Total RNA isolation from plant

User manual

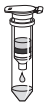
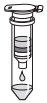
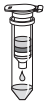
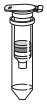
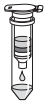
NucleoSpin® RNA Plant

February 2011 / Rev. 06

MACHEREY-NAGEL

Total RNA isolation from plant

Protocol-at-a-glance (Rev. 06)

		NucleoSpin® RNA Plant	
1	**Homogenize sample**		100 mg
2	**Lyse cells**		350 µL RA1 3.5 µL ß-mercaptoethanol or 350 µL RAP 3.5 µL ß-mercaptoethanol Mix
3	**Filtrate lysate**	 	11,000 x *g* 1 min
4	**Adjust RNA binding conditions**		350 µL 70 % ethanol Mix
5	**Bind RNA**	 	Load sample 11,000 x *g* 30 s
6	**Desalt silica membrane**	 	350 µL MDB 11,000 x *g* 1 min
7	**Digest DNA**		95 µL DNase reaction mixture RT, 15 min
8	**Wash and dry silica membrane**		1st wash: 200 µL RA2 2nd wash: 600 µL RA3 3rd wash: 250 µL RA3 1st and 2nd:  11,000 x *g*, 30 s 3rd:  11,000 x *g*, 2 min
9	**Elute highly pure RNA**	 	60 µL RNase-free H_2O 11,000 x *g* 1 min

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1 Components

1.1 Kit contents

	NucleoSpin® RNA Plant		
	20 preps	**50 preps**	**250 preps**
REF	**740949.20**	**740949.50**	**740949.250**
Lysis Buffer RA1	10 mL	25 mL	125 mL
Lysis Buffer RAP	10 mL	25 mL	125 mL
Wash Buffer RA2	15 mL	15 mL	80 mL
Wash Buffer RA3 (Concentrate)*	5 mL	12.5 mL	3 x 25 mL
Membrane Desalting Buffer MDB	10 mL	25 mL	125 mL
Reaction Buffer for rDNase	3 mL	7 mL	30 mL
rDNase, RNase-free (lyophilized)*	1 vial (size C)	1 vial (size D)	5 vials (size D)
RNase-free H_2O	5 mL	15 mL	65 mL
NucleoSpin® Filters (violet rings)	20	50	250
NucleoSpin® RNA Plant Columns (light blue rings – plus Collection Tubes)	20	50	250
Collection Tubes (2 mL)	60	150	750
Collection Tubes (1.5 mL)	20	50	250
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* For preparation of working solutions and storage conditions see section 3.

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

Reagents

- 96–100 % ethanol (to prepare Wash Buffer RA3)
- 70 % ethanol (to adjust RNA binding conditions)
- Reducing agent (ß-mercaptoethanol, **or** DTT (dithiothreithol), **or** TCEP (BisTris (Bis-(2-hydroxyethyl)-imino-tris(hydroxymethyl)-methane)) as supplement for Lysis Buffer RA1

Consumables

- 1.5 mL microcentrifuge tubes
- Sterile RNase-free pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization (see section 2.3)
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® RNA Plant** kit is used for the first time. 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 on the internet at ***www.mn-net.com***.

2 Product description

2.1 The basic principle

One of the most important aspects in the isolation of RNA is to prevent degradation of the RNA during the isolation procedure. With the **NucleoSpin® RNA Plant** method, the cells are first disrupted by grinding in the presence of liquid N_2. Complete denaturation is then achieved by incubation in a solution containing large amounts of chaotropic ions. This lysis buffer immediately inactivates RNases – which are present in virtually all biological materials – and creates appropriate binding conditions which favor adsorption of RNA to the silica membrane. Contaminating DNA is removed by an rDNase solution which is directly applied onto the silica membrane during the preparation (RNase-free rDNase is supplied with the kit). 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 H_2O (supplied).

The **NucleoSpin® RNA Plant** kit contains two different lysis buffers, RA1 (guanidinium thiocyanate) and RAP (guanidinium-HCl), respectively. In most cases, use of Buffer RA1 is recommended for lysis due to the stronger denaturing properties of the thiocyanate. The presence of peculiar metabolites in a variety of plant tissues or fungi, however, requires the use of an alternative buffer, because they may lead to solidification of the lysate, resulting in a non-processible slurry. In such cases, Buffer RAP is the buffer of choice.
Besides Buffer RA1 and Buffer RAP, MACHEREY-NAGEL offers alternatively a lysis buffer with a high detergent concentration, Buffer RL1 (see ordering information).

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

Simultaneous isolation of RNA and DNA (NucleoSpin® RNA/DNA Buffer Set*)

The NucleoSpin® RNA/DNA Buffer Set (see ordering information) is a support set for RNA and DNA isolation in conjunction with NucleoSpin® RNA II, NucleoSpin® RNA XS, NucleoSpin® RNA Plant, or NucleoSpin® RNA/Protein.

This patented technology enables successive elution of DNA and RNA from one NucleoSpin® Column with low salt buffer and water respectively. DNA and RNA are immediately ready for downstream applications.

* DISTRIBUTION AND USE OF THE NUCLEOSPIN® RNA/DNA BUFFER SET IN THE USA IS PROHIBITED FOR PATENT REASONS.

2.2 Kit specifications

- **NucleoSpin® RNA Plant** is recommended for the isolation of total RNA from plant cells and tissues or filamentous fungi.
- Generally, 1–10 % of the eluate of total RNA prepared from 10 mg of plant tissue is sufficient as template for RT-PCR. If possible, intron-spanning primers should be used for RT-PCR. Hands-on time for RNA preparation from plant tissue with **NucleoSpin® RNA Plant** is less than 30 min.
- **NucleoSpin® Filters** for homogenization and reduction of lysate viscosity are included in the kit.
- The kit allows purification of up to 70 µg of pure RNA, suitable for applications like reverse transcriptase-PCR (RT-PCR), Northern blotting, primer extension, or RNase protection assays.
- rDNase is supplied with the kit. DNA contaminations are efficiently removed by on-column digestion with rDNase. Anyhow, traces of DNA might be detected in very sensitive applications. For most demanding applications a subsequent digestion with rDNase in the eluate is possible. The NucleoSpin® RNA II / RNA Plant system is checked by the following procedure: One million HeLa cells are subjected to RNA isolation according to the protocol. RNA eluate is used as template for PCR detection of a 1 kbp fragment in a 30 cycle reaction. Generally, no PCR fragment is obtained if the DNase is applied. However, a strong PCR fragment is obtained if DNase is omitted. The eventuality of DNA detection with PCR increases with:
  1. the number of DNA copies per preparation: single copy target < plastidial/mitochondrial target < plasmid transfected into cells,
  2. decreasing of PCR amplicon size.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® RNA Plant
Format	Mini spin column
Sample material	< 100 mg tissue
Fragment size	> 200 b
Typical yield	3–70 µg from 100 mg plant material
A_{260}/A_{280}	1.9–2.1
Elution volume	60 µL
Preparation time	30 min/6 preps
Binding capacity	200 µg

2.3 Handling, preparation, and storage of starting materials

RNA is not protected against digestion 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 flash frozen in liquid N_2 immediately and stored at -70 °C or processed as soon as possible. Samples can be stored in Lysis Buffer RA1 after disruption at -70 °C for up to one year, at 4 °C for up to 24 hours or up to several hours at room temperature. Frozen samples are stable up to 6 months. Frozen samples in Buffer RA1 should be thawed slowly before starting with the isolation of total RNA.

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

Plant tissues are often solid and must therefore be broken up mechanically as well as lysed. Depending on the disruption method, the viscosity of the lysed sample has to be reduced further for optimal results. It is essential for efficient RNA preparation that all the RNA contained in the sample is released from the cells by disruption and that the viscosity of the sample is reduced by homogenization.

The most commonly used technique for disruption of plant tissues is grinding with a **pestle and mortar**. Grind the sample to a fine powder in the presence of liquid N_2. Take care that the sample does not thaw during or after grinding or weighing and add the frozen powder to an appropriate aliquot of Buffer RA1 respectively RAP containing ß-mercaptoethanol and mix immediately. The broken-up tissue must then be homogenized with a **NucleoSpin® Filter** or by passing ≥ 5 through a 0.9 mm syringe needle.

Thawing of undisrupted plant tissue should only be done in the presence of Buffer RA1 during simultaneous mechanical disruption, e.g. with a **rotor-stator homogenizer**. This ensures that the RNA is not degraded by RNases before the preparation has started. The spinning rotor disrupts and simultaneously homogenizes the sample by mechanical shearing of DNA within seconds up to minutes (homogenization time depends on sample). Take care to keep the rotor tip submerged in order to avoid excess foaming. Select a suitably sized homogenizer (5–7 mm diameter rotors can be used for homogenization in microcentrifuge tubes).

2.4 Elution procedures

It is possible to adapt elution method and volume of water used for the subsequent application of interest. In addition to the standard method described in the individual protocols (recovery rate about 70–90 %) there are several modifications 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 reelution.

Eluted RNA should immediately be put on ice and always kept on ice for optimal stability because almost omnipresent RNases (general lab ware, fingerprints, dust) will degrade RNA. For short-term storage freeze at -20 °C, for long-term storage freeze at -70 °C.

2.5 Yields with different samples

Table 2: Typical yields of total RNA per 50 mg sample

Specie	Organ	Yield
Allium cepa (onion)	Germ bud	13 µg
Allium sativum (garlic)	Leaf	13 µg
Arabidopsis thaliana (Thale cress)	Leaf	15 µg
Beta vulgaris (sugar beet)	Leaf	17 µg
Brassica napus (rapeseed)	Leaf Blossom Stalk	9 µg 9 µg 7 µg
Capsicum annuum (red pepper)	Leaf	8 µg
Cucumis melo (cucumber)	Leaf	15 µg
Gladiolus spec.	Leaf	7 µg
Hordeum vulgare (barley)	Leaf	3 µg
Lactuca sativa (lettuce)	Leaf	4 µg
Lycopersicum esculentum (tomato)	Leaf	10 µg
Mucor rouxii (fungus)	Mycelium	6 µg
Nicotiana tabacum (tabacco)	Leaf Root tip Stalk Blossom	24 µg 12 µg 18 µg 33 µg
Secale cereale (rye)	Leaf	12 µg
Taraxacum officinale (dadelion)	Leaf	10 µg
Thymus herba-barona (thyme)	Leaf	15 µg
Triticum aestivum (wheat)	Leaf	4 µg
Viola tricolor (viola)	Leaf	9 µg
Zea mays (maize)	Leaf	18 µg

3 Storage conditions and preparation of working solutions

Attention:

Buffers RA1, RA2, and MDW contain guanidinium thiocyanate; RAP contains guanidinium-HCl. Wear gloves and goggles!

- Store lyophilized **rDNase (RNase-free)** at 4 °C on arrival (stable up to 1 year).
- All other kit components should be stored at room temperature (18–25 °C) and are stable up to one year. Storage at lower temperatures may cause precipitation of salts.
- Check that 70% ethanol is available as additional solution to adjust RNA binding conditions in the Buffer RA1 lysate.

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

- **rDNase (RNase-free):** Add indicated volume of RNase-free H_2O (see table below) to the rDNase vial and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. Dispense into aliquots and store at -18 °C. The frozen working solution is stable for 6 months. Do not freeze/thaw the aliquots more than three times.
- **Wash Buffer RA3:** Add the indicated volume of 96–100 % ethanol (see table below) to Buffer RA3 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer RA3 at room temperature (18–25 °C) for up to one year.

	NucleoSpin® RNA Plant		
	20 preps	**50 preps**	**250 preps**
REF	**740949.10**	**740949.50**	**740949.250**
Wash Buffer RA3 (Concentrate)	5 mL Add 20 mL ethanol	12.5 mL Add 50 mL ethanol	3 x 25 mL Add 100 mL ethanol to each vial
rDNase, RNase-free (lyophilized)	1 vial (size C) Add 230 µL RNase-free H_2O	1 vial (size D) Add 540 µL RNase-free H_2O	5 vials (size D) Add 540 µL RNase-free H_2O to each vial

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® RNA Plant** kits contain hazardous contents.

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

Component ***Inhalt***	**Hazard contents** ***Gefahrstoff***	**Hazard symbol** ***Gefahrstoffsymbol***		**Risk phrases** ***R-Sätze***	**Safety phrases** ***S-Sätze***
rDNase, RNase-free	rDNase, lyophilized *rDNase (lyophilisiert)*	 Xn	May cause sensitization by inhalation and skin contact *Sensibilisierung durch Einatmen und Hautkontakt möglich*	R 42/43	S 22–24
RA1	Guanidinium thiocyanate *Guanidinium-thiocyanat*	 Xn*	Harmful by inhalation, in contact with the skin, and if swallowed *Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut*	R 20/21/22	S 13
RA2	Guanidinium thiocyanate *Guanidinium-thiocyanat*	 Xn*	Flammable - Harmful by inhalation, in contact with skin, and if swallowed *Entzündlich - Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut*	R 10-20/21/22	S 7-13-16
RAP	Guanidine hydrochloride *Guanidin-hydrochlorid*	 Xn*	Harmful if swallowed - Irritating to eyes and skin *Gesundheitsschädlich beim Verschlucken - Reizt die Augen und die Haut*	R 22-36/38	
MDB	Guanidinium thiocyanate <10% + ethanol <10% *Guanidinium-thiocyanat <10% + Ethanol <10%*		Flammable *Entzündlich*	R 10	S 7–16

* Hazard labeling not necessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

Risk phrases / *R-Sätze*

R 10	Flammable *Entzündlich*
R 20/21/22	Harmful by inhalation, in contact with skin, and if swallowed *Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut*
R 22	Harmful if swallowed *Gesundheitsschädlich beim Verschlucken*
R 36/38	Irritating to eyes and skin *Reizt die Augen und die Haut*
R 42/43	May cause sensitisation by inhalation and skin contact *Sensibilisierung durch Einatmen und Hautkontakt möglich*

Safety phrases / *S-Sätze*

S 7	Keep container tightly closed *Behälter dicht geschlossen halten*
S 13	Keep away from food, drink, and animal feedstuffs *Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten*
S 16	Keep away from sources of ignition – No Smoking! *Von Zündquellen fernhalten – Nicht rauchen*
S 22	Do not breathe dust *Staub nicht einatmen*
S 24	Avoid contact with the skin *Berührung mit der Haut vermeiden*

5 Protocols

5.1 Total RNA isolation from plant tissue or filamentous fungi

Before starting the preparation:

- Check if Wash Buffer RA3 and rDNase were prepared according to section 3.

1 Homogenize sample

Grind up to **100 mg tissue** under liquid N_2 (for handling and preparation methods see section 2.3).

Grind sample

2 Lyse cells

Add **350 µL Buffer RA1** and **3.5 µL ß-mercaptoethanol** (ß-ME) to 100 mg tissue and vortex vigorously.

If the lysate solidifies upon addition of Buffer RA1, use **350 µL Buffer RAP** instead.

Note: As alternative to ß-ME the reducing agent DTT or TCEP may be used. Use a final concentration of 10–20 mM DTT or TCEP within the Lysis Buffer RA1 or RAP (e.g., add 7–14 µL of a 500 mM DTT or TCEP solution).

+ 350 µL RA1
+ 3.5 µL ß-ME

or

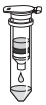
+ 350 µL RAP
+ 3.5 µL ß-ME

3 Filtrate lysate

Reduce viscosity and clear the lysate by filtration through **NucleoSpin® Filter (violet ring)**: Place NucleoSpin® Filter in a Collection Tube (2 mL), apply the mixture, and centrifuge for **1 min** at **11,000 x *g***. Transfer the filtrate to a new 1.5 mL microcentrifuge tube (nor provided).

Important note: Do not disturb the pellet of cell debris at the bottom of the collecting tube, which may be visible after centrifugation.

In case of visible pellet formation (depending on sample amount and nature) transfer supernatant without any formed pellet to a new 1.5 mL microcentrifuge tube (not provided).



11,000 x *g*
1 min

4 Adjust RNA binding conditions

Discard the NucleoSpin® Filter and add **350 µL ethanol (70 %)** to the homogenized lysate and mix by pipetting up and down (5 times).

Alternatively, transfer flow-through into a new 1.5 mL microcentrifuge tube (not provided), add **350 µL ethanol (70 %)**, and mix by vortexing (2 x 5 s).

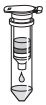
+ 350 µL 70 % ethanol

Mix

After addition of ethanol a stringy precipitate may become visible which will not affect the RNA isolation. Be sure to disaggregate any precipitate by mixing and load all of the precipitate on the column as described in step 5. Do not centrifuge the ethanolic lysate before loading it onto the column in order to avoid pelleting the precipitate.

5 Bind DNA

For each preparation take one **NucleoSpin® RNA Plant Column (light bue ring)** placed in a Collection Tube and load the lysate. Centrifuge for **30 s** at **11,000 x *g***. Place the column in a new Collection Tube (2 mL).



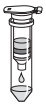
Load lysate

30 s 11,000 x *g*

Maximum loading capacity of NucleoSpin® RNA Plant Columns is 750 µL. Repeat the procedure if larger volumes are to be processed.

6 Desalt silica membrane

Add **350 µL MDB** (Membrane Desalting Buffer) and centrifuge at **11,000 x *g*** for **1 min** to dry the membrane.



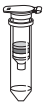
+ 350 µL MDB

1 min 11,000 x *g*

Salt removal will make the following rDNase digest much more effective. If the column outlet has come into contact with the flow-through for any reason, discard the flow-through and centrifuge again for 30 s at 11,000 x g.

7 Digest DNA

Prepare **DNase reaction mixture** in a sterile 1.5 mL microcentrifuge tube (not provided): For each isolation, add **10 µL reconstituted rDNase** (see section 3) to **90 µL Reaction Buffer for rDNase**. Mix by flicking the tube.



+ 95 µL rDNase reaction mixture

RT
15 min

Apply **95 µL DNase reaction mixture** directly onto the center of the silica membrane of the column. Incubate at **room temperature** for **15 min**.

8 Wash and dry silica membrane

1st wash

Add **200 µL Buffer RA2** to the NucleoSpin® RNA Plant Column. Centrifuge for **30 s** at **11,000 x *g***. Place the column into a new Collection Tube (2 mL).

+ 200 µL RA2

11,000 x *g*
30 s

Buffer RA2 will inactivate the rDNase.

2nd wash

Add **600 µL Buffer RA3** to the NucleoSpin® RNA Plant Column. Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the Collection Tube.

+ 600 µL RA3

11,000 x *g*
30 s

3rd wash

Add **250 µL Buffer RA3** to the NucleoSpin® RNA Plant Column. Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane completely. Place the column into a nuclease-free Collection Tube (1.5 mL, supplied).

+ 250 µL RA3

11,000 x *g*
2 min

If for any reason, the liquid level in the Collection Tube has reached the NucleoSpin® RNA Plant Column after centrifugation, discard flow-through, and centrifuge again.

9 Elute highly pure RNA

Elute the RNA in **60 µL RNase-free H_2O**, (supplied) and centrifuge at **11,000 x *g*** for **1 min**.

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

For alternative elution procedures see section 2.4.

+ 60 µL RNase-free H_2O

11,000 x *g* 1 min

5.2 Support protocol NucleoSpin® RNA Plant: rDNase digestion in solution

Comments on DNA removal:

The on-column rDNase digestion in the standard protocol is already very efficient and thus resulting in a minimal residual DNA content of the purified RNA. This DNA will not be detectable in most downstream applications. Despite this, there are still certain applications which require even lower contents of residual DNA. However, removal of DNA to a completely undetectable level is challenging and the efficiency of an on-column DNA digestion is sometimes not sufficient for downstream applications requiring lowest residual content of DNA.

A typical example for such a demanding application is an RT-PCR reaction in which the primer molecules do not differentiate between cDNA (derived from RNA) and contaminating genomic DNA. Especially, if

- high copy number targets are analyzed (e.g. multi gene family, mitochondrial, plastidal or plasmid targets (from transfections))
- the target gene is of a very low expression level
- the amplicon is relatively small (< 200 bp)

DNA digestion in solution can efficiently destroy contaminating DNA. However, stringent RNase control and subsequent repurification of the RNA (in order to remove buffer, salts, DNase and digested DNA) are usually required.

The high quality, recombinant RNase-free DNase (rDNAse) in the NucleoSpin® RNA kits facilitates such a digestion in solution in order to remove even traces of contaminating DNA.

- Check if rDNase was prepared according to section 3.

A 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.)

B Incubate sample

Incubate for **10 min** at **37 °C**.

C Repurify RNA

Repurify RNA with a suitable RNA cleanup procedure, for example by use of the NucleoSpin® RNA Clean-up / RNA Clean-up XS kit (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 for sample containing 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.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded/ no RNA obtained	*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.
Poor RNA quality or yield	*Reagents not applied or restored properly* • Reagents not properly restored. Add the indicated volume of RNase-free H_2O to rDNase vial and 96 % ethanol to Buffer RA3 Concentrate and mix. Reconstitute and store lyophilized rDNase according to instructions given in section 3. • Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added. • No ethanol has been added after lysis. Binding of RNA to the silica membrane is only effective in the presence of ethanol. *Kit storage* • Reconstitute and store lyophilized rDNase according to instructions given in section 3. • Store other kit components at room temperature. Storage at low temperatures may 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. *Sample material* • Sample material not stored properly. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid N_2. Samples should always be kept at -70 °C. Never allow tissues to thaw before addition of Buffer RA1. Perform disruption of samples in liquid N_2. • Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption and use **NucleoSpin® Filters** for easy homogenization of disrupted starting material.
Clogged NucleoSpin® Column	*Sample material* • Too much starting material used. Overloading may lead to decreased overall yield. Reduce amount of sample material or use larger volume of Buffer RA1. • Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption and use NucleoSpin® Filters for easy homogenization of disrupted starting material.
Contamination of RNA with genomic DNA	*rDNase not active* • Reconstitute and store lyophilized rDNase according to instructions given in section 3. *rDNase solution not properly applied* • Pipette rDNase solution directly onto the center of the silica membrane. *Too much cell material used* • Reduce quantity of cells or tissue used.

Contamination of RNA with genomic DNA *(continued)*	*DNA detection system too sensitive* The amount of DNA contamination is effectively reduced during the on-column digestion with rDNase. Anyhow, 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 NucleoSpin® RNA II/Plant system is checked by the following procedure: One million HeLa cells are subjected to RNA isolation according to the protocol. RNA eluate is used as template for PCR detection of a 1 kbp fragment in a 30 cycle reaction. Generally, no PCR product is obtained while skipping the DNase digest usually leads to positive PCR results. 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 support protocol 5.2 for subsequent rDNase digestion in solution.**
Suboptimal performance of RNA in downstream experiments	*Carry-over of ethanol or salt* • Do not let the flow-through touch the column outlet after the second Buffer RA3 wash. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Buffer RA3 completely. • Check if Buffer RA3 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Buffer RA3. *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.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® RNA Plant	740949.20/.50/.250	20/50/250
NucleoSpin® RNA Clean-up	740948.10/.50/.250	10/50/250
NucleoSpin® RNA XS	740902.10/.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® RNA/DNA Buffer Set*	740944	Suitable for 100 preps
NucleoSpin® TriPrep*	740966.10/.50/.250	10/50/250
Buffer RA1	740961 740961500	50 mL 500 mL
Buffer RAP	740936.50 740936.500	50 mL 500 mL
Buffer RL1	740385.50 740385.125	50 mL 125 mL
rDNase Set	740963	1 set
NucleoSpin® Filters	740606	50
Collection Tubes (2 mL)	740600	1000

* DISTRIBUTION AND USE OF NUCLEOSPIN® RNA/DNA BUFFER SET and NUCLEOSPIN® TRIPREP IN THE USA IS PROHIBITED FOR PATENT REASONS.

6.3 References

Vogelstein B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76: 615-619.

6.4 Product use restriction/warranty

NucleoSpin® RNA Plant kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform

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