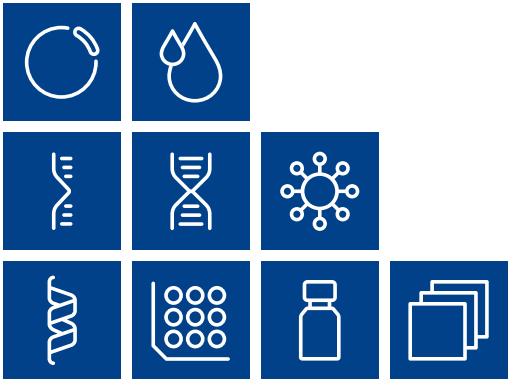

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