Bioanalysis

MACHEREY-NAGEL

User manual

Viral RNA and DNA isolation from plants

■ NucleoSpin® Plant Pathogen

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1 Components

1.1 Kit contents

	NucleoSpin® Plant Pathogen	
REF	**10 preps 740170.10**	**50 preps 740170.50**
Lysis Buffer PFL	8 mL	30 mL
Reduction Buffer PFR	5 mL	5 mL
Binding Buffer PFB	10 mL	30 mL
Wash Buffer PFW1	8 mL	30 mL
Wash Buffer PFW2 (concentrate)*	6 mL	25 mL
RNase-free H_2O	13 mL	13 mL
NucleoSpin® Plant Pathogen Columns (light blue rings)	10	50
Collection Tubes (2 mL)	30	150
Collection Tubes (1.5 mL)	10	50
Leaflet	1	1

* 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)

Consumables

- Disposable pipette tips
- NucleoSpin® Bead Tubes Type G (optional, see section 6.2 for ordering information)
- NucleoSpin® Filter Columns (optional, see section 6.2 for ordering information)

Equipment

- Manual pipettes
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization (see section 2.4)
- Thermoshaker or water bath
- 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® Plant Pathogen** 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® Plant Pathogen** kit is designed for the isolation of viral nucleic acid (RNA, DNA) from diverse plant material, e.g. tobacco, pepper, cauliflower, broccoli, radish, hemp, and hop.

First, plant material is mechanically disrupted (e.g., with a pipette tip or with 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 transfer of the lysate supernatant, a binding solution is added which creates appropriate binding conditions which favor adsorption of RNA/DNA to the silica membrane. Washing steps with two different buffers remove salts, metabolites and macromolecular cellular components. Pure RNA/DNA is finally eluted under low ionic strength conditions with RNase-free water. The eluate contains viral nucleic acids in case of infected plant material was used as sample as well as plant nucleic acids.

The RNA preparation using **NucleoSpin® Plant Pathogen** 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® Plant Pathogen** is recommended for the isolation of RNA from diverse plant tissues like leaves, stems, roots. The kit is not suitable for the isolation of small RNA (< 200 nt).
- Typically, 10 – 100 mg sample input is recommended per preparation.
- The kit allows the isolation of up to 20 µg RNA/DNA, suitable for downstream applications such as qRT-PCR, cDNA synthesis, Northern blotting and others.

Kit specifications at a glance	
Parameter	NucleoSpin® Plant Pathogen
Format	Mini spin column
Use	For research use only
Target	RNA/DNA (viral and plant)
Handling	Centrifugation
Sample material	≤ 100 mg plant material
Fragment size	> 200 nt
Typical yield	up to 20 µg total nucleic acid
A_{260}/A_{280}	1.9 – 2.1 (might vary for strongly infected plants)

Kit specifications at a glance	
A_{260}/A_{230}	~ 2 (might vary for strongly infected plants)
Typical RIN (RNA Integrity Number)	7 – 9 (for sample with strong virus symptoms, RIN might vary)
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.

Plant material crushing with a pipet tip

To release sufficient virus particle from infected plant material it is typically sufficient to crush plant material (without lysis buffer) within a 1.5 mL tube with a disposable pipet tip by grinding and crushing until some microliter of leaf juice appear. After addition of lysis buffer and mixing the supernatant is transfered into a fresh tube before addition of binding buffer.

Mortar, pestle and liquid nitrogen

This common sample disruption method can be used for most sample types. It typically gives excellent RNA quality; however, this method is not recommended due to the risk of cross contamination from sample to sample.

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.

When using bead tubes for sample disruption the subsequent filtration with a NucleoSpin® Filter (see ordering information) is recommended in order to remove debris from the lysate.

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 until: see package label. Storage at lower temperatures may cause precipitation of salts.

Such precipitates can be easily dissolved by incubating the bottle at 40 °C before use.

Before starting any NucleoSpin® Plant Pathogen 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**
Wash Buffer PFW2 (concentrate)	6 mL Add 24 mL ethanol	25 mL Add 100 mL ethanol

4 Safety instructions

When working with the **NucleoSpin® Plant Pathogen** 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® Plant Pathogen** 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

5.1 Nucleic acid isolation from plant material

Before starting the preparation:

- Check if Wash Buffer PFW2 was prepared according to section 3.
- Set thermoshaker or water bath to 56 °C.
- During storage, especially at low temperatures, a precipitate may form in some buffers. Such precipitates can be easily dissolved by incubating the bottle at 40 °C before use.

1 Homogenize sample – release of viruses

Option A: Sample crushing with pipet tip

Place approximately 10 – 100 mg sample material (typically plant leaf) into a 1.5 mL tube (not provided).

Grind sample

Using a disposable pipet tip, grind the sample by circular and stinging movements until several microliter plant juice appears.

Note: Appearance of plant juice might not be obvious for partially dried samples. For dry sample materials the homogenization option B (bead tubes) is recommended.

Add **300 µL Buffer PFL** to the sample.

300 µL PFL

Add **12 µL Buffer PFR** to the tube.

12 µL PFR

Mix

Note: A Premix of PFL and PFR can be prepared for several samples on a daily basis.

Vortex the tube for approximately 10 seconds.

Incubate lysis tube for **5 min** at **56 °C**. Mix occasionally or use the thermoshaker device.

56 °C, 5 min

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

14,000 x *g*, 1 min

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.

Transfer approximately 300 µL supernatant into a fresh tube (not provided).

Transfer sample

Option B: Bead Tubes (not provided)

300 µL PFL
12 µL PFR

Add **300 µL Buffer PFL** into **NucleoSpin® Bead Tubes Type G**.

Add **12 µL Buffer PFR** to the tube.

Transfer sample

Transfer sample to the NucleoSpin® Bead Tube Type G.

Agitate
2 × 30 s

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).

56 °C, 5 min

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

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.

Filtrate Lysate

Load lysate

Insert a **NucleoSpin® RNA Plant and Fungi Filter Column** (not provided, please inquire) into a Collection Tube (see ordering information).

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

14,000 x *g*,
30 s

Load the clear **lysate** from the bead tube onto the column.

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.

Transfer approximately 300 µL supernatant into a fresh tube (not provided).

2 Adjust nucleic acid binding conditions

300 µL PFB

RT, 5 min

Add **300 µL Buffer PFB** to the lysate and mix by pipetting.

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

3 Bind nucleic acid

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

Load the sample **(typically approx. 600 µL)** but not more than 650 µL onto the NucleoSpin Plant Pathogen column.

Load 650 µL sample

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

14,000 x *g*, 30 s

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

14,000 x *g*, 30 s

4 Wash and dry silica membrane

1st wash

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

500 µL PFW1

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

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

14,000 x *g*, 1 min

2nd wash

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

500 µL PFW2

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

14,000 x *g*, 1 min

Discard flowthrough and reuse collection tube.

3rd wash

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

500 µL PFW2

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

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

14,000 x *g*, 1 min

<u>Optional:</u>. For some plant samples comprising less than 10 mg plant material the third wash step might be omitted.

Add 500 µL Wash Buffer PFW2 onto the column.

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

Discard collection tube with flowthrough.

5 Elute nucleic acid

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

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

50 µL RNase-free H_2O

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

RT, 1 min

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

14,000 x *g*, 1 min

If higher nucleic acid 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

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestion
Clogged NucleoSpin® Plant Pathogen Column	*Too much sample material* • Use less sample material • and / or centrifuge for 3 min at 20,000 x *g* prior to addition of ethanol to the lysate • and / or centrifuge the column for 3 min at 20,000 xg after loading the ethanolic lysate onto the column .
Poor RNA/DNA quality or yield	*Highly symptomatic, partially dried or coloured leaves* • Samples in advanced stages of viral infection might show reduced nucleic acid yield. If possible, use material showing less viral infection symptoms. *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/DNA purity and or colored silica membrane / eluate	*Washing steps not sufficient* • Perform an additional wash step with Buffer PFW1.

Problem	Possible cause and suggestion
Poor RNA quality or yield	*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. *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
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.

Problem	Possible cause and suggestion
Low A_{260}/A_{230} ratio	*Carry-over of contaminants* • Carefully load the lysate to the NucleoSpin® RNA Plant Pathogen 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.
Centrifuge not capable to reach 14,000 xg	• Use 11,000 x *g* or max speed of your centrifuge. Increase centrifugation time if necessary.
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.

6.2 Ordering Information

Product	REF	Preps/Pack of
NucleoSpin® Plant Pathogen	740170.10/.50	10/50
Lysis Buffer PFL	740122.30	30 mL
Reduction Buffer PFR	740123.5	5 mL
Wash Buffer PFW1	740119.30	30 mL
Wash Buffer PFW2 (concentrate)	740939/.1	50 mL / 200 mL
NucleoSpin® Bead Tubes Type G	740817.50	50
MN 96 Bead Plate Type G	740855.1/.4	1 × 96 / 4 × 96
Collection Tubes (2 mL)	740600	1000

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.

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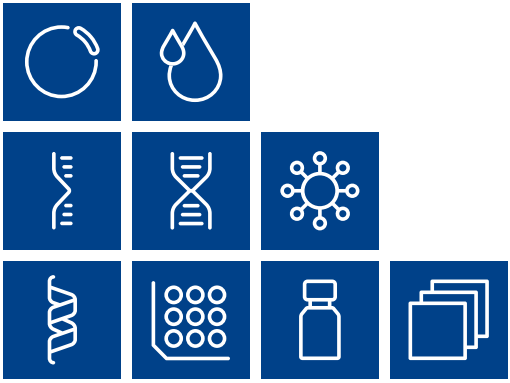

Plasmid DNA
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RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
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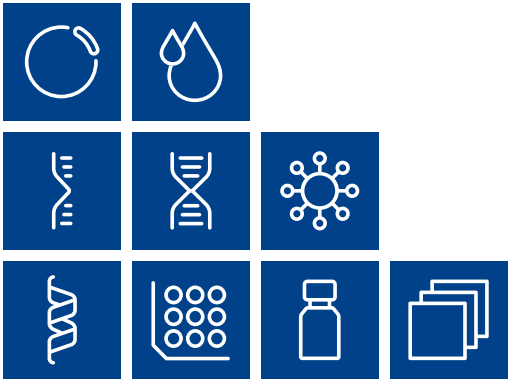

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