BIOKÉ
sharing knowledge

Plesmanlaan 1d
2333 BZ Leiden
The Netherlands
T. +31 (0)71 568 10 00
T. Belgium: 0800 71640
F. +31 (0)71 568 10 10
info@bioke.com
www.bioke.com

RNA Clean-up

User Manual

NucleoSpin® RNA Clean-up

February 2010 / Rev. 02

MACHEREY-NAGEL

Table of contents

1 Components

1.1 Kit contents

	NucleoSpin® RNA Clean-up		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740948.10**	**740948.50**	**740948.250**
Lysis Buffer RA1	10 ml	25 ml	125 ml
Wash Buffer RA2	15 ml	15 ml	80 ml
Wash Buffer RA3 (Concentrate)*	5 ml	12.5 ml	3 x 25 ml
RNase-free H_2O	5 ml	15 ml	65 ml
NucleoSpin® RNA 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 the user

Reagents

- 96 – 100% ethanol (to prepare Wash Buffer RA3 and to adjust RNA binding conditions)

Consumables

- 1.5 ml microcentrifuge tubes
- Sterile RNase-free tips

Equipment

- Manual pipettors
- 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** 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*.

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 ideal for the clean-up of total RNA from RNA preparations which contain inacceptable amounts of RT-PCR inhibitors (e.g., RNA prepared with phenol-chloroform based methods).
- The kit is further recommended for the isolation of RNA from small amounts of cultured cells whenever copurification of some genomic DNA is acceptabel. The kits allow purification of pure RNA with an A_{260}/A_{280} ratio generally exceeding 1.9 (measured in TE buffer (pH 7.5)).
- **NucleoSpin® RNA Clean-up** kits are recommended for the clean-up of RNA from enzymatic reactions like *in vitro* transcribed RNA, amplification reactions, 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 for example 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	
Parameter	**NucleoSpin® RNA Clean-up**
Sample material	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 – 200 µg RNA input)	85 – 95%
Elution volume	40 – 120 µl
Binding capacity	200 µg
Preparation time	Approx. 20 min/6 preps
Spin column type	Mini spin column

- The standard protocol (section 5.1) allows the clean-up of up to 200 µg of RNA per NucleoSpin® RNA Binding Column or the isolation of total RNA from up to 1 x 10^5 cultured cells (section 5.2).

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 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. 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 N_2 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 adjust the elution method and the volume of RNase-free water used for the subsequent application of interest. In addition to the standard method described in the individual protocols (recovery rate about 70 – 90%) 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 placed 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:

Buffers RA1 and RA2 contain guanidine thiocyanate. Wear gloves and goggles!

- 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:

- **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		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740948.10**	**740948.50**	**740948.250**
Wash 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
RA1	Guanidinium thiocyanate	✖ Xn*	Harmful by inhalation, in contact with skin, and if swallowed	R 20/21/22	S 13
RA2	Guanidinium thiocyanate	✖ Xn*	Flammable - Harmful by inhalation, in contact with skin, and if swallowed	R 10-20/21/22	S 13-16

Risk phrases

R 10	Flammable
R 20/21/22	Harmful by inhalation, in contact with skin, and if swallowed

Safety phrases

S 13	Keep away from food, drink, and animal feedstuffs
S 16	Keep away from sources of ignition – No Smoking!

* 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

Before starting the preparation:

- Check if Wash Buffer RA3 was prepared according to section 3.

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 Buffer RA1-ethanol premix with a ratio of 1:1.

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

Prepare premix:

Mix 300 µl RA1 with 300 µl ethanol (96 – 100%)

3 Adjust RNA binding conditions

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

If a 200 µl RNA sample is processed, add 1200 µl Buffer RA1-ethanol premix.

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

+ 6 vol. premix

Mix

4 Bind RNA

For each preparation, take one NucleoSpin® RNA Binding Column (light blue ring) placed in a Collection Tube and load the lysate (700 µl).

Load 700 µl lysate

Centrifuge for **30 s** at **8,000 x *g***. Discard Collection Tube with flow-through and place the column in a new Collection Tube.

8,000 x *g*
30 s

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

5 Wash and dry silica membrane

+ 700 µl RA3

1st wash

Add **700 µl Buffer RA3** to the NucleoSpin® RNA Binding Column. Centrifuge for **30 s** at **8,000 x *g***. Discard flow-through and reuse Collection Tube.

8,000 x *g*
30 s

2nd wash

Add **350 µl Buffer RA3** to the NucleoSpin® RNA Binding Column. Centrifuge for **2 min** at **8,000 x *g***.

+ 350 µl RA3

Transfer the NucleoSpin® RNA Binding Column to a nuclease-free Collection Tube (1.5 ml, supplied). Open the lid of the column and let the membrane dry for 3 min.

8,000 x *g*
2 min

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

The procedure ensures complete removal of ethanol from the column.

6 Elute RNA

+ 60 µl RNase-free H_2O

Elute the RNA in **60 µl RNase-free H_2O**, (supplied) and centrifuge at **8,000 x *g***. for **1 min**.

8,000 x *g*
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.

5.2 RNA isolation from up to 10^5 cells

Before starting the preparation:

- Check if Wash Buffer RA3 was prepared according to section 3.

1 Sample preparation

As sample material use **up to 10^5 cells** in a volume of up to **100 µl**.

Fill up sample to 100 µl (e.g. with PBS)

2 Cell lysis

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

+ 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.

+ 300 µl ethanol (96 – 100%)

Mix

4 Bind RNA

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

Centrifuge for **30 s** at **8,000 x *g***. Discard Collection Tube with flow-through and place the column in a new Collection Tube.

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

Load lysate

8,000 x *g*
30 s

5 Wash and dry silica membrane

1st wash — **+ 250 µl RA2**

Add **250 µl Buffer RA2** to the NucleoSpin® RNA Binding Column. Centrifuge for **30 s** at **8,000 x *g***. Discard flow-through and reuse Collection Tube — **8,000 x *g*, 30 s**

2nd wash — **+ 700 µl RA3**

Add **700 µl Buffer RA3** to the NucleoSpin® RNA Binding Column. Centrifuge for **30 s** at **8,000 x *g***. Discard flow-through and reuse Collection Tube. — **8,000 x *g*, 30 s**

3rd wash

Add **350 µl Buffer RA3** to the NucleoSpin® RNA Binding Column. Centrifuge for **2 min** at **8,000 x *g***.

Transfer the NucleoSpin® RNA Binding Column to a nuclease-free Collection Tube (1.5 ml, supplied). Open the lid of the column and let the membrane dry for 3 min. — **+ 350 µl RA3**, **8,000 x *g*, 2 min**

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

The procedure ensures complete removal of ethanol from the column.

6 Elute RNA

Elute the RNA in **60 µl RNase-free H_2O**, (supplied) and immediately centrifuge at **8,000 x *g***. for **1 min**. — **+ 60 µl RNase-free H_2O**, **8,000 x *g*, 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.

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. Binding of RNA to the silica membrane is only effective in the presence of ethanol. *Kit storage* • 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. *Sample material* • Sample material not stored properly. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid N_2. Samples should always be kept at -70°C. Never allow tissues to thaw before addition of lysis buffer. Perform disruption of samples in liquid N_2.
Contamination of RNA with genomic DNA	• 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 rDNase containing NucleoSpin® RNA kits (see ordering information).

Problem	Possible cause and suggestions
Suboptimal performance of RNA in downstream experiments	*Carry-over 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 Wash Buffer RA3. • A 2 min centrifugation with a subsequent 3 min drying with open lid is sufficent for an extensive removal of ethanol from the column. Residual ethanol will typically be around 1%. Increasing the drying step with open lid from 3 min to 20 min will decrease the residual ethanol content commonly to below 0.1%, but also RNA recovery will be reduced 5 – 20%. *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. *RNA concentration is too low* • For highest RNA concentration and most sensitive downstream applications, NucleoSpin® RNA Clean-up XS is recommended. NucleoSpin® RNA Clean-up XS allows elution in only 5 – 20 µl volume (see ordering information).
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).

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® RNA Clean-up	740948.10	10 preps
	740948.50	50 preps
	740948.250	250 preps
NucleoSpin® RNA Clean-up XS	740903.10	10 preps
	740903.50	50 preps
	740903.250	250 preps
NucleoSpin® RNA II	740955.20	20 preps
	740955.50	50 preps
	740955.250	250 preps
NucleoSpin® RNA XS	740902.10	10 preps
	740902.50	50 preps
	740902.250	250 preps
NucleoSpin® RNA L	740962.20	20 preps
NucleoSpin® RNA Plant	740949.20	20 preps
	740949.50	50 preps
	740949.250	250 preps
NucleoSpin® 8 RNA	740698	12 x 8 preps
	740698.5	60 x 8 preps
NucleoSpin® 96 RNA	740709.2	2 x 96 preps
	740709.4	4 x 96 preps
	740709.24	24 x 96 preps
NucleoMag 96 RNA	744350.1	1 x 96 preps
	744350.4	4 x 96 preps
NucleoSpin® miRNA	740971.10	10 preps
	740971.50	50 preps
	740971.250	250 preps
NucleoSpin® TriPrep*	740966.10	10 preps
	740966.50	50 preps
	740966.250	250 preps

* DISTRIBUTION AND USE OF NUCLEOSPIN® TRIPREP IN THE USA IS PROHIBITED FOR PATENT REASONS.

Product	Cat. No.	Pack of
NucleoSpin® RNA/Protein	740933.10	10 preps
	740933.50	50 preps
	740933.250	250 preps
NucleoSpin® FFPE RNA	740969.10	10 preps
	740969.50	50 preps
	740969.250	250 preps
Buffer RA1	740961	50 ml
	740961.500	500 ml
rDNase Set	740963	1 set

Visit *www.mn-net.com* for more detailed product information.

6.3 Product use restriction / warranty

NucleoSpin® RNA Clean-up 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** kit 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

Last updated: 12/2006, Rev. 02