

MACHEREY-NAGEL

User manual



■ NucleoSpin® RNA Clean-up

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1 Components

1.1 Kit contents

NucleoSpin® RNA Clean-up			
REF	10 preps 740948.10	50 preps 740948.50	250 preps 740948.250
Lysis Buffer RA1	10 mL	25 mL	125 mL
Wash Buffer RA2	15 mL	15 mL	80 mL
Wash Buffer RA3 (Concentrate)*	6 mL	12 mL	3 × 25 mL
RNase-free H ₂ O	13 mL	13 mL	60 mL
NucleoSpin® RNA Clean-up Columns (light blue rings – plus Collection Tubes)	10	50	250
Collection Tubes (2 mL)	10	50	250
Collection Tubes (1.5 mL)	10	50	250
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1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol (to prepare Wash Buffer RA3 and to adjust RNA binding conditions)

Consumables

- 1.5 mL microcentrifuge tubes
- Sterile RNase-free tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this user Manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the internet at www.mn-net.com.

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

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

2 Product description

2.1 The basic principle

One of the most important aspects in the isolation and handling of RNA is to prevent degradation of the RNA during the isolation procedure. With the **NucleoSpin® RNA Clean-up** kit, RNA containing samples are mixed with a solution containing large amounts of chaotropic ions. This solution 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. Simple washing steps remove salts, metabolites, organics like phenol, and macromolecular cellular components. Pure RNA is finally eluted under low ionic strength conditions with RNase-free water (supplied).

The RNA clean up preparation using **NucleoSpin® RNA Clean-up** kits can be performed at room temperature (15–25 °C). 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.

2.2 Kit specifications

- **NucleoSpin® RNA Clean-up** kits are ideal for the clean up of total RNA from RNA preparations which contain unacceptable amounts of RT-PCR inhibitors (e.g., RNA prepared with phenol-chloroform based methods).
- The kit is further recommended for the isolation of RNA from small amounts of cultured cells whenever copurification of some genomic DNA is acceptable. The kit allows for purification of pure RNA with an A_{260}/A_{280} ratio generally exceeding 1.9 (measured in TE buffer (pH 7.5)).
- **NucleoSpin® RNA Clean-up** kits are recommended for the clean up of RNA from enzymatic reactions such as *in vitro* transcribed RNA, amplification reactions, biotinylated RNA, or fluorescent (Cy dye) labeled RNA.
- The purified RNA is ready to use for applications such as enzymatic labeling reactions (e.g., dye incorporation), reverse transcriptase-PCR (RT-PCR), as well as for most other downstream applications.
- The standard protocol (section 5.1) allows for the cleanup of up to 200 µg of RNA per NucleoSpin® RNA Clean-up Column or the isolation of total RNA from up to 1×10^5 cultured cells (section 5.2).

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® RNA Clean-up
Technology	Silica membrane technology
Format	Mini spin columns
Sample material	< 100 µL RNA sample with single column loading containing up to 200 µg RNA < 200 µL RNA sample with double column loading containing up to 200 µg RNA Up to 10 ⁵ cells
Fragment size	> 200 nt
Typical recovery (0.1 – 200 µg RNA input)	85 – 95 %
A ₂₆₀ /A ₂₈₀	1.9 – 2.1
Elution volume	40 – 120 µL
Preparation time	Approx. 20 min/6 preps
Binding capacity	200 µg
Use	For research use only

2.3 Handling, preparation, and storage of starting materials

RNA intended to be used as sample for the **NucleoSpin® RNA Clean-up** procedure should be handled with the same care as any RNA sample. The stability of prepurified RNA samples (e.g., RNA isolated with phenol based protocols) depends very much on the performed procedure. RNA in biological samples 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 biological samples are flash frozen in liquid N₂ immediately and stored at -70 °C or processed as soon as possible. Samples can be stored in lysis buffer 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 lysis buffer should be thawed slowly before starting with the isolation of total RNA.

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

2.4 Elution procedures

It is possible to adjust the elution method and the volume of RNase-free 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 placed 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 RA1 and RA2 contain chaotropic salt. Wear gloves and goggles!

CAUTION: Buffers RA1 and RA2 contain guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- All kit components should be stored at 15–25 °C and are stable until: see package label. Storage at lower temperatures may cause precipitation of salts.
- Check that 96–100 % ethanol is available as additional solution in the lab.

Before starting any **NucleoSpin® RNA Clean-up** protocol, prepare the following:

- **Wash Buffer RA3:** Add the indicated volume of 96–100 % ethanol (see table below) to Wash Buffer RA3 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer RA3 at room temperature for up to one year.

NucleoSpin® RNA Clean-up			
REF	10 preps 740948.10	50 preps 740948.50	250 preps 740948.250
Wash Buffer RA3 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	3 × 25 mL Add 100 mL ethanol to each bottle

4 Safety instructions

When working with the **NucleoSpin® RNA Clean-up** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at www.mn-net.com/msds).



Caution: Guanidinium thiocyanate in buffer RA1 and buffer RA2 can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® RNA Clean-up** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocols

5.1 RNA Clean-up

Before starting the preparation:

- Check if Wash Buffer RA3 was prepared according to section 3.

1 Sample preparation

Fill up RNA samples smaller than 100 μL with RNase-free water to **100 μL** .



Fill up RNA sample to 100 μL with water

RNA samples from 100–200 μL should be filled up with RNase-free water to 200 μL .

2 Preparation of lysis-binding buffer premix

Prepare a Buffer RA1-ethanol premix with a ratio of 1:1.

For each **100 μL RNA sample** mix **300 μL Buffer RA1** and **300 μL of ethanol (96–100 %)**.

If multiple samples are processed, the preparation of a master-premix is recommended (e.g., 2 mL Buffer RA1 + 2 mL 98 % ethanol for approximately 6 preparations).

Prepare premix:

**Mix
300 μL RA1 with
300 μL ethanol
(96–100 %)**

3 Adjust RNA binding conditions

To **100 μL RNA sample** add **600 μL (6 volumes) of Buffer RA1-ethanol-premix**. Mix sample with premix by vortexing.



**+ 6 vol. premix
Mix**

If a 200 μL RNA sample is processed, add 1200 μL Buffer RA1-ethanol premix.

After addition of ethanol, a stringy precipitate may become visible which will not affect the RNA isolation. Be sure to mix thoroughly and apply sample as homogeneous solution onto the column.

4 Bind RNA

For each preparation, take one NucleoSpin® RNA Clean-up Column (light blue ring) placed in a Collection Tube and load the lysate (700 μL).



Load 700 μL lysate

Centrifuge for **30 s** at **8,000 x g**. Discard Collection Tube with flowthrough and place the column in a new Collection Tube.



**8,000 x g,
30 s**

Maximal loading capacity of NucleoSpin® RNA Clean-up Columns is 750 μL . Repeat the procedure if larger volumes are to be processed.

5 Wash and dry silica membrane**1st wash**

Add **700 µL Buffer RA3** to the NucleoSpin® RNA Clean-up Column. Centrifuge for **30 s** at **8,000 x g**. Discard flowthrough and reuse Collection Tube.

**+ 700 µL RA3****8,000 x g,
30 s****2nd wash**

Add **350 µL Buffer RA3** to the NucleoSpin® RNA Clean-up Column. Centrifuge for **2 min** at **8,000 x g**.

Transfer the NucleoSpin® RNA Clean-up Column to a nuclease-free Collection Tube (1.5 mL, supplied). Open the lid of the column and let the membrane dry for 3 min.

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

The procedure ensures complete removal of ethanol from the column.

**+ 350 µL RA3****8,000 x g,
2 min****6 Elute RNA**

Elute the RNA in **60 µL RNase-free H₂O** (supplied) and centrifuge at **8,000 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 volumes.

For further alternative elution procedures see section 2.4.

**+ 60 µL
RNase-free H₂O****8,000 x g,
1 min**

5.2 RNA isolation from up to 10⁵ cells

Before starting the preparation:

- Check if Wash Buffer RA3 was prepared according to section 3.

1 Sample preparation

As sample material use **up to 10⁵ cells** in a volume of up to **100 µL**.



Fill up sample to
100 µL
(e.g. with PBS)

2 Cell lysis

Add **300 µL Buffer RA1** and vortex vigorously in order to lyse the cells.

+ **300 µL RA1**
Vortex

3 Adjust RNA binding conditions

Add **300 µL ethanol (96 – 100 %)** to the lysate and mix by vortexing or pipetting up and down.



+ **300 µL ethanol**
(96 – 100 %)
Mix

After addition of ethanol, a stringy precipitate may become visible which will not affect the RNA isolation. Be sure to mix thoroughly and apply sample as homogeneous solution onto the column.

4 Bind RNA

For each preparation, take one NucleoSpin® RNA Clean-up Column (light blue) placed in a Collection Tube and load the lysate (700 µL).

Centrifuge for **30 s** at **8,000 x g**. Discard Collection Tube with flowthrough and place the column in a new Collection Tube.



Load lysate

8,000 x g,
30 s

Maximal loading capacity of NucleoSpin® RNA Clean-up Columns is 750 µL. Repeat the procedure if larger volumes are to be processed.

5 Wash and dry silica membrane**1st wash**

Add **250 µL Buffer RA2** to the NucleoSpin® RNA Clean-up Column. Centrifuge for **30 s** at **8,000 x g**. Discard flowthrough and reuse Collection Tube.



+ 250 µL RA2

8,000 x g,
30 s**2nd wash**

Add **700 µL Buffer RA3** to the NucleoSpin® RNA Clean-up Column. Centrifuge for **30 s** at **8,000 x g**. Discard flowthrough and reuse Collection Tube.



+ 700 µL RA3

8,000 x g,
30 s**3rd wash**

Add **350 µL Buffer RA3** to the NucleoSpin® RNA Binding Column. Centrifuge for **2 min** at **8,000 x g**.

Transfer the NucleoSpin® RNA Clean-up Column to a nuclease-free Collection Tube (1.5 mL, supplied). Open the lid of the column and let the membrane dry for 3 min.

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

The procedure ensures complete removal of ethanol from the column.



+ 350 µL RA3

8,000 x g,
2 min**6 Elute RNA**

Elute the RNA in **60 µL RNase-free H₂O**, (supplied) and immediately centrifuge at **8,000 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 volumes.

For further alternative elution procedures see section 2.4.

+ 60 µL RNase-free H₂O8,000 x g,
1 min

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded/no RNA obtained	<i>RNase contamination</i>
	<ul style="list-style-type: none"> • 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	<i>Reagents not applied or restored properly</i>
	<ul style="list-style-type: none"> • Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added. • No ethanol has been added. Binding of RNA to the silica membrane is only effective in the presence of ethanol.
	<i>Kit storage</i>
Poor RNA quality or yield	<ul style="list-style-type: none"> • Store kit components at room temperature. Storage at low temperatures may cause salt precipitation. If salt precipitates are visible, warm up to 37 °C until all salt precipitates are dissolved. • Keep bottles tightly closed in order to prevent evaporation or contamination.
	<i>Sample material</i>
Contamination of RNA with genomic DNA	<ul style="list-style-type: none"> • Sample material not stored properly. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid N₂. Samples should always be kept at -70 °C. Never allow tissues to thaw before addition of lysis buffer. Perform disruption of samples in liquid N₂.
	<ul style="list-style-type: none"> • The NucleoSpin® RNA Clean-up procedure does not comprise a DNA digestion step. Therefore, the extent of DNA contamination mainly depends on the sample material. If lowest level of DNA contamination is desired, use one of the rDNase contained in NucleoSpin® RNA kits (see ordering information).

Problem	Possible cause and suggestions
Suboptimal performance of RNA in downstream experiments	<p><i>Carry over of ethanol or salt</i></p> <ul style="list-style-type: none"> • Do not let the flowthrough touch the column outlet after the second wash using Wash Buffer RA3. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Wash Buffer RA3 completely. • Check if Wash Buffer RA3 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Wash Buffer RA3. • A 2 min centrifugation with a subsequent 3 min drying with open lid is sufficient for an extensive removal of ethanol from the column. Residual ethanol will typically be around 1 %. Increasing the drying step with open lid from 3 min to 20 min will decrease the residual ethanol content commonly to below 0.1 %, but also RNA recovery will be reduced 5–20 %.
	<p><i>Store isolated RNA properly</i></p> <ul style="list-style-type: none"> • 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.
	<p><i>RNA concentration is too low</i></p> <ul style="list-style-type: none"> • For highest RNA concentration and most sensitive downstream applications, NucleoSpin® RNA Clean-up XS is recommended. NucleoSpin® RNA Clean-up XS allows elution in only 5–20 µL volume (see ordering information).
Higher RNA yield than theoretically possible	<ul style="list-style-type: none"> • If performing clean-up of samples containing less than approximately 300 ng, RNA subsequent quantification by A_{260} measurement may simulate yields larger than the RNA input. This may be due to absorbance of silica abrasion. In order to prevent incorrect A_{260} quantification of small RNA amounts, centrifuge the elution tube for 30 s at 8.000–11.000 x g and withdraw an aliquot for measurement without disturbing any sediment or use a silica abrasion insensitive RNA quantification method (e.g., RiboGreen® fluorescent dye).

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® RNA Clean-up	740948.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® RNA Clean-up XS	740903.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® RNA	740955.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® RNA Plus	740984.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® RNA XS	740902.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® RNA Plus XS	740990.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® RNA Midi	740962.20	20 preps
NucleoSpin® miRNA	740971.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® RNA Blood	740200.10 / 50	10 / 50 preps
NucleoSpin® totalRNA FFPE	740982.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® totalRNA FFPE XS	740969.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® RNA Plant and Fungi	740120.10 / .50 / .250	10 / 50 / 250 preps
NucleoBond® RNA Soil	740140.20	20 preps
NucleoBond® RNA Soil Mini	740142.10 / .50	10 / 50 preps
NucleoSpin® RNA Stool	740130.10 / .50	10 / 50 preps

Visit www.mn-net.com for more detailed product information.

6.3 Product use restriction/warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the customer and are not a part of a contract of sale or of this warranty.

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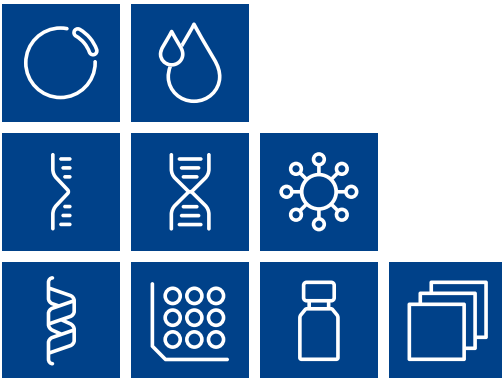
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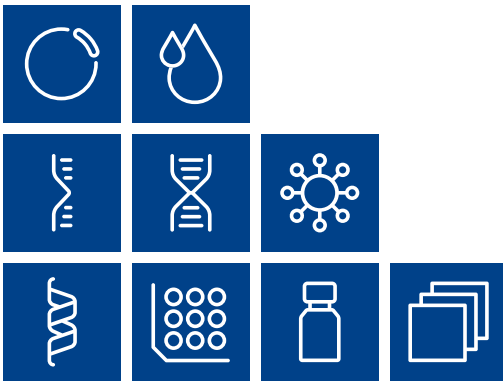
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Plasmid DNA
Clean up
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DNA
Viral RNA and DNA
Protein
High throughput
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