

RNA Clean up

User manual

NucleoSpin[®] RNA Clean-up Maxi

June 2018 / Rev. 01

RNA Clean up

Protocol at a glance (Rev.01)



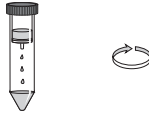
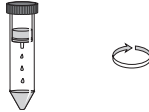
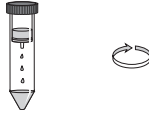
NucleoSpin® RNA Clean-up Maxi		
1 Sample preparation		Fill up RNA sample to 7.5 mL sample RNase-free water
2 Adjust RNA binding conditions		+ 7.5 mL RNA Clean-up Buffer RCU Mix
3 Bind RNA		Load sample 4,000 x g, 2 min
4 Wash and dry silica membrane		1 st wash + 7.5 mL Wash Buffer RA2 2 nd wash + 7.5 mL Wash Buffer RA3 3 rd wash + 7.5 mL Wash Buffer RA3 4,000 x g, 2 min after each washing step
5 Elute RNA		+ 5 mL RNase-free H ₂ O 4,000 x g, 2 min <i>or alternatively</i> + 1 mL RNase-free H ₂ O 4,000 x g, 2 min Repeat 2 more times

Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents, consumables, and equipment to be supplied by user	4
1.3	RNase-free work environment	5
1.4	About this user manual	5
2	Product description	6
2.1	The basic principle	6
2.2	Kit specifications	6
2.3	Handling, preparation, and storage of starting materials	7
2.4	Elution procedures	7
2.5	Stability of isolated RNA	7
3	Storage conditions and preparation of working solutions	8
4	Safety instructions	9
5	Protocol	11
6	Appendix	13
6.1	rDNase digestion in solution	13
6.2	Troubleshooting	14
6.3	Ordering information	14
6.4	Product use restriction / warranty	15

1 Components

1.1 Kit contents

NucleoSpin® RNA Clean-up Maxi	
REF	20 preps 740910.20
Clean-up Buffer RCU (concentrate)*	60 mL
Wash Buffer RA2	160 mL
Wash Buffer RA3 (concentrate)*	100 mL
RNase-free H ₂ O	60 mL
NucleoSpin® RNA Maxi Column (plus collection tubes)	20
Collection Tube (50 mL)	40
User manual	1

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

Reagents

- 96–100% ethanol (to prepare Clean-up Buffer RCU and Wash Buffer RA3, non-denatured ethanol recommended)

Consumables

- 15 mL or 50 mL tubes (to prepare sample for column loading)
- Sterile RNase-free tips

Equipment

- Manual pipettors
- Vortex mixer
- Centrifuge for 50 mL tubes
- Personal protection equipment (e.g. lab coat, gloves, goggles)

Note: Reducing agents (e.g. β -mercaptoethanol) often used for RNA isolation is not required for NucleoSpin RNA Clean-up Maxi preparations.

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

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 (50 mL, for column flow through and 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.

1.4 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® RNA Clean-up Maxi** 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.

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 Clean-up Maxi kit is designed to clean up large amounts of RNA from either crude preparations or from enzyme reactions.

One of the most important aspects during the isolation of RNA is to prevent degradation of the RNA. With the NucleoSpin® RNA Clean-up Maxi kit, RNA containing samples are mixed with a solution containing large amounts of chaotropic ions and ethanol. In this mixture, RNases are immediately inactivated and creates appropriate binding conditions to allow adsorption of RNA to the silica membrane. Three washing steps with two different wash buffers remove impurities. Pure RNA is finally eluted at low ionic strength conditions with RNase-free water (supplied).

The RNA clean-up using NucleoSpin® RNA Clean-up Max kits can be performed at room temperature.

The eluate should be treated with care because RNA is very sensitive to trace contaminations of RNases, often found on general lab ware, fingerprints and dust. Keep RNA frozen at -20 °C for short-term or -70 °C for long-term storage to ensure RNA stability.

2.2 Kit specifications

- **NucleoSpin® RNA Clean-up Maxi** kit is recommended for the clean-up and concentration of prepurified RNA samples. Typical sample material covers milligram amounts of prepurified RNA (e.g. phenol-purified RNA) and RNA from reaction mixtures (e.g. DNase-treated samples, in-vitro transcribed samples).
- The isolated RNA is ready to use in diverse downstream applications.
- RNA isolated with the NucleoSpin® RNA Clean-up Maxi kit is of high integrity. Obtained RIN (RNA Integrity Number) or RQN (RNA Quality Number) is predominantly determined by the integrity of the RNA within the sample.
- RNA molecules longer than approximately 200 nucleotides will typically recovered with rates of > 90%. RNA molecules shorter than approximately 50 bp show lower recovery rates.

Table 1: Kit specifications at a glance

Technology	Silica membrane technology
Format	Maxi column
Sample material	1 to 35 mg crude RNA
Fragment size	> 200 nt
Typical recovery	> 85 %
A _{260/280}	1.9–2.1
A _{260/230}	1.5–2.5
Typical RIN (RNA Integrity Number)	equal to integrity of RNA input
Elution volume	1–5 mL
Preparation time	25–30 min/6 preps
Binding capacity	> 30 mg RNA per column

2.3 Handling, preparation, and storage of starting materials

RNA intended to be used as sample for the **NucleoSpin® RNA Clean-up Maxi** procedure should be handled with the same care as any RNA sample. The stability of prepurified RNA 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. Similarly, prepurified RNA should be kept cold or frozen up to the point it is mixed with the Clean-up Buffer RCU.

2.4 Elution procedures

Elution volumes in the range of 3–5 mL are recommended. The default elution volume is 5 mL. Alternatively, 3 x 1 mL can be used for samples > 5 mg RNA input.

2.5 Stability of isolated RNA

Eluted RNA should immediately be put and always kept on ice during work for optimal stability! Contamination with almost omnipresent RNases (general lab ware, fingerprints, dust) may be a risk for isolated 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: Clean-up Buffer RCU and Wash Buffer RA2 contain chaotropic salt. Wear gloves and goggles!

CAUTION: Buffers RCU and RA2 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.
- Check that 96–100 % ethanol is available as additional solution in the lab

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

- **Clean-up Buffer RCU:** Add the indicated volume of 96–100 % ethanol (see table below) to the RCU concentrate.
- **Wash Buffer RA3:** Add the indicated volume of 96–100 % ethanol (see table below) to Buffer RA3 Concentrate.

Mark the label of each bottle to indicate that ethanol was added. Clean-up Buffer RCU can be stored at room temperature (18–25 °C) for at least six month, wash Buffer RA3 for at least one year.



NucleoSpin® RNA Clean-up Maxi	
REF	20 preps 740910.20
Clean-up Buffer RCU (concentrate)	60 mL Add 180 mL ethanol
Wash Buffer RA3	100 mL Add 400 mL ethanol

4 Safety instructions

The following components of the **NucleoSpin® RNA Clean-up Maxi** 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	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Inhalt	Gefahrstoff	GHS-Symbol	H-Sätze	P-Sätze
RCU	Guanidinium thiocyanate 45–60 % <i>Guanidinthiocyanat 36–50 %</i> CAS 593-84-0	 WARNING ACHTUNG	302, 412	264W, 273, 301+312, 330
RA2	Guanidinium thiocyanate 30–45 % and ethanol 20–35 % <i>Guanidinthiocyanat 30–45 % und Ethanol 20–35 %</i> CAS 593-84-0, 64-17-5	 WARNING ACHTUNG	302, 315, 319	264W, 280sh, 301+312, 330

Hazard phrases

H 302	Harmful if swallowed. <i>Gesundheitsschädlich bei Verschlucken.</i>
H 315	Causes skin irritation. <i>Verursacht Hautreizungen.</i>
H 319	Causes serious eye irritation. <i>Verursacht schwere Augenreizung.</i>
H 412	Harmful to aquatic life with long lasting effects. <i>Schädlich für Wasserorganismen, mit langfristiger Wirkung.</i>

Precaution phrases

P 264W	Wash with water thoroughly after handling. <i>Nach Gebrauch mit Wasser gründlich waschen.</i>
P 273	Avoid release to the environment. <i>Freisetzung in die Umwelt vermeiden.</i>
P 280sh	Wear protective gloves / eye protection. <i>Schutzhandschuhe / Augenschutz tragen.</i>
P 301+312	IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell. <i>BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt anrufen.</i>
P 330	Rinse mouth. <i>Mund ausspülen.</i>



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 Protocol

Before starting the preparation:

- Check if Clean-up Buffer RCU and Wash Buffer RA3 were prepared according to section 3.

1 Sample preparation

Provide up to **7.5 mL sample** containing up to 35 mg crude RNA in a 15 mL or 50 mL tube (not provided).

Note: Fill up RNA samples smaller than 7.5 mL with RNase-free water to 7.5 mL



**7.5 mL
sample**

2 Adjust RNA binding conditions

Add **7.5 mL RNA Clean-up Buffer RCU** to the sample and mix well by moderate vortexing or by pipetting up and down several times.



**7.5 mL
RNA
Clean-up
Buffer
RCU**

3 Bind RNA

Transfer the whole mixture (~15 mL) to the NucleoSpin® RNA Clean-up Maxi Column preassembled with a 50 mL Collection Tube (provided).

Centrifuge for **2 min at 4,000 x g**.

Discard the flow through with collection tube and place the column into a fresh 50 mL Collection Tube (provided).



**2 min,
4,000 x g**

4 Wash and dry silica membrane

1st wash

Add **7.5 mL Wash Buffer RA2** onto the column.

Centrifuge for **2 min at 4,000 x g**.

Note: The flow through may remain in the collection tube.



**7.5 mL
Wash
Buffer RA2**

**2 min,
4,000 x g**

2nd wash

Add **7.5 mL Wash Buffer RA3** onto the column.

Centrifuge for **2 min at 4,000 x g**.

Note: The flow through may remain in the collection tube.



**7.5 mL
Wash
Buffer RA3**

**2 min,
4,000 x g**

3rd wash

Add again **7.5 mL Wash Buffer RA3** onto the column.

Centrifuge for **2 min at 4,000 x g**.

Discard flow through with collection tube.

Place the column into a fresh 50 mL Collection Tube (provided).

Note: If for any reason, the flow through liquid in the Collection Tube has contacted the column after centrifugation, discard flow through and centrifuge again.



**7.5 mL
Wash
Buffer RA3**

**2 min,
4,000 x g**

5 Elute RNA

Add **5 mL RNase-free H₂O** and centrifuge **2 min at 4,000 x g**.
or alternatively

Add **1 mL RNase-free H₂O** and centrifuge **2 min at 4,000 x g**.

Add another **1 mL RNase-free H₂O** and centrifuge **2 min at 4,000 x g**.

Add another **1 mL RNase-free H₂O** and centrifuge **2 min at 4,000 x g**.

Note: Optional elution volume depends on RNA amount. For highest RNA recovery, a three-step elution is recommended. Alternatively, RNA can be eluted with a single elution step using 5 mL water. This reduces handling time, but might result in reduced yield and RNA concentration.



**5 mL
RNase-
free H₂O**

**2 min,
4,000 x g**

*or alter-
natively*



**+ 1 mL
RNase-
free H₂O**

**4,000 x g,
2 min**

**Repeat
2 more
times**

6 Appendix

6.1 rDNase digestion in solution

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 required.

In order to obtain good results, i.e. keep the RNA integrity, the RNA has to be provided in an RNase-free solution, preferably water. Then, the high quality, recombinant, RNase-free rDNase (REF 740963) can be used for such a DNA digestion in solution in order to remove even traces of contaminating DNA. Subsequently, RNA can be cleaned-up with NucleoSpin® RNA Clean-up Maxi kit.

A: Digest DNA (Reaction setup)

Add 100 µL Reaction Buffer for rDNase and 10 µL rDNase per 1000 µL RNA solution.

Gently swirl the tube in order to mix the solution. Spin down gently (1 s at 1,000 x g) to collect every droplet of the solution at the bottom of the tube.

B Incubate sample

Incubate for 10 min at 37 °C.

C Repurify RNA

Repurify RNA with a NucleoSpin® RNA Clean-up Maxi following section 5.

6.2 Troubleshooting

Problem	Possible cause and suggestion
Low RNA recovery.	<ul style="list-style-type: none"> Highly degraded RNA. Some RNA types, e.g. Toroula yeast RNA, is commonly highly degraded with fragment length of < 50 nt. Such highly degraded RNA might cause reduced recovery rate if less than 10 mg RNA is used as sample. Use more than 10 mg for such RNA types.
Unexpected ratio $A_{260/280}$	<ul style="list-style-type: none"> RNA type. Ratio $A_{260/280}$ is base dependent. E.g. Poly-A+ RNA has a $A_{260/280}$ ratio of 3.3–3.7 and a $A_{260/230}$ ratio of 3.5–4.1. Consider RNA base composition for interpretation of absorbance ratios.
Discrepancy between spectrophotometric and fluorescent dye based quantification methods.	<ul style="list-style-type: none"> Some RNA types (e.g. highly fragmented RNA like commercial toroula yeast RNA and homopolymeric RNA like poly-A+ RNA) have a reduced affinity to fluorescent dye based quantification methods like RiboGreen assays or Bioanalyzer assays. Consider this.
Appearance of white precipitate upon mixing of sample with buffer RCU.	<ul style="list-style-type: none"> RNA precipitation. Using highly concentrated RNA samples, RNA precipitate might appear upon addition of buffer RCU. Mix thoroughly, do not centrifuge this mixture at this point, but apply the complete mixture onto the NucleoSpin® RNA Maxi column.

6.3 Ordering information

Product	REF	Preps / Pack of
NucleoSpin® RNA Clean-up Maxi	740910.20	20
rDNase Set	740963	1 set
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 Midi	740962.20	20

6.4 Product use restriction / warranty

NucleoSpin® RNA Clean-up Maxi 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 Clean-up Maxi kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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 as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish an extra copy.

MACHEREY-NAGEL does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; against defects in products or components not manufactured by MACHEREY-NAGEL, or against damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL's sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agent 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 the contract of sale or of this warranty.

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 may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Please contact:

MACHEREY-NAGEL Germany

Tel.: +49 (0) 24 21 969-270

e-mail: TECH-BIO@mn-net.com

Trademarks:

NucleoSpin is a registered trademark of MACHEREY-NAGEL GmbH & Co KG

All used names and denotations can be brands, trademarks or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information, i.e. it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment. Regarding these products or services we can not grant any guarantees regarding selection, efficiency or operation.



www.mn-net.com

MACHEREY-NAGEL



MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6-8 · 52355 Düren · Germany

DE / International:

Tel.: +49 24 21 969-0
 Fax: +49 24 21 969-199
 E-mail: info@mn-net.com

CH:

Tel.: +41 62 388 55 00
 Fax: +41 62 388 55 05
 E-mail: sales-ch@mn-net.com

FR:

Tel.: +33 388 68 22 68
 Fax: +33 388 51 76 88
 E-mail: sales-fr@mn-net.com

US:

Tel.: +1 484 821 0984
 Fax: +1 484 821 1272
 E-mail: sales-us@mn-net.com