

# RNA isolation

## User manual

NucleoSpin<sup>®</sup> RNA Plus

July 2014 / Rev. 01

**MACHEREY-NAGEL**


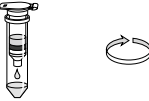
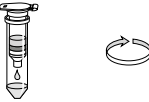




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# RNA isolation

## Protocol-at-a-glance (Rev.01)

### NucleoSpin® RNA Plus

1	<b>Homogenize sample and lyse sample</b>		350 µL LBP
2	<b>Remove gDNA and filtrate lysate</b>		11,000 x <i>g</i> , 30 s
3	<b>Adjust RNA binding conditions</b>		100 µL BS Mix
4	<b>Bind RNA</b>		Load sample 11,000 x <i>g</i> , 15 s
5	<b>Wash and dry silica membrane</b>		1 <sup>st</sup> wash 200 µL WB1 2 <sup>nd</sup> wash 600 µL WB2 3 <sup>rd</sup> wash 250 µL WB2
	1 <sup>st</sup> and 2 <sup>nd</sup>		11,000 x <i>g</i> , 15 s
	3 <sup>rd</sup>		11,000 x <i>g</i> , 2 min
6	<b>Elute RNA</b>		30 µL RNase-free H <sub>2</sub> O 11,000 x <i>g</i> , 1 min 30 µL RNase-free H <sub>2</sub> O 11,000 x <i>g</i> , 1 min

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## Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents, consumables, and equipment to be supplied by user	5
1.3	RNase-free work environment	5
1.4	About this user manual	6
2	Product description	7
2.1	The basic principle	7
2.2	Kit specifications	7
2.3	Handling, preparation, and storage of starting materials	9
2.4	Elution procedures	11
3	Storage conditions and preparation of working solutions	12
4	Safety instructions	13
5	NucleoSpin® RNA Plus protocol	15
6	Appendix	18
6.1	rDNase digestion in solution	18
6.2	Troubleshooting	20
6.3	Ordering information	23
6.4	Product use restriction/warranty	24

# 1 Components

## 1.1 Kit contents

<b>NucleoSpin® RNA Plus</b>			
<b>REF</b>	<b>10 preps 740984.10</b>	<b>50 preps 740984.50</b>	<b>250 preps 740984.250</b>
Lysis Buffer LBP	5 mL	25 mL	125 mL
Binding Solution BS	1.5 mL	6 mL	30 mL
Wash Buffer WB1	3 mL	12 mL	60 mL
Wash Buffer WB2 (Concentrate)*	6 mL	12 mL	50 mL
RNase-free H <sub>2</sub> O	13 mL	13 mL	60 mL
NucleoSpin® gDNA Removal Column (yellow rings)	10	50	250
NucleoSpin® RNA Plus Columns (light blue rings – plus Collection Tubes)	10	50	250
Collection Tubes (2 mL)	20	100	500
Collection Tubes (1.5 mL)	10	50	250
User manual	1	1	1

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

### Reagents

- 96–100% ethanol (to prepare Wash Buffer WB2, non-denatured ethanol recommended)

### Consumables

- 1.5 mL or 2.0 mL microcentrifuge tubes (to prepare sample lysate)
- Sterile RNase-free tips

### Equipment

- Manual pipettors
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization (see section 2.3)
- Personal protection equipment (e.g., lab coat, gloves, goggles)
- RNase-free working environment

*Note: Reducing agents (e.g.  $\beta$ -mercaptoethanol) often used for RNA isolation is typically not required for NucleoSpin<sup>®</sup> RNA Plus preparations.*

## 1.3 RNase-free work environment

Kit components have been tested to ensure they are RNase-free. However, a RNase-free working environment is also a critical factor for performing successful RNA isolation and handling. Therefore, general recommendations to avoid RNase contamination should be followed:

- Maintain a separate area, dedicated pipettors and materials when working with RNA.
- Wear gloves when handling RNA and reagents to avoid contact with skin, which is a source of RNases. Change gloves frequently.
- Use sterile RNase-free plastic tubes. Collection Tubes (2 mL, for column flow-through and 1.5 mL for elution) are provided in the kit. Tubes for lysate preparation have to be supplied by user.
- Use RNase-free water contained in kit for elution.
- Keep all kit components sealed when not in use and all tubes tightly closed when possible.

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\* For preparation of working solutions and storage conditions see section 3.

## 1.4 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® RNA Plus** 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](http://www.mn-net.com)**.

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

## **2 Product description**

### **2.1 The basic principle**

The **NucleoSpin® RNA Plus** kit is designed to purify RNA from a variety of cell and tissue types. This kit introduces the

NucleoSpin® gDNA Removal Column. The probability of DNA detection with PCR increases with:

1. the number of DNA copies per preparation: single copy target < plasmid / mitochondrial target < plasmid transfected into cells.
2. decreasing PCR amplicon size.

**Table 1: Kit specifications at a glance**

Parameter	NucleoSpin® RNA Plus
Technology	Two column silica-membrane system: 1. Column for DNA removal 2. Column for RNA isolation
Format	Mini spin column
Sample material	< 1 x 10 <sup>7</sup> cultured cells, < 10 <sup>9</sup> bacterial cells, < 10 <sup>8</sup> yeast cells, < 30 mg tissue
Fragment size	> 200 nt
Typical yield	HeLa cells 5 x 10 <sup>6</sup> : 40–60 µg Mouse liver 20 mg: 80–100 µg Mouse kidney 20 mg: 40–70 µg Mouse spleen 5 mg: 30–60 µg
A <sub>260</sub> /A <sub>280</sub>	1.9–2.1
A <sub>260</sub> /A <sub>230</sub>	1.8–2.5
Typical RIN (RNA integrity number)	> 9
Elution volume	30–120 µL
Preparation time	20 min/6 preps
Binding capacity	200 µg



## 2.3 Handling, preparation, and storage of starting materials

It is important, to use an appropriate amount of sample material in order to obtain optimal RNA yield and purity. The maximum amount of sample material that can be used with the NucleoSpin® RNA Plus kit depends on type of sample and its RNA and DNA content.

Maximal amount of sample material to be used per preparation (approximate values):

- Eukaryotic cells (e.g. HeLa cells):  $10^7$  cells
- Animal tissue: 30 mg (wet weight)
- Plant tissue: 100 mg (wet weight)
- Microorganisms (e.g. yeast): 30 mg

### Sample harvest and RNase-inhibition

RNA is not protected against digestion until the sample material is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents.

Sample harvest methods:

- Use freshly harvested sample for immediate lysis and RNA purification.
- Samples can be stored in lysis buffer after disruption at  $-70\text{ }^{\circ}\text{C}$  for up to one year, at  $4\text{ }^{\circ}\text{C}$  for up to 24 hours or up to several hours at room temperature. Frozen samples in lysis buffer should be thawed slowly before starting with the isolation of RNA.
- Flash freeze sample in liquid  $\text{N}_2$  immediately upon harvest and store at  $70\text{ }^{\circ}\text{C}$ . Frozen samples are stable up to 6 months. Mortar and pestle can be used to pulverize the sample in a frozen state. Make sure that the sample does not thaw prior to contact with lysis buffer.
- Samples can be submerged and stored in RNA $later$ ®. Before using such samples, remove excess RNA $later$ ® solution from the tissue before use.

### Disrupting and homogenizing sample material

#### Cell lysis of adherent growing cells in a culture dish:

Completely aspirate cell-culture medium and immediately add Lysis Buffer to the cell-culture dish. Avoid incomplete removal of the cell-culture medium in order to allow full lysis activity of the lysis buffer. Mixing cultured cells with lysis buffer is usually sufficient for complete lysis.

**To trypsinize adherent growing cells:**

Aspirate cell-culture medium, and add an equal amount of PBS in order to wash the cells. Aspirate PBS. Add 0.1–0.3% trypsin in PBS and incubate for an appropriate time to detach the cells from the dish surface. After cell detachment, add cell culture medium, transfer cells to an appropriate tube (not supplied), and pellet by centrifugation for 5 min at 300 x g. Remove supernatant and continue with the addition of lysis buffer to the cell pellet.

**Animal tissues** are often solid and must therefore be broken up mechanically as well as lysed. It is essential for efficient RNA preparation that all the RNA contained in the sample is released from the cells by disruption, and if viscosity is present the sample needs to be homogenized further to reduce the viscosity.

The most common technique used for disruption of animal tissues is grinding with a pestle and mortar. Grind the sample to a fine powder in the presence of liquid N<sub>2</sub>. Take care that the sample does not thaw during or after grinding or weighing and add the frozen powder to an appropriate aliquot of lysis buffer and mix immediately. The broken-up and lysed tissue is then applied to the NucleoSpin® gDNA Removal Column (included in the kit) in order to bind DNA and remove insoluble matter.

Thawing of undisrupted animal tissue should be exclusively done in the presence of lysis buffer during simultaneous mechanical disruption, for example, 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 prior to RNA isolation (homogenization time depends on sample). Take care to keep the rotor tip submerged in order to avoid excess foaming. Select a suitable sized homogenizer (5–7 mm diameter rotors can be used for homogenization in microcentrifuge tubes).

**Bacteria and yeasts** have to be incubated in lysozyme or lyticase / zymolase solutions, respectively. By this treatment, the robust cell walls of these organisms are digested or at least weakened, which is essential for effective cell lysis by lysis buffer. For microorganisms with extremely resistant cell walls – like some Gram-positive bacterial strains – it may be necessary to optimize the conditions of the treatment with lytic enzymes or the cultivation conditions. After lysis in lysis buffer, homogenization is achieved by the use of a NucleoSpin® gDNA Removal Column. Alternatively, bacteria and yeast cells can be broken up by addition of ceramic beads in presence of lysis buffer and mechanical agitation with a bead mill.

**Plant material** can be disrupted e.g., with mortar and pestle in a frozen state (liquid N<sub>2</sub>) prior to addition of lysis buffer, or using Dispomix® (e.g. Xiril / VWR), or gentleMACS™ Dissociator (Miltenyi Biotec) immediately after addition of lysis buffer.

## 2.4 Elution procedures

It is possible to adapt elution method and volume of water used for the subsequent application of interest. There are several elution procedures possible.

- **High yield:** Perform two elution steps with 30  $\mu$ L. 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.
- **Convenient elution:** Elute once with 60  $\mu$ L water.

Eluted RNA should immediately be put 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.

### 3 Storage conditions and preparation of working solutions

#### Attention:

*Buffers LBP and WB1 contain chaotropic salt. Wear gloves and goggles!*

*CAUTION: Buffers LBP and WB1 contain chaotropic salt 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 room temperature (18–25 °C) and are stable for at least one year. Storage at lower temperatures may cause precipitation of salts.

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

- **Wash Buffer WB2:** Add the indicated volume of 96–100 % ethanol (see table below) to Buffer WB1 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer WB2 can be stored at room temperature (18–25 °C) for at least one year.

NucleoSpin® RNA Plus			
REF	10 preps 740984.10	50 preps 740984.50	250 preps 740984.250
Wash Buffer WB2 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol

- Binding Solution BS is ready-to-use.
- Reducing agent (e.g.  $\beta$ -mercaptoethanol) is not necessary for NucleoSpin® RNA Plus preparations.

## 4 Safety instructions



The following components of the **NucleoSpin® RNA Plus** kit contain hazardous contents.

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

### GHS classification

Only harmful features do not need to be labeled with H and P phrases until 125 mL or 125 g.

*Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.*

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>GHS Symbol</i>	<i>H-Sätze</i>	<i>P-Sätze</i>
LBP	Guanidinium thiocyanate 30–60 % <i>Guanidiniumthiocyanat</i> 30–60 %	 Warning <i>Achtung</i>	302, 412, EUH031	260, 273, 301+312, 330
WB1	Guanidine hydrochloride 24–36 % + ethanol 20-35 % <i>Guanidinhydrochlorid</i> 24–36 % + <i>Ethanol</i> 20–35 %	 Warning <i>Achtung</i>	226, 302,	210, 233, 301+312, 330, 403+235

### Hazard phrases

- H 226 Flammable liquid and vapour.  
*Flüssigkeit und Dampf entzündbar.*
- H 302 Harmful if swallowed.  
*Gesundheitsschädlich bei Verschlucken.*
- H 412 Harmful to aquatic life with long lasting effects.  
*Schädlich für Wasserorganismen, mit langfristiger Wirkung.*
- EUH031 Contact with acids liberates toxic gas.  
*Entwickelt bei Berührung mit Säure giftige Gase.*

### Precaution phrases

- P 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.  
*Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.*
- P 233 Keep container tightly closed  
*Behälter dicht verschlossen halten.*
- P 260 Do not breathe vapours.  
*Dampf nicht einatmen.*
- P 273 Avoid release to the environment.  
*Freisetzung in die Umwelt vermeiden.*
- P 301+312 IF SWALLOWED: Call a POISON CENTER/ doctor/.../if you feel unwell.  
*BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /... anrufen.*

**Precaution phrases**

P 330 Rinse mouth.  
*Mund ausspülen.*

P 403+235 Store in a well ventilated place. Keep cool.  
*Kühl an einem gut belüfteten Ort aufbewahren.*

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

## 5 NucleoSpin® RNA Plus protocol

### Before starting the preparation:

- Check if Wash Buffer WB2 was prepared according to section 3.

#### 1 Homogenize and lyse sample

Add **350 µL Buffer LBP** per sample and disrupt the sample according to one of the methods described in section 2.3.

*Note: Lysis tube is not included in the kit. Addition of reducing agent (e.g. β-mercaptoethanol, DTT, or TCEP) is not necessary. The sample material should be broken up and lysed as completely as possible.*



**+ 350 µL LBP**

#### 2 Remove gDNA and filtrate lysate

Place NucleoSpin® gDNA Removal Column (yellow ring) in a Collection Tube (2 mL; provided), transfer the homogenized lysate to the NucleoSpin® gDNA Removal Column, and centrifuge for **30 s at 11,000 x g**.

Discard the column and continue with the flow-through.

*Note: Ensure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.*

*If (in rare cases) the flow-through contains obvious undissolved sediment, recover flow-through without sediment. Optimize mechanical sample disruption for subsequent preparations.*



**11,000 x g,  
30 s**

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### 3 Adjust RNA binding conditions

Add **100 µL Binding Solution BS** to the flow-through and mix well by moderate vortexing or by pipetting up and down several times.

*Note: If mixing is done by vortexing, be careful in order to avoid spilling, because the Collection Tube does not contain a lid.*

*After addition of Binding Solution BS 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 the following step. Do not centrifuge the lysate after addition of Binding Solution before loading it onto the column in order to avoid pelleting the precipitate.*



**+ 100 µL BS  
Mix**

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### 4 Bind RNA

Transfer the whole lysate (~ 450 µL) to the NucleoSpin® RNA Plus Column (light blue ring) placed in a Collection Tube. Centrifuge for **15 s at 11,000 x g**.

*Note: Flow-through (~ 450 µL) may stay in the Collection Tube. Alternatively, discard the flow-through and reuse collection tube or place the column into a new 2 mL collection tube (not provided).*



**Load lysate**



**11,000 x g,  
15 s**



## 5 Wash and dry silica membrane

### 1<sup>st</sup> wash

Add **200 µL Buffer WB1** to the NucleoSpin® RNA Plus Column. Centrifuge for **15 s** at **11,000 x g**. Discard the flow-through with collection tube and place the column into a new 2 mL Collection Tube (provided).

+ 200 µL WB1

11,000 x g,  
15 s



### 2<sup>nd</sup> wash

Add **600 µL Buffer WB2** to the NucleoSpin® RNA Plus Column. Centrifuge for **15 s** at **11,000 x g**. Discard flow-through and place the column back into the Collection Tube.



+ 600 µL  
WB2

11,000 x g,  
15 s

*Note: Make sure that residual buffer from the previous steps is washed away with Buffer WB2 especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim flush it with Buffer WB2.*

### 3<sup>rd</sup> wash

Add **250 µL Buffer WB2** to the NucleoSpin® RNA Plus 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, provided).



+ 250 µL  
WB2

11,000 x g,  
2 min



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

## 6 Elute RNA

Add **30 µL RNase-free H<sub>2</sub>O** and centrifuge at **11,000 x g** for **1 min**.

+ 30 µL  
RNase-free  
H<sub>2</sub>O

11,000 x g,  
1 min

Add again **30 µL RNase-free H<sub>2</sub>O** and centrifuge at **11,000 x g** for **1 min**.



+ 30 µL  
RNase-free  
H<sub>2</sub>O

11,000 x g,  
1 min



*For further alternative elution procedures see section 2.4.*

## **6 Appendix**

### **6.1 rDNase digestion in solution**

The passage of the lysed sample through NucleoSpin® gDNA Removal Column according to the stan

### **C Repurify RNA**

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

#### **Ethanol precipitation, exemplary:**

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

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

*Note: Choose long incubation times if the sample contains low RNA concentration. Short incubation times are sufficient if the sample contains high RNA concentration.*

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

Wash RNA pellet with **70 % ethanol**.

Dry RNA pellet and resuspend RNA in RNase-free H<sub>2</sub>O.

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## 6.2 Troubleshooting

<b>Problem</b>	<b>Possible cause and suggestions</b>
RNA is degraded/ no RNA obtained	<i>RNase contamination</i> <ul style="list-style-type: none"><li>• 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.</li></ul>
	<i>Insufficient sample quality</i> <ul style="list-style-type: none"><li>• Control sample harvest, storage and lysis. Make sure that samples are harvested, stored and lysed adequately in order to preserve RNA integrity.</li></ul>
Poor RNA quality or yield	<i>Reagents not applied or restored properly</i> <ul style="list-style-type: none"><li>• Reagents not properly restored. Add the indicated volume of 96 % ethanol to Buffer WB2 Concentrate and mix.</li><li>• Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added.</li><li>• No Binding Solution BS has been added after lysis. Binding of RNA to the silica membrane is only effective in the presence of Binding Solution.</li></ul>
	<i>Kit storage</i> <ul style="list-style-type: none"><li>• Store kit components at room temperature. Storage at low temperatures may cause salt precipitation.</li><li>• Keep bottles tightly closed in order to prevent evaporation or contamination.</li></ul>

*Ionic strength and pH influence  $A_{260}$  absorption as well as ratio  $A_{260}/A_{280}$*

Poor RNA  
quality or yield  
(continued)

- 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 Chomczynski, 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  $-70\text{ }^\circ\text{C}$ . Never allow tissues to thaw before addition of Buffer LBP. Perform disruption of samples in liquid  $N_2$ .
  - Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption.
- 

*Carry-over of guanidinium thiocyanate*

Low  $A_{260}/A_{230}$   
ratio

- Carefully load the lysate to the NucleoSpin<sup>®</sup> RNA Plus 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.
- 

*Sample material*

Clogged  
NucleoSpin<sup>®</sup>  
Column/  
Poor RNA  
quality  
or yield

- Too much starting material used. Overloading may lead to decreased overall yield. Reduce amount of sample material or use larger volume of lysis buffer.
  - Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption and use NucleoSpin<sup>®</sup> gDNA Removal Column for DNA removal and for easy homogenization of disrupted starting material.
  - Increase *g*-force and centrifugation time if necessary.
-

*Too much cell material used*

- Reduce quantity of cells or tissue used.

*DNA detection system too sensitive*

Contamination  
of RNA with  
genomic DNA

The amount of DNA contamination is effectively reduced by the NucleoSpin® gDNA Removal Column. However, 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 < plasmid/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 6.1 for subsequent rDNase digestion in solution.**

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*Carry-over of ethanol or salt*

Suboptimal  
performance  
of RNA in  
downstream  
experiments

- Do not let the flow-through touch the column outlet after the second Buffer WB2 wash. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Buffer WB2 completely.
- Check if Buffer WB2 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Buffer WB2.

*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.
-

### 6.3 Ordering information

<b>Product</b>	<b>REF</b>	<b>Pack of</b>
NucleoSpin® RNA Plus	740984.10/.50/.250	10/50/250
NucleoSpin® RNA	740955.10/.50/.250	10/50/250
NucleoSpin® RNA Midi	740962.20	20
NucleoSpin® miRNA	740971.10/.50/.250	10/50/250 preps
NucleoSpin® RNA/Protein	740933.10/.50/.250	10/50/250
NucleoSpin® TriPrep*	740966.10/.50/.250	10/50/250
NucleoSpin® totalRNA FFPE XS	740969.10/.50/.250	10/50/250
NucleoSpin® totalRNA FFPE	740982.10/.50/.250	10/50/250
NucleoSpin® RNA Blood	740200.10/.50	10/50
NucleoSpin® RNA Blood Midi	740210.20	20
NucleoSpin® 8 RNA Blood	740220/.5	12 x 8/60 x 8
NucleoSpin® 96 RNA Blood	740225.2/.4	2 x 96/4 x 96
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
rDNase Set	740963	1
Collection Tubes (2 mL)	740600	1000

Visit [www.mn-net.com](http://www.mn-net.com) for more detailed product information.

\* DISTRIBUTION AND USE OF NUCLEOSPIN® TRIPREP IN THE USA IS PROHIBITED FOR PATENT REASONS.

## 6.4 Product use restriction/warranty

**NucleoSpin® RNA Plus** 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).

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