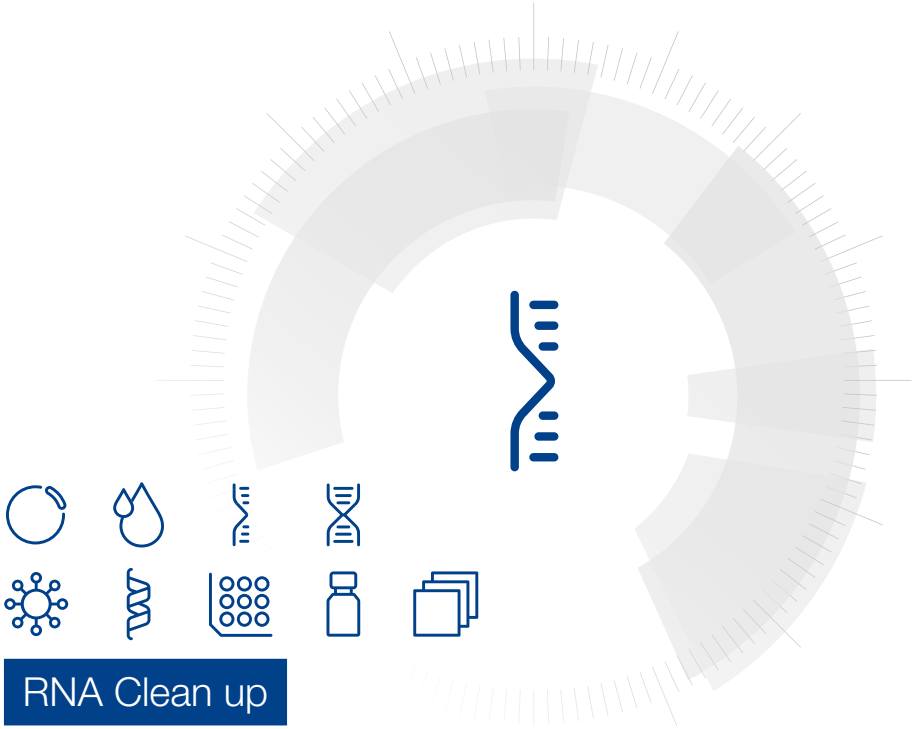
Bioanalysis

MACHEREY-NAGEL

User manual



RNA Clean up

■ NucleoSpin® RNA Clean-up Maxi

May 2023 / Rev. 03

RNA Clean up

Protocol at a glance (Rev. 03)

		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	 	1st wash + 7.5 mL Wash Buffer RA2 2nd wash + 7.5 mL Wash Buffer RA3 3rd 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_2O 4,000 x *g*, 2 min *or alternatively* + 1 mL RNase-free H_2O 4,000 x *g*, 2 min Repeat 2 more times

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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_2O	60 mL
NucleoSpin® RNA Clean-up Maxi Column (plus collection tubes)	20
Collection Tube (50 mL)	40
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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. ß-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 strenght 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.

For more information, visit our E-Training website: ***www.mn-net.com/clean-up***

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	3 – 5 mL
Preparation time	25 – 30 min/6 preps
Binding capacity	35 mg RNA per column
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 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 the 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 × 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 15 – 25 °C until: see package label. NucleoSpin® RNA Clean up Maxi Columns have to be stored at 4 °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 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 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

When working with the **NucleoSpin® RNA Clean up Maxi** 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 RCU 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 Maxi** 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 acceptablemanner and in accordance with all local and regulatory requirements.

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***.

2 min, 4,000 x *g*

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

4 **Wash and dry silica membrane**

1st wash

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

7.5 mL Wash Buffer RA2

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

2 min, 4,000 x *g*

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

2nd wash

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

7.5 mL Wash Buffer RA3

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

2 min, 4,000 x *g*

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

3rd wash

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

7.5 mL Wash Buffer RA3

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

Discard flow through with collection tube.

2 min, 4,000 x *g*

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.

5 **Elute RNA**

Add **5 mL RNase-free H_2O** and centrifuge **2 min at 4,000 x *g***.

5 mL RNase-free H_2O

or alternatively

Add **1 mL RNase-free H_2O** and centrifuge **2 min at 4,000 x *g***.

2 min, 4,000 x *g*

Add another **1 mL RNase-free H_2O** and centrifuge **2 min at 4,000 x *g***.

or alternatively

Add another **1 mL RNase-free H_2O** and centrifuge **2 min at 4,000 x *g***.

+ 1 mL RNase-free H_2O

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.

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.	• 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 $A_{260/280}$	• 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.	• 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 fluorecent dye based quantification methods like RiboGreen assays or Bioanalyzer assays. Consider this.
Appearance of white precipitate upon mixing of sample with buffer RCU.	• RNA precipitation. Using highly concentrated RNA samples, RNA precipitate might appear upon addition of buffer RCU. Mix thouroghly, 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

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 costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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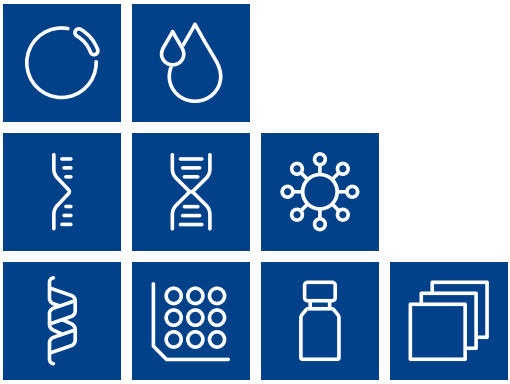

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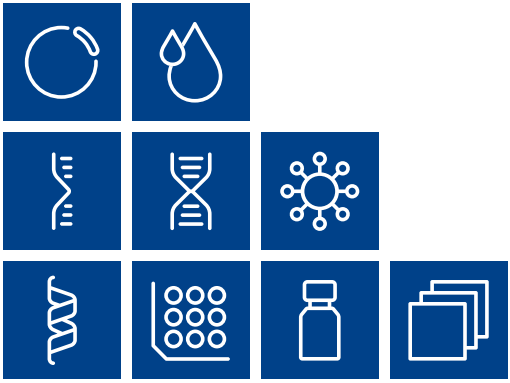

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