RNA Clean-up

User Manual

NucleoSpin® RNA Clean-up XS

March 2008 / Rev. 01

MACHEREY-NAGEL

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1 Components

1.1 Kit contents

	NucleoSpin® RNA Clean-up XS		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740903.10**	**740903.50**	**740903.250**
Clean-up Buffer RCU (Concentrate)*	1 ml	5 ml	25 ml
Wash Buffer RA3 (Concentrate)*	2 ml	7 ml	2 x 20 ml
H_2O, RNase-free	5 ml	15 ml	25 ml
NucleoSpin® RNA XS 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 Consumables and equipment to be supplied by user

Consumables

- 96-100% ethanol
- 1.5 microcentrifuge tubes (for sample supply)
- Manual pipettors and disposable pipette tips

Equipment

- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Personal protection equipment (lab coat, gloves, goggles)

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

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 20x up to 50x 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 $A_{260/280}$ 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	
	NucleoSpin® RNA Clean-up XS
Sample size	up to 90 µg RNA up 300 µl RNA solution
Recovery rate	85 – 95%
Elution volume	5 – 30 µl
Binding capacity	> 90 µg
Maximum loading volume	600 µl
Time/prep	15 min / 6 preps
Spin column type	NucleoSpin® XS columns

2.3 Handling, preparation, and storage of starting materials

Crude RNA samples are unprotected against digestion by RNases. Therefore keep such samples frozen, preferably at –70°C. Thaw samples immediately before starting the **NucleoSpin® RNA Clean-up XS** 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 weakly 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:

Buffer RCU contains guanidine thiocyanate. Wear gloves and goggles!

- All kit components should be stored at room temperature (20-25°C) and are stable up to one year. Storage at lower temperatures may cause precipitation of salts.

- Check that 96-100% ethanol is available for preparation of the Clean-up Buffer RCU.

Before starting with any **NucleoSpin® RNA Clean-up XS** 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 (20-25°C) for up to one year.

- **Wash Buffer RA3**: Add the indicated volume of 96-100% ethanol to the Wash Buffer RA3 Concentrate. See table below or bottle label for necessary volumes. Store Buffer RA3 at room temperature (20-25°C) for up to one year.

	NucleoSpin® RNA Clean-up XS		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740903.10**	**740903.50**	**740903.250**
Clean-up Buffer RCU (Concentrate)	1 ml add 3 ml ethanol	5 ml add 15 ml ethanol	25 ml add 75 ml ethanol
Wash Buffer RA3 (Concentrate)	2 ml add 8 ml ethanol	7 ml add 28 ml ethanol	2 x 20 ml add 80 ml ethanol to each bottle

4 Safety instructions – risk and safety phrases

The following components of the NucleoSpin® RNA Clean-up XS kit contain hazardous contents.

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

Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
Buffer RCU	Guanidine thiocyanate	 Xn*	Harmful by inhalation, in contact with skin and if swallowed.	R 20/21/22	S 13

Risk Phrases

R 20/21/22 Harmful by inhalation, in contact with the skin and if swallowed

Safety Phrases

S 13 Keep away from food, drink and animal feedstuffs

* Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

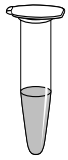
5 Protocols

5.1 RNA clean-up and concentration of RNA with NucleoSpin® RNA Clean-up XS

Before starting with the preparation, make sure that Buffer RCU and RA3 have been prepared according to section 3!

1 Supply sample

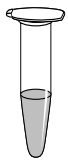
Provide up to 300 µl sample containing up to 90 µg RNA – such as prepurified RNA (e.g. phenol purified) or RNA from reaction mixtures (e.g. labelling reactions) – in a microcentrifuge tube (not provided).



For appropriate sample amounts see section 2.3.

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 sec**. If necessary, spin down gently (approx. 1 sec at 1,000 x *g*) to clear the lid.



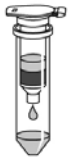
add 1 vol. RCU to sample

mix (2 x 5 sec)

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 sec** at **11,000 × *g***.

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



load sample mix

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

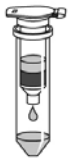
30 sec 11,000 × *g*

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 lager 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 sec at 2,000 x g *prior to centrifugation for 30 sec at 11,000 x* g*.*

4 1st wash

Add **400 µl Buffer RA3** to the NucleoSpin® RNA XS Column. **Centrifuge** for **30 sec** at **11,000 x *g***. Discard flow-through and place the column back into the Collection Tube.

+ 400 µl RA3



30 sec
11,000 × *g*

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).

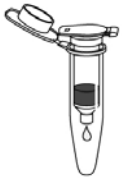
+ 200 µl RA3

2 min
11,000 x *g*

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

5 Elute highly pure RNA

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



+ 10 µl H_2O
(RNase-free)

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

For further details on alternative elution procedures see section 2.4.

30 sec
11,000 x *g*

5.2 Support protocol: DNA digestion in crude RNA extracts and subsequent clean-up with NucleoSpin® RNA Clean-up XS

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, Cat.-No. 740963), 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 sec at 1,000 x *g*) to collect every droplet of the solution at the bottom of the tube.

Note: Dissolve lyophilized rDNase (rDNase Set, Cat.-No. 740963) in 540 µl RNase-free H_2O as described in the corresponding User Manual.

2 Incubation

Incubate for **10 min** at **37°C**.

3 Repurification of 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. *Kit storage* • Store kit components as described in section 3. • Keep bottles tightly closed in order to prevent evaporation or contamination. *Ionic strength and pH influence A_{260} absorption as well as ratio $A_{260/280}$* • For adsorption measurement, use 5 mM Tris pH 8.5 as diluent. Please see also: - Manchester, K L. 1995. Value of A260/A280 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 was not stored properly. Keep thawed samples on ice before addition of Buffer RCU.

Problem	Possible cause and suggestions
Contamination of RNA with genomic DNA	*Sample material already contaminated with DNA* • Digest contaminating DNA in an RNA sample according to section 5.2.
Suboptimal performance of RNA in downstream experiments	*Carryover of ethanol or salt* • Do not let the flow-through 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 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.
Discrepancy between A_{260} quantification values and PCR quantification values	*Silica abrasion from the membrane* • When processing nanogramm amounts of RNA, an RNA quantification via A_{260} absorption measurement may be compromised by the low sensitivity of the method. When performing absorption measurements close to the detection limit of the photometer, the measurement can be influenced by trace amounts of silica abrasion. In order to prevent incorrect A_{260}-quantification of small RNA amounts, centrifuge the eluate for 30 sec at >11,000 x *g* and take an aliquot for measurement. Alternatively, a silica abrasion insensitive RNA quantification method (e.g. RiboGreen fluorescent dye) can be applied.
Unexpected $A_{260/280}$ ratio	*Measurement not in the range of photometer detection limit* • In order to obtain a significant $A_{260/280}$ 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 $A_{260/280}$ ratios.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® RNA Clean-up XS	740903.10/ .50/ .250	10/ 50/ 250
NucleoSpin® RNA XS	740902.10/ .20/ .50/ .250	10/ 50/ 250
NucleoSpin® RNA II	740955.10/ .20/ .50/ .250	10/ 20/ 50/ 250
NucleoSpin® RNA L	740962.20	20
NucleoSpin® RNA/Protein	740933.10/ .50/ .250	10/ 50/ 250
NucleoSpin® RNA Clean-up	740948.10/ .50/ .250	10/ 50/ 250
NucleoSpin® RNA/DNA Buffer Set	740944	suitable for 100 preps
Buffer RCU	740961	50 ml
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 were developed, designed, distributed 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 XS kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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Last updated 12/2006, Rev. 02