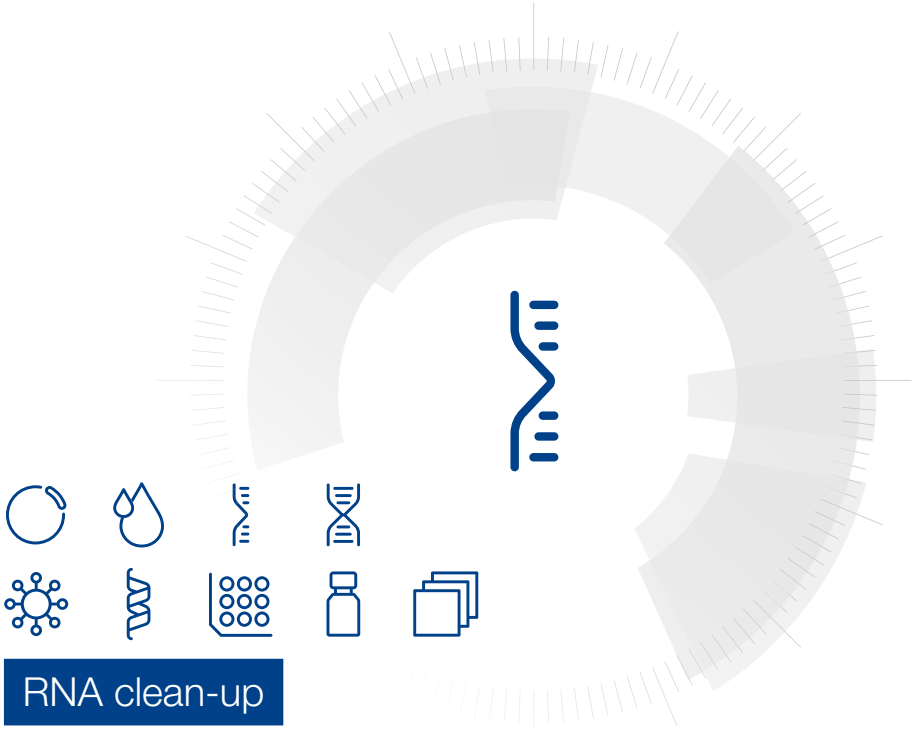
Bioanalysis

MACHEREY-NAGEL

User manual



RNA clean-up

■ NucleoSpin® RNA Clean-up XS

March 2021 / Rev. 05

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1 Components

1.1 Kit contents

	NucleoSpin® RNA Clean-up XS		
REF	**10 preps 740903.10**	**50 preps 740903.50**	**250 preps 740903.250**
Clean-up Buffer RCU (Concentrate)*	5 mL	5 mL	5 x 5 mL
Wash Buffer RA3 (Concentrate)*	6 mL	12 mL	50 mL
RNase-free H_2O	13 mL	13 mL	13 mL
NucleoSpin® RNA Clean-up XS Binding 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
User manual	1	1	1

* 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 and prepare Clean-up Buffer RCU and Wash Buffer RA3)

Consumables

- 1.5 mL microcentrifuge tubes
- Sterile RNase-free tips

Equipment

- Manual pipettors
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Personal protection equipment (e.g., 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 Clean-up XS** 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

A major aspect of RNA clean up is preventing degradation of the RNA during the clean up procedure. The **NucleoSpin® RNA Clean-up XS** method achieves this by mixing the crude RNA extract with a binding buffer, containing chaotropic ions, and ethanol. This buffer immediately inactivates RNases (which are present in virtually all biological materials) and creates appropriate binding conditions to allow adsorption of RNA to the silica membrane. Two washing steps with a single buffer remove any impurities. Pure RNA is finally eluted at low ionic strength conditions with RNase-free water (supplied) in a volume as small as 5 µL.

The RNA clean up procedure using **NucleoSpin® RNA Clean-up XS** kit can be performed at room temperature. The eluate should be treated with care because RNA is very sensitive to trace contaminations of RNases, often present on general lab ware, fingerprints, and dust. To ensure RNA stability, we recommend keeping the RNA solution frozen at -20 °C for short-term or -70 °C for long-term storage.

2.2 Kit specifications

- The **NucleoSpin® RNA Clean-up XS** kit is recommended for the clean up and concentration of prepurified RNA samples. Typical sample material covers nanogramm to microgramm amounts of prepurified RNA (e.g., phenol-purified RNA) and RNA from reaction mixtures (e.g., DNase treated samples).
- The innovative column design with a funnel shaped thrust ring and a small silica membrane area allows sample volumes of up to 300 µL and elution of RNA in as little as 5–30 µL. Thus, **highly concentrated RNA** is eluted and is ready for common downstream applications (e.g., RT-PCR). RNA enrichment of 20 x up to 50 x can be achieved (e.g., input: 300 µL sample containing crude RNA (10 ng/µL); output: 5 µL eluate containing pure RNA (510 ng/µL); enrichment of factor 51 (MACHEREY-NAGEL in-house data)).
- The **RNA recovery rate** is typically 85–95 %.
- High quality RNA (RNA Integrity Number (RIN) > 9 according to Agilent 2100 Bioanylzer assays) can be obtained from high quality RNA samples. The RIN of the processed sample is typically equal (±0.3) to the RIN of the input sample. RNA quality always depends on the sample quality, see section 6.3 for further aspects.
- The **NucleoSpin® RNA Clean-up XS** kit allows clean up and concentration of RNA with an A_{260}/A_{280} ratio generally exceeding 1.9 (measured in TE buffer pH 7.5). Due to the high RNA purity, large amounts of eluates can be used as template in RT-PCR without inhibition (e. g., 8 µL of 10 µL eluates as template in a 20 µL qRT-PCR setup generating stronger signal compared to reactions with less template in a LightCycler™ PCR with the Sigma SYBR® Green Quantitative RT-PCR Kit).

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® RNA Clean-up XS
Technology	Silica membrane technology
Format	Mini spin columns – XS design
Sample material	< 300 µL RNA solution containing < 90 µg RNA
Fragment size	> 200 nt
Typical recovery	85–95 %
A_{260}/A_{280}	1.9–2.1
Elution volume	5–30 µL
Preparation time	Approx. 20 min/6 preps
Binding capacity	110 µg

2.3 Handling, preparation, and storage of starting materials

RNA intended to be used as sample for the **NucleoSpin® RNA Clean-up XS** 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.

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

2.4 Elution procedures

A high RNA concentration in the elution fraction is desirable for all typical downstream applications. In particular with regard to limited volumes of reaction mixes, high RNA concentration can be a crucial criterion. Due to a high default elution volume, standard kits often result in low concentrated RNA, if only small samples are processed.

Such RNA often even requires a subsequent concentration to be suitable for the desired application.

In contrast to standard kits, **NucleoSpin® RNA Clean-up XS** allows an efficient elution in a very small volume resulting in highly concentrated RNA.

Elution volumes in the range of 5–30 µL are recommended, the default volume is 10 µL.

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: *Buffers RCU contains chaotropic salt. Wear gloves and goggles!*

CAUTION: Buffer RCU contains 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 room temperature (18–25 °C) and are stable up to 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** protocol, prepare the following:

- **Clean-up Buffer RCU:** Add the indicated volume of 96–100 % ethanol to the Clean-up Buffer RCU Concentrate. See table below or bottle label for necessary volumes. Store Buffer RCU at room temperature (18–25 °C) for up to one year.
- **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 (18–25 °C) for up to one year.

	NucleoSpin® RNA Clean-up		
REF	**10 preps 740903.10**	**50 preps 740903.50**	**250 preps 740903.250**
Clean-up Buffer RCU (Concentrate)	5 mL Add 15 mL ethanol	5 mL Add 15 mL ethanol	5 x 5 mL Add 15 mL ethanol to each bottle
Wash Buffer RA3 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol

4 Safety instructions

The following components of the **NucleoSpin® RNA Clean-up XS** kits 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 ***Inhalt***	**Hazard contents** ***Gefahrstoff***	**GHS symbol** ***GHS-Symbol***	**Hazard phrases** ***H-Sätze***	**Precaution phrases** ***P-Sätze***
RCU	guanidinium thiocyanate 45–60 % *Guanidinthiocyanat 45–60 %* CAS 593-84-0	 WARNING *ACHTUNG*	302, 412	264W, 273, 301+312, 330

Hazard phrases

H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 412	Harmful to aquatic life with long lasting effects. *Schädlich für Wasserorganismen, mit langfristiger Wirkung.*

Precaution phrases

P 264W	Wash with water thoroughly after handling. *Nach Gebrauch mit Wasser gründlich waschen.*
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.*
P 330	Rinse mouth. *Mund ausspülen.*

 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)***.

* Hazard labeling not neccessary 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.

5 Protocols

5.1 RNA clean up and concentration of RNA

Before starting the preparation:

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

1 Sample preparation

Provide up to **300 µL sample** containing up to 90 µg RNA – such as prepurified RNA (e.g., phenol purified) or RNA from reaction mixtures (e.g., labelling reactions) – in a microcentrifuge tube (not provided).

For appropriate sample amounts see section 2.2.

Note: Fill up RNA samples smaller than 100 µL with RNase-free water to 100 µL. RNA samples from 100–200 µL should be filled up with RNase-free water to 200 µL.

2 Adjust RNA binding conditions

Add **one volume of Buffer RCU** to the sample (e.g., 100 µL RCU to 100 µL sample) and mix **2 x 5 s**. If necessary, spin down gently (approx. 1 s at 1,000 x *g*) to clean the lid.

+ 1 vol. RCU

Mix (2 x 5 s)

3 Bind RNA

Take one NucleoSpin® RNA XS Column (light blue ring) placed in a Collection Tube for each preparation. Load up to **300 µL sample mix** to the column. Centrifuge for **30 s at 11,000 x *g***.

Load sample mix

For volumes exceeding 300 µL, load the sample mix in two subsequent centrifugation steps onto the column.

11,000 x *g*, 30 s

Place the column in a new Collection Tube (2 mL).

Maximal loading capacity of NucleoSpin® RNA XS Columns is 600 µL. However, for maximum performance loading at most 300 µL onto the column for one centrifugation step is recommended. For larger volumes, load the sample mix in two (or more if necessary) successive centrifugation steps. Repeat the procedure if larger volumes are to be processed. For high demanding applications, the recovery rate can further be increased as follows: Centrifuge 30 s at 2,000 x g prior to centrifugation for 30 s at 11,000 x g.

4 Wash and dry silica membrane

1st wash

Add **400 µL Buffer RA3** to the NucleoSpin® RNA XS Column. Centrifuge for **30 s** at **11,000 x *g***. Discard flowthrough and place the column back into the Collection Tube.

+ 400 µL RA3

11,000 x *g*, 30 s

2nd wash

Add **200 µL Buffer RA3** to the NucleoSpin® RNA XS Column. Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane. Place the column into a nuclease-free Collection Tube (1.5 mL, supplied).

+ 200 µL RA3

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

11,000 x *g*, 2 min

5 Elute RNA

Elute the RNA in **10 µL RNase-free H_2O**, (supplied) and centrifuge at **11,000 x *g***. for **30 s**.

+ 10 µL RNase-free H2O

If higher RNA concentrations or higher elution volumes are desired, elution volume may be varied in the range of 5–30 µL.

11,000 x *g*, 30 s

For further details on alternative elution procedures see section 2.4.

5.2 DNA digestion in crude RNA extracts and subsequent clean up

Several commonly used RNA purification methods co-purify DNA to a considerable extent (e.g., phenol based RNA purification). This often requires a subsequent removal of contaminating DNA and clean up of the RNA from the reaction mixture.

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 MACHEREY-NAGEL rDNase Set (to be ordered separately, see ordering information), contains high quality, recombinant RNase-free DNase (rDNase) and reaction buffer. It is optimized for a highly efficient digestion in order to remove even traces of contaminating DNA.

1 Digest DNA (reaction setup)

Prepare enzyme-buffer premix: Add **1 µL rDNase** to **10 µL Reaction Buffer for rDNase**.

Add 1 / 10 volume of enzyme-buffer premix to the crude RNA extract (e.g., to 10 µL RNA extract add 1 µL of the premix comprising buffer and enzyme).

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

Note: Dissolve lyophilized rDNase (rDNase Set, see ordering information) in 540 µL RNase-free H_2O as described in the corresponding user manual.

2 Incubate sample

Incubate for **10 min** at **37 °C**.

3 Repurify RNA

Repurify RNA with the NucleoSpin® RNA Clean up XS kit according to section 5.1.

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* • Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added. • No ethanol has been added to Clean-up Buffer RCU. Binding of RNA to the silica membrane is only effective in the presence of ethanol. Adjust binding conditions by adding ethanol to Clean-up Buffer RCU Concentrate as described in section 3. • Store kit components at room temperature (18–25 °C). Storage at lower temperatures may cause salt precipitation. If precipitation occurs, incubate the bottle for several minutes at about 30–40 °C and mix well until the precipitate is redissolved. • Keep bottles tightly closed in order to prevent evaporation or contamination. *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. Keep thawed samples on ice before addition of Buffer RCU.
Contamination of RNA with genomic DNA	*Sample material already contaminated with DNA* • Digest contaminating DNA in an RNA sample according to section 5.2.

Problem	Possible cause and suggestions
Suboptimal performance of RNA in downstream experiments	*Carry-over of ethanol or salt* • 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. • Depending on the robustness of the used RT-PCR system, RT-PCR might be inhibited if complete eluates are used as template for RT-PCR. Use less eluate as template. *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.
Higher RNA yield than theoretically possible	• 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).
Unexpected A_{260}/A_{280} ratio	*Measurement not in the range of photometer detection limit* • In order to obtain a significant A_{260}/A_{280} ratio it is necessary that the initially measured A_{260} and A_{280} values are significantly above the detection limit of the photometer used. An A_{280} value close to the background noise of the photometer will cause unexpected A_{260}/A_{280} ratios.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® RNA Clean-up XS	740903.10 740903.50 740903.250	10 preps 50 preps 250 preps
NucleoSpin® RNA XS	740902.10 740902.50 740902.250	10 preps 50 preps 250 preps
NucleoSpin® RNA	740955.20 740955.50 740955.250	20 preps 50 preps 250 preps
NucleoSpin® RNA Midi	740962.20	20 preps
NucleoSpin® RNA/Protein	740933.10 740933.50 740933.250	10 preps 50 preps 250 preps
NucleoSpin® TriPrep	740966.10 740966.50 740966.250	10 preps 50 preps 250 preps
NucleoSpin® RNA Clean-up	740948.10 740948.50 740948.250	10 preps 50 preps 250 preps
NucleoSpin® miRNA	740971.10 740971.50 740971.250	10 preps 50 preps 250 preps
NucleoSpin® RNA Blood	740200.10 740200.50	10 preps 50 preps
NucleoSpin® RNA Plant	740949.10 740949.50 740949.250	10 preps 50 preps 250 preps
NucleoSpin® FFPE RNA	740969.10 740969.50 740969.250	10 preps 50 preps 250 preps
NucleoSpin® RNA/DNA Buffer Set	740944	Suitable for 100 preps
rDNase Set	740963	1 set
NucleoSpin® Filters	740606	50
Collection Tubes (2 mL)	740600	1000

6.3 Literature

Fleige S, Pfaffl MW.: RNA integrity and the effect on the real-time qRT-PCR performance. Mol Aspects Med. 2006 Apr-Jun; 27(2–3):126–39. Epub 2006 Feb 15. Review.

Imbeaud S, Graudens E, Boulanger V, Barlet X, Zaborski P, Eveno E, Mueller O, Schroeder A, Auffray C.: Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces. Nucleic Acids Res. 2005 Mar 30;33(6):e56.

Miller CL, Diglisic S, Leister F, Webster M, Yolken RH.: Evaluating RNA status for RT-PCR in extracts of postmortem human brain tissue. Biotechniques. 2004 Apr; 36(4):628–33.

Schoor O, Weinschenk T, Hennenlotter J, Corvin S, Stenzl A, Rammensee HG, Stevanovic S.: Moderate degradation does not preclude microarray analysis of small amounts of RNA. Biotechniques. 2003 Dec; 35(6):1192–6, 1198–201.

6.4 Product use restriction / warranty

NucleoSpin® RNA Clean-up XS 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.

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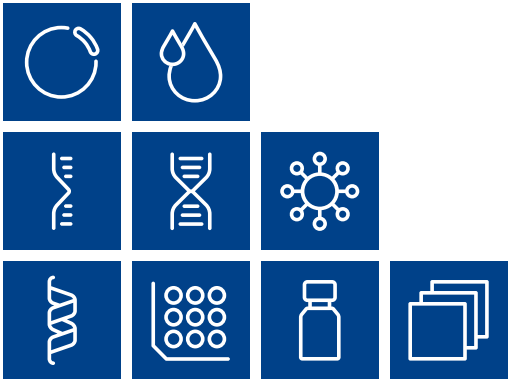

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