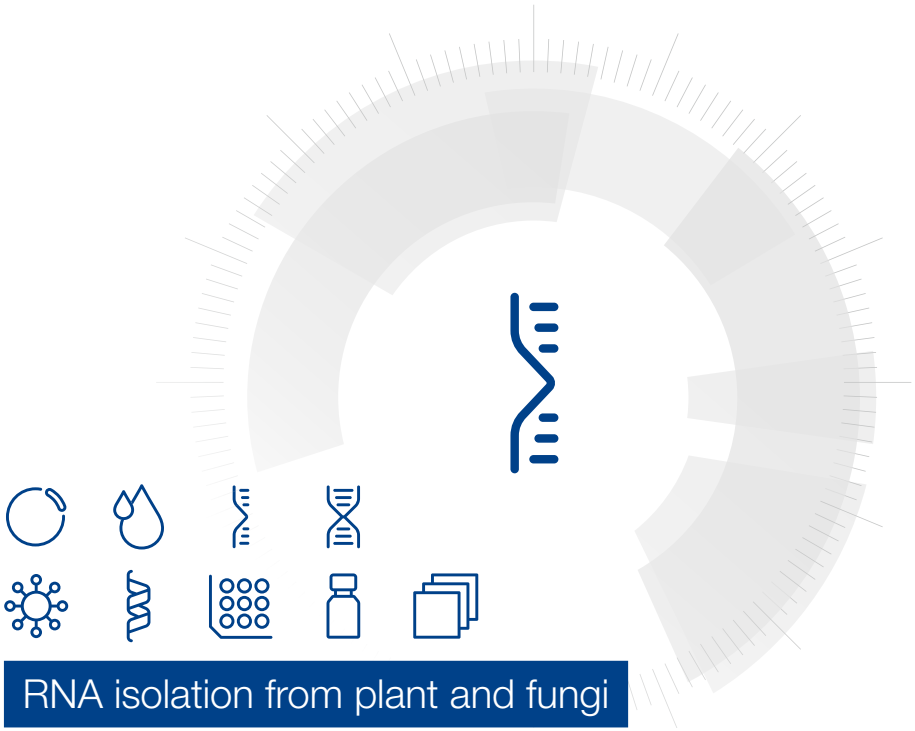
Bioanalysis

MACHEREY-NAGEL

# User manual



## RNA isolation from plant and fungi

■ NucleoSpin® RNA Plant and Fungi

May 2018 / Rev. 03

# RNA isolation from plant and fungi

## Protocol at a glance (Rev. 03)

| | | NucleoSpin® RNA Plant and Fungi |
|---|---|---|
| **1 Homogenize and lyse sample** |   | 500 µL PFL<br>10–50 µL PFR<br>Mix<br>56 °C, 5 min<br>14,000 x *g*,<br>1 min |
| **2 Filtrate lysate** |   | Load lysate<br>14,000 x *g*,<br>1 min |
| **3 Adjust RNA binding conditions** |  | 500 µL PFB<br>Mix<br>RT, 5 min |
| **4 Bind RNA** |   | Load 650 µL sample<br>14,000 x *g*,<br>30 s<br>Load residual sample<br>14,000 x *g*,<br>30 s |
| **5 Wash silica membrane** |   | 1st wash 500 µL PFW1<br>2nd wash 500 µL PFW2<br>3rd wash 500 µL PFW2<br>14,000 x *g*,<br>1 min<br>after each washing step |
| **6 Elute RNA** |   | 50 µL RNase-free $H_2O$<br>RT, 1 min<br>14,000 x *g*,<br>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 |
| User Manual | 1 | 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)
- 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**<br>**740120.10** | **50 preps**<br>**740120.50** | **250 preps**<br>**740120.250** |
| Wash Buffer PFW2 (concentrate) | 6 mL<br>Add 24 mL ethanol | 25 mL<br>Add 100 mL ethanol | 3 x 25 mL<br>Add 100 mL ethanol to each bottle |

# 4 Safety instructions

The following components of the **NucleoSpin® RNA Plant and Fungi** kit contain hazardous contents.

*Wear gloves and goggles and follow the safety instructions given in this section.*

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.
*Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.*

| Component<br>*Inhalt* | Hazard contents<br>*Gefahrstoff* | GHS symbol<br>*GHS-Symbol* | Hazard phrases<br>*H-Sätze* | Precaution phrases<br>*P-Sätze* |
|---|---|---|---|---|
| PFL | Guanidine hydrochloride 36–50 %<br>*Guanidinhydrochlorid 36–50 %*<br>CAS 50-01-1 |  <br>WARNING<br>*ACHTUNG* | 302, 319 | 264W, 280sh, 301+312, 330 |
| PFB | Lithium chloride 40–70 %<br>*Lithiumchlorid 40–70 %*<br>CAS 7447-41-8 |  <br>WARNING<br>*ACHTUNG* | 302, 315, 319 | 264W, 280sh, 301+312, 330 |

### Hazard phrases

H 302 Harmful if swallowed.
*Gesundheitsschädlich bei Verschlucken.*

H 315 Causes skin irritation.
*Verursacht Hautreizungen.*

H 319 Causes serious eye irritation.
*Verursacht schwere Augenreizung.*

### Precaution phrases

P 264W Wash with water thoroughly after handling..
*Nach Gebrauch mit Wasser gründlich waschen.*

P 280sh Wear protective gloves / eye protection.
*Schutzhandschuhe / Augenschutz tragen.*

P 301+312 IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell.
*BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt anrufen.*

P 330 Rinse mouth.
*Mund ausspülen.*

 The symbol shown on labels refers to further safety information in this section.
*Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.*

For further information please see Material Safety Data Sheets (***www.mn-net.com***).
*Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (**www.mn-net.com**).*

# 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*<br>• 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*<br>• Clear fruit tissue sample lysates and perform the heat incubation with the clear supernatant only.<br>*Sample with high starch content was heat incubated*<br>• Samples such as potato tubers, maize kernels, wheat kernels and similar should not be incubated at elevated temperatures during the RNA purification procedure<br>• 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*<br>• Perform an additional wash step with Buffer PFW2. |

| 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 $A_{260}/A_{280}$*<br>• For adsorption measurement, use 5 mM Tris pH 8.5 as diluent. Please see also:<br>- Manchester, K L. 1995. Value of $A_{260}$ / $A_{280}$ ratios for measurement of purity of nucleic acids. Biotechniques 19, 208–209.<br>- 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*<br>• 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.<br>• 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.<br>• 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*<br>• Reduce quantity of sample material used.<br>*DNA detection system too sensitive*<br>• 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:<br>- the number of DNA copies per preparation: single copy target < plastidial / mitochondrial target < plasmid transfected into cells<br>- decreasing of PCR amplicon size.<br>• Use larger PCR targets (e.g., > 500 bp) or intron spanning primers if possible.<br>• 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*<br>• 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.<br>• Check if wash buffer has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by wash buffer.<br>*Store isolated RNA properly*<br>• 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*<br>• 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

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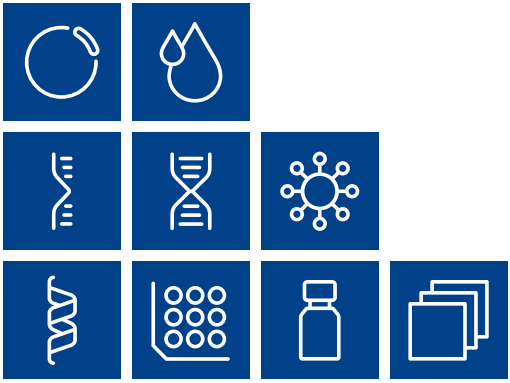

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