

RNA Clean-up

User manual

NucleoSpin[®] RNA Clean-up

October 2004/Rev. 01

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1 Kit contents

NucleoSpin® RNA Clean-up			
Cat. No.	10 preps 740948.10	50 preps 740948.50	250 preps 740948.250
Buffer RA1	10 ml	25 ml	125 ml
Buffer RA2	15 ml	15 ml	80 ml
Buffer RA3 (concentrate)*	5 ml	12.5 ml	3 x 25 ml
H ₂ O (RNase-free)	5 ml	15 ml	65 ml
NucleoSpin® RNA Binding Columns (light blue-plus collecting tube)	10	50	250
NucleoSpin® Collecting tubes	10	50	250
1.5 ml elution tubes	10	50	250
Protocol	1	1	1

* 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. 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 recommended for the clean-up of total RNA from RNA preparations which contain unacceptable amounts of RT-PCR inhibitors, often found in e.g. RNA prepared with phenol-chloroform based methods.
- It is further recommended for the isolation of RNA from small amounts of cultured cells whenever copurification of some genomic DNA is acceptable. For the isolation of RNA from cells and tissue with lowest DNA contamination of the isolated RNA we recommend DNase I containing NucleoSpin® RNA kits (see ordering information). The kits allow purification of pure RNA with an $A_{260/280}$ ratio generally exceeding 1.9 (measured in TE buffer (pH 7.5)).
- NucleoSpin® RNA Clean-up kits are further recommended for the clean-up of RNA from enzymatic reactions like *in vitro* transcribed RNA, amplification reactions (e.g. ExpressArt® Amino-Allyl-mRNA Amplification Kit, artus GmbH, Hamburg, Germany), biotinylated RNA or fluorescent (Cy dye) labeled RNA.
- The purified RNA is ready to use for applications like enzymatic labelling reactions (e.g. dye incorporation), reverse transcriptase-PCR* (RT-PCR*), and for DNA/RNA based chip hybridisations (e.g. MWG rat microarray, MWG, Ebersberg, Germany or Human Genome U133A Array, Affymetrix, USA).
- Integrity of purified RNA, originally isolated from e.g. eukaryotic cells, is examined by denaturing agarose gel electrophoresis: rRNA bands are sharp, with the 28S band being about twice as intense as the 18S band.

Table 1: Kit specifications at a glance

Sample size	up to 100 µl RNA sample with single column loading
	up to 200 µl RNA sample with double column loading
	up to 10 ⁵ cells
Average RNA retrieval for clean-up procedure (0,1 – 20 µg RNA input)	85 – 95%
Elution volume	40 - 120 µl
Binding capacity	100 µg
Time/prep	approx. 20 min / 6 preps
Spin column type	mini

- The standard protocol (section 4.1) allows the clean-up of up to 100 µg of RNA per NucleoSpin[®] RNA Binding Column or the isolation of total RNA from up to 1 x 10⁵ cultured cells (section 4.2).

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 take as for any RNA sample. The stability of prepurified RNA samples (e.g. 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 adapt elution method and volume of water used for the subsequent application of interest. In addition to the standard method described in the protocol (recovery rate about 70-90%), section 5, 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 put 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:

Buffer RA1 and RA2 contain 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% is available as additional solution in the lab.

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



- **Buffer RA3:** Add the indicated volume of 96 – 100% ethanol to the buffer concentrate. Store Buffer RA3 at room temperature (20-25°C) for up to one year.

NucleoSpin® RNA Clean-up			
	10 preps	50 preps	250 preps
Cat. No.	740948.10	740948.50	740948.250
Buffer RA3 (concentrate)	5 ml add 20 ml ethanol	12.5 ml add 50 ml ethanol	3 x 25 ml add 100 ml ethanol to each bottle

4 Safety instructions – risk and safety phrases

The following components of the NucleoSpin® RNA Clean-up kits 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 RA1	guanidine thiocyanate	 Xn*	Harmful by inhalation, in contact with skin and if swallowed	R 20/21/22 S 13
Buffer RA2	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 (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

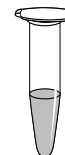
5 Protocols

5.1 RNA Clean-up

1 Sample preparation

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.



fill up RNA sample to 100 µl with water

2 Preparation of lysis-binding buffer premix

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

For each 100 µl RNA sample mix 300 µl of 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 RA1 + 2 ml 98% ethanol for approximately 6 preparations).

Prepare premix:

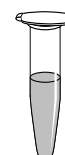
mix 300 µl RA1 with 300 µl ethanol (~98%)

3 Adjust RNA binding conditions

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

If 200 µl of RNA sample are processed, add 1200 µl of 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 homogenous solution onto the column.



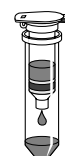
+ 6 vol. premix mix

4 Bind RNA

For each preparation, take one NucleoSpin® RNA Binding Column (light blue) placed in a 2 ml centrifuge tube and load the lysate (700 µl).

Centrifuge **for 30 s at 8,000 × g**. Discard collecting tube with flow-through and place the column in a new collecting tube.

Maximal loading capacity of RNA Binding Columns is 750 µl. Repeat the procedure if larger volumes are to be processed.



load 700 µl lysate

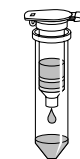


**30 s
8,000 × g**

5 Wash and Dry silica membrane

1st wash

Add **700 µl Wash buffer RA3** to the RNA Binding Column. Centrifuge for **30 s** at **8,000 × g**. Discard flow-through and reuse collecting tube.



+ 700 µl RA3

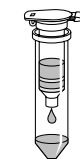
30 s
8,000 × g



2nd wash

Add **350 µl Wash buffer RA3** to the RNA Binding Column. Centrifuge for **2 min** at **8,000 × g**.

Transfer the spin column to a fresh 1.5 ml elution tube. Open the lid of the spin column and let it sit for 3 min.



+ 350 µl RA3

2 min
8,000 × g



If for any reason, the liquid level in the collecting tube has reached the RNA Binding Column after centrifugation, discard flow-through and centrifuge again.

The procedure ensures complete removal of ethanol from the column.

6 Elute highly pure RNA

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



+ 60 µl H₂O (RNase-free)

1 min
8,000 × g



For further alternative elution procedures see section 2.4.

5.2 RNA isolation from up to 10⁵ cells

1 Sample preparation

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



up to 100 µl
sample
volume

2 Cell lysis

Add 300 µl buffer RA1 and vortex vigorously in order to lyse the cells.

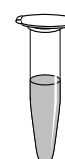
add 300 µl RA1

vortex

3 Adjust RNA binding conditions

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

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



add 300 µl
ethanol
(96 – 100%)

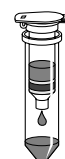
mix

4 Bind RNA

For each preparation, take one NucleoSpin® RNA Binding Column (light blue) placed in a 2 ml centrifuge tube and load the lysate (700 µl).

Centrifuge **for 30 s at 8,000 × g**. Discard collecting tube with flow-through and place the column in a new collecting tube.

Maximal loading capacity of RNA Binding Columns is 750 µl. Repeat the procedure if larger volumes are to be processed.



load lysate

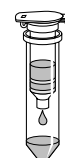


30 s
8,000 × g

5 Wash and Dry silica membrane

1st wash

Add **250 µl buffer RA2** to the NucleoSpin® RNA Binding Column. Centrifuge for **30 s at 8,000 × g**. Discard flow-through and reuse collection tube



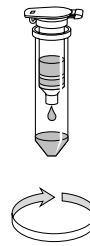
+ 250 µl RA2



30 s
8,000 × g

2nd wash

Add **700 µl Wash buffer RA3** to the RNA Binding Column. Centrifuge for **30 s** at **8,000 × g**. Discard flow-through and reuse collecting tube.



+ 700 µl RA3

30 s
8,000 × g

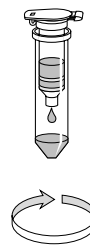
3rd wash

Add **350 µl Wash buffer RA3** to the RNA Binding Column. Centrifuge for **2 min** at **8,000 × g**.

Transfer the spin column to a fresh 1.5 ml elution tube. Open the lid of the spin column and let stand for 3 min.

If for any reason, the liquid level in the collecting tube has reached the RNA Binding Column after centrifugation, discard flow-through and centrifuge again.

The procedure ensures complete removal of ethanol from the column.



+ 350 µl RA3

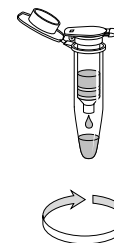
2 min
8,000 × g

6 Elute highly pure RNA

Elute the RNA in **60 µl H₂O (RNase-free)** (supplied) and centrifuge at **8,000 × 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 H₂O
(RNase-free)

1 min
8,000 × g

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded/ no RNA obtained	<p data-bbox="416 443 727 477"><i>RNase contamination</i></p> <ul data-bbox="416 499 1382 689" 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	<p data-bbox="416 741 1015 775"><i>Reagents not applied or restored properly</i></p> <ul data-bbox="416 797 1362 965" 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. <p data-bbox="416 1010 571 1043"><i>Kit storage</i></p> <ul data-bbox="416 1066 1326 1223" style="list-style-type: none"> • Store kit components at room temperature. Storage at low temperatures may cause salt precipitation. • Keep bottles tightly closed in order to prevent evaporation or contamination. <p data-bbox="416 1267 651 1301"><i>Sample material</i></p> <ul data-bbox="416 1323 1382 1491" 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.
Contamination of RNA with genomic DNA	<ul data-bbox="416 1559 1382 1727" 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 DNase I containing NucleoSpin® RNA kits (see ordering information).

Problem	Possible cause and suggestions
Suboptimal performance of RNA in downstream experiments	<p data-bbox="414 268 798 302"><i>Carryover of ethanol or salt</i></p> <ul data-bbox="414 324 1391 784" style="list-style-type: none"> <li data-bbox="414 324 1391 459">• 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. <li data-bbox="414 481 1391 593">• 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. <li data-bbox="414 616 1391 784">• A 2 min centrifugation with a subsequent 3 min drying with open lid is sufficient for an extensive removal of ethanol from the column. Increasing the drying step with open lid from 3 min to 20 min will decrease the residual ethanol content commonly to below 0.1%. <p data-bbox="414 828 798 862"><i>Store isolated RNA properly</i></p> <ul data-bbox="414 884 1391 1052" style="list-style-type: none"> <li data-bbox="414 884 1391 1052">• 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	<ul data-bbox="414 1120 1391 1478" style="list-style-type: none"> <li data-bbox="414 1120 1391 1478">• 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 sec. 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	Cat. No.	Pack of
NucleoSpin® RNA II	740955.20	20
NucleoSpin® RNA II	740955.50	50
NucleoSpin® RNA II	740955.250	250
NucleoSpin® RNA L	740962.20	20
NucleoSpin® RNA Plant	740949.20	20
NucleoSpin® RNA Plant	740949.50	50
NucleoSpin® RNA Plant	740949.250	250
NucleoSpin® 8 RNA	740698	12 x 8
NucleoSpin® 8 RNA	740698.5	60 x 8
NucleoSpin® 96 RNA	740709.2	2 x 96
NucleoSpin® 96 RNA	740709.4	4 x 96
NucleoSpin® 96 RNA	740709.24	24 x 96
Lysis buffer RA1	740961	50 ml
Lysis buffer RA1	740961.500	500 ml
DNase I set	740963	1 set
NucleoSpin® Filter	740606	50
NucleoSpin® collection tubes	740600	1000
NucleoSpin® 96 RNA Filter Plate	740711	4 plates

6.3 Product use restriction / warranty

NucleoSpin® RNA Clean-up kits 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** 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.

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