Total RNA Isolation

User Manual

NucleoSpin® 8 RNA
NucleoSpin® 96 RNA

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MACHEREY-NAGEL

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1 Components

1.1 Kit contents

	NucleoSpin® 8 RNA	
	12 x 8 preps	60 x 8 preps
Cat. No.	740698	740698.5
Lysis Buffer RA1	50 ml	2 x 90 ml
Wash Buffer RA2	80 ml	360 ml
Wash Buffer RA3 (Concentrate)[1]	40 ml	2 x 90 ml
Wash Buffer RA4 (Concentrate)[1]	30 ml	3 x 65 ml
Reaction Buffer for rDNase	20 ml	5 x 20 ml
rDNase, RNase-free (lyophilized)[1]	2 vials	10 vials
RNase-free H_2O	30 ml	2 x 65 ml
NucleoSpin® RNA Binding Strips (blue rings)	12	60
Collection Tubes (1.5 ml)[2]	8	40
MN Wash Plates (including six Paper Sheets)[3]	2	5
Racks of Tube Strips with Cap Strips[4]	3	15
MN Square-well Blocks	2	2
Elution Plates U-Bottom (including one Self-adhering PE Foil)	1	5
User Manual	1	1

[1] For preparation of working solutions and storage conditions see section 3.

[2] For rDNase working solution during automated use

[3] Is not used when following the centrifuge protocol in section 6.3 for the isolation of total RNA.

[4] Set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips.

1.1 Kit contents *continued*

	NucleoSpin® 96 RNA		
	2 x 96 preps	4 x 96 preps	24 x 96 preps[1]
Cat. No.	740709.2	740709.4	740709.24
Lysis Buffer RA1	60 ml	125 ml	6 x 125 ml
Wash Buffer RA2	160 ml	360 ml	6 x 360 ml
Wash Buffer RA3 (Concentrate)[2]	90 ml	2 x 90 ml	12 x 90 ml
Wash Buffer RA4 (Concentrate)[2]	65 ml	2 x 65 ml	12 x 65 ml
Reaction Buffer for rDNase	2 x 20 ml	4 x 20 ml	24 x 20 ml
rDNase, RNase-free (lyophilized)[2]	4 vials	8 vials	48 vials
RNase-free H_2O	65 ml	2 x 65 ml	12 x 65 ml
NucleoSpin® RNA Binding Plates (blue rings)	2	4	24
Collection Tubes (1.5 ml)[3]	8	16	96
MN Wash Plates (including six Paper Sheets)[4]	2	4	24
MN Square-well Blocks	4	6	36
Elution Plate U-Bottom (including one Self-adhering PE Foil)[4]	2	4	24
Round-well Blocks Low (including Self-adhering PE Foil)	2	4	24
User Manual	1	1	6

[1] The kit for 24 x 96 preparations (Cat. No. 740709.24) consists of 6 x Cat. No. 740709.4.

[2] For preparation of working solutions and storage conditions see section 3.

[3] For rDNase working solution during automated use

[4] Is not used when following the centrifuge protocol in section 6.4 for the isolation of total RNA.

1.2 Reagents to be supplied by user

- 96 - 100% ethanol (see section 3)
- ß-mercaptoethanol (3 µl per preparation)
- NucleoSpin® RNA Filter Strips (NucleoSpin® 8 RNA) or NucleoSpin® RNA Filter Plates (NucleoSpin® 96 RNA) (optional; see section 2.4)

1.3 Required hardware

NucleoSpin® 8 RNA

The NucleoSpin® 8 RNA kit can be used **manually** with the NucleoVac 96 Vacuum Manifold (see ordering information) or similar suitable vacuum manifolds (see section 1.4) by using the Starter Set A containing Column Holders A and NucleoSpin® Dummy Strips (see ordering information).

For **automation** on laboratory platforms with standard 96-well plate vacuum chambers the use of the Starter Set A is also required.

For **centrifugation** a microtiterplate centrifuge is required which is able to accommodate the NucleoSpin® RNA Binding Strips stacked on a round or square-well block and reaches accelerations of 5,600 - 6,000 x *g* (bucket height: 85 mm). Furthermore, Starter Set C (see ordering information), containing Column Holders C, MN Square-well Blocks, and Rack of Tube Strips are required.

NucleoSpin® 96 RNA

The NucleoSpin® 96 RNA kit can be used with either the NucleoVac 96 Vacuum Manifold (see ordering information) or other common vacuum manifolds (see section 1.4). When using NucleoSpin® 96 RNA with less than 96 samples, Self-adhering PE Foil (see ordering information) should be used in order to close and protect non-used wells of the NucleoSpin® 96 RNA Binding Plate to guarantee a proper vacuum.

For **centrifugation** a microtiterplate centrifuge is required which is able to accommodate the NucleoSpin® RNA Binding Plate stacked on a round or square-well block and reaches accelerations of 5,600 - 6,000 x *g* (bucket height: 85 mm).

1.4 Suitable vacuum manifolds

The **NucleoSpin® 8/96 RNA** kits can be used with the NucleoVac 96 Vacuum Manifold or other common vacuum devices. For further details see Table 1.

Table 1: Suitable vacuum manifolds

Vacuum manifold	Suitability	Additional equipment
NucleoVac 96	Yes	Starter Set A for NucleoSpin® 8 RNA kit
Qiagen/QIAvac 96[1]	Yes	MN Frame (see ordering information) Starter Set A for NucleoSpin® 8 RNA kit
Promega/Vac-Man® 96[2]	Yes	Starter Set A for NucleoSpin® 8 RNA kit

[1] In general the QIAvac 96 is suitable for the use with the NucleoSpin® RNA Binding Plate. Nevertheless, it is recommended to use the MN Frame to adjust the proper height of the MN Wash Plate and Elution Plate in order to ensure best performance.

[2] MN Wash Plate cannot be used.

2 Product description

2.1 The basic principle

One of the most important aspects in the isolation of RNA is to prevent degradation of RNA during the isolation procedure. With the **NucleoSpin® 8/96 RNA** kits, cells or tissue are lysed by incubation in a solution containing large amounts of chaotropic salt. This lysis buffer immediately inactivates RNases which are present in virtually all biological materials and creates in combination with Buffer RA4 appropriate binding conditions which favor adsorption of RNA to the silica membrane. Contaminating DNA, which is also bound to the silica membrane, is removed by DNase which is directly applied onto the silica membrane during the preparation (RNase-free recombinant DNase is supplied with the kit). Salts, proteins, and other cellular components are removed by simple washing steps with three different buffers. Finally, pure RNA is eluted under low ionic strength conditions with RNase-free water (supplied).

2.2 Kit specifications

- The **NucleoSpin® 8/96 RNA** kits are designed for fast 8-well/96-well small-scale purification of total RNA from tissue or cells. Fresh, frozen or stabilized sample material can be processed.
- The **NucleoSpin® 8/96 RNA** kits can be used fully automated on common laboratory workstations (see section 2.6).
- The **NucleoSpin® 8/96 RNA** kits can be used manually under vacuum or under centrifugation.
- The kits provide reagents and consumables for purification of up to 100 µg highly-pure total RNA suitable for direct use in standard molecular biology applications, like RT-PCR, TaqMan, Northern Blot, or microarray analysis.
- The **NucleoSpin® 8/96 RNA** kits yield RNA of highest purity and integrity.
- Using the **NucleoSpin® 8/96 RNA** kits allows for simultaneous processing of up to 96 samples typically within less than 70 minutes. Actual automated processing time depends on the configuration of workstation used.

2.3 Yield and quality of total RNA

- **NucleoSpin® 8/96 RNA** can be used under vacuum or in a centrifuge. In a centrifuge, however, slightly higher yields are possible because of the higher amount of starting material that can be processed and the reduced dead volume of the membrane.

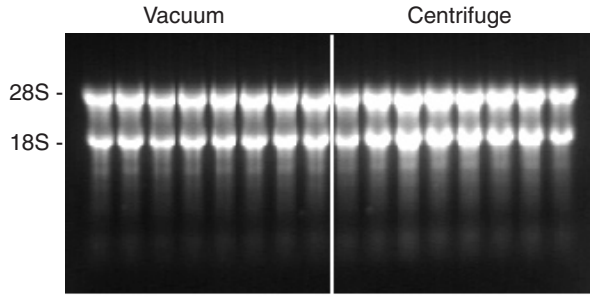


Figure 1: Total RNA was purified from 10 mg of liver tissue using NucleoSpin® 96 RNA. 100 µl of RNase-free H_2O was dispensed onto the silica membrane for elution. Thereby, 100 µl of RNA eluate were recovered by centrifugation, and 80 µl of RNA eluate were recovered using vacuum processing. 20/100 µl or 20/80 µl of each eluate were analyzed on a 1% formaldehyde agarose gel.

- Typical amounts of starting material and anticipated yields are shown in Table 2. Please note that yield of total RNA depends strongly on the starting material and on complete lysis/homogenization. Results may vary. For more information about the lysis/homogenization process see section 2.4.

Table 2: Kit specifications at a glance

	NucleoSpin® 8/96 RNA			
	Animal tissue		Cell culture	
Parameter	**Vacuum**	**Centrifuge**	**Vacuum**	**Centrifuge**
Max. sample size	10 - 30 mg	30 mg	2 x 10^6 cells	1 x 10^7 cells
Typical RNA yield	Up to 40 µg	Up to 100 µg	Up to 20 µg	Up to 100 µg
Elution volume	50 - 130 µl			
Max. binding capacity	100 µg			
RNA integrity	Sharp rRNA bands with no substantial degradative bands visibile; 28S:18S ~ 2.1 Excellent RNA Integrity Number (RIN) values typically: 9 - 10 for vells and ≥ 7 for tissue			
RNA purity	A_{260}/A_{280} 1.8 - 2.2			
Preparation time	70 min/96 preps			

- The final concentration of eluted RNA is 50 - 500 ng/µl (depending on elution volume and starting material). Suitable elution volumes range from 50 µl to 130 µl. For RNA purity typically, the A_{260}/A_{280} ratio is 1.9 - 2.1.

- The residual content of genomic DNA is less than 0.003% after isolation from more than 5 x 10^5 cells, as determined by quantitative PCR (see Figure 2).

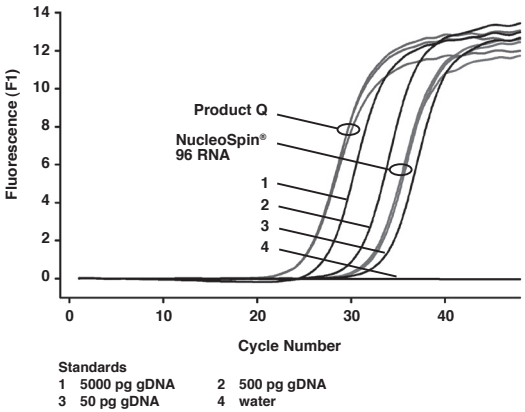


Figure 2: Total RNA was prepared manually under centrifugation from 5 x 10^5 HeLa cells according to the standard protocol for NucleoSpin® 96 RNA (including rDNase treatment) or product Q (does not include DNase treatment). PCR was performed (primer 28S, LightCycler-FastStart™ DNA Master SYBR® Green) to determine the amount of residual genomic DNA in the eluted RNA. The NucleoSpin® 96 RNA samples show a CT of 32.4 (equaling 140 pg gDNA), the samples purified with product Q show a CT of 25.2 (equaling 20.6 ng gDNA, averages from 3 runs each). Product Q shows an about 150 times higher amount of residual gDNA.

- Isolated RNA is of highest quality and integrity (see Figure 3) indicating a highly efficient inactivation of RNases and gentle purification.

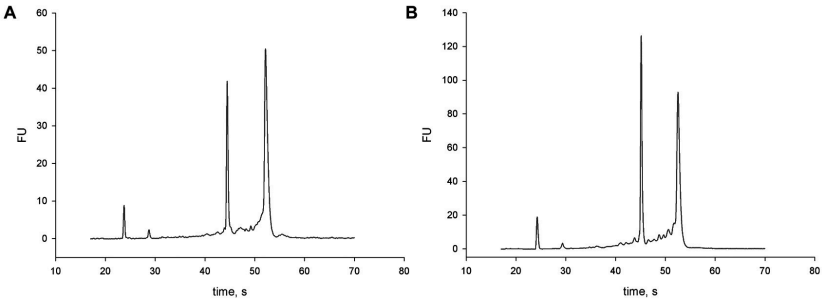


Figure 3: Typical electropherograms obtained from the Agilent Bioanalyzer 2100 using RNA Nano 6000 reagents. RNA was isolated from cells (A) or frozen liver tissue (B). RNA integrity numbers (RIN) are 9.7 for RNA isolated from cells and 9.1 for RNA isolated from frozen liver tissue.

2.4 Storage and homogenization of samples

RNA is not protected against digestion until the sample material is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents. It is therefore important that samples are either immediately frozen and stored at -70°C, put in a RNA stabilizing reagent (not included in the kit), or processed as soon as harvested. After disruption samples can be stored at -70°C in Lysis Buffer RA1. Frozen samples are stable for up to 6 months. Frozen samples in Buffer RA1 should be thawed completely and centrifuged before starting with the isolation of total RNA.

If larger cell numbers or large amounts of tissue are used for optimal homogenization and removal of particles the filtration of the RA1 lysate through the NucleoSpin® RNA Filter Strips or the NucleoSpin® RNA Filter Plate is recommended under vacuum or centrifugation (see ordering information).

Cell culture

- Up to 2 x 10^6 cells can be processed under vacuum. Using a centrifuge up to 1 x 10^7 cells can be processed. Transfer the cell suspension to a suitable square-well block (not included in the kit) and centrifuge for 5 min at 500 x *g*. The supernatant has to be removed completely. Lyse cells by addition of 300 µl Buffer RA1. Following lysis add 300 µl of Buffer RA4 to adjust RNA binding conditions.

- For adherent cell cultures in 96-well format, make sure that the culture medium is completely removed. Lyse cells by addition of 130 µl Buffer RA1. Following lysis add 130 µl of Buffer RA4 to adjust binding conditions.

- If using more than 1 x 10^6 cells, it is recommended to use a commercial homogenizer after lysis with Buffer RA1 in order to reduce the viscosity. To prevent the NucleoSpin® RNA Binding Strips/Plate from clogging it is also recommended to filtrate the lysate through the NucleoSpin® RNA Filter Strips/Plate (not included in the kit) before applying them to the NucleoSpin® RNA Binding Strips/Plate.

Tissue

- Depending on the type of sample up to 30 mg (see also Table 2) can be processed. Add 300 µl Buffer RA1 for homogenization of tissue. Following homogenization add 300 µl of Buffer RA4 to adjust binding conditions.

- For higher throughput in the 96-well format, add Lysis Buffer RA1 to frozen or stabilized tissue collected in a Round- or MN Square-well Block or Tube Strips (not included in the kit, see ordering information) and immediately disrupt tissue in an appropriate homogenizer.

- If working with tissue or nuclease-rich cells, add 1% ß-mercaptoethanol to Buffer RA1. ß-mercaptoethanol supports the inhibition of RNases.

- For sample homogenization and removal of cell debris, it is recommended to filter the lysate through the NucleoSpin® RNA Filter Strips/Plate (not included in the kit) before applying them to the NucleoSpin® RNA Binding Strips/Plate. Alternatively: Centrifuge homogenized tissue samples for 5 min at maximum *g*-forces, transfer supernatant to suitable plate and proceed with the standard protocol by adding Buffer RA4.

- Binding capacity of the membrane is up to 100 µg. Depending on type of tissue and homogenization yield will differ and has to be tested individually. The following table gives some typical results.

Table 3: Yields of total RNA with NucleoSpin® 8/96 RNA

Sample source	Max. starting material	Max. yield of total RNA
Spleen	20 mg	50 µg
Kidney	30 mg	45 µg
Brain	30 mg	20 µg
Liver	30 mg	80 µg
Yield of total RNA depends strongly on the tissue and the effectiveness of lysis/homogenization. Therefore, results may vary.		

Methods for sample homogenization

- Commercial homogenizers, for example, Geno/Grinder 2000 (SPEXCertiPrep® group, *www.spexcsp.com*) can be used for sample homogenization.

Use of NucleoSpin® RNA Filter Strips/Plate

- For sample homogenization and to prevent clogging of the NucleoSpin® RNA Binding Strips/Plate the use of the NucleoSpin® RNA Filter Strips/Plate is recommended under vacuum or centrifugation.

Centrifugation

NucleoSpin® 8 RNA: Insert desired number of NucleoSpin® RNA Filter Strips into the Column Holder C (see ordering information) and place it on an MN Square-well Block (see ordering information). Transfer lysates in Buffer RA1 to the wells of the NucleoSpin® RNA Filter Strips.

NucleoSpin® 96 RNA: Place the NucleoSpin® RNA Filter Plate on an MN Square-well Block (see ordering information) and apply the sample lysate in Buffer RA1 to the wells of the NucleoSpin® RNA Filter Plate.

Centrifuge at 5,600 - 6,000 x *g* until all samples have passed the filter (approx. 5 min). Start the RNA purification procedure with the filtrate collected in the MN Square-well Block.

Vacuum

Insert spacers "Square-well Block" into the NucleoVac 96 Vacuum Manifold. Put an MN Square-well Block in the manifold and close manifold with the manifold lid.

NucleoSpin® 8 RNA: Insert desired number of NucleoSpin® RNA Filter Strips into the Column Holder A (see ordering information) and place it on top of the manifold.

NucleoSpin® 96 RNA: Place the NucleoSpin® RNA Filter Plate on top of the manifold.

Transfer the sample lysate in Buffer RA1 to the filter and apply vacuum until the lysates have passed the filter. Start the RNA purification procedure with the filtrate collected in the MN Square-well Block. Please note that the dead volume of the NucleoSpin® RNA Filter Strip/Plate is rather large compared to the processing under centrifugation, thus processing under vacuum is only recommended when complete automation is desired.

2.5 Elution of pure total RNA

Due to dead volume of the silica membrane please notice that the difference between the dispensed elution buffer and the recovered elution buffer containing total RNA is approx. 20 µl. Elution of RNA is possible using a dispense volume of 50 - 130 µl nuclease free water. Higher RNA concentrations are obtained when using a dispense volume of 50 µl. Higher elution efficiencies are obtained when using dispense volumes of > 100 µl.

Recovered elution volume = Dispensed elution volume – 20 µl

Elution is possible under vacuum and in a centrifuge **without any cross-contamination** (see Figure 4). To achieve this, vacuum settings during the elution have to be adjusted carefully (smooth elution) to avoid splattering of liquid.

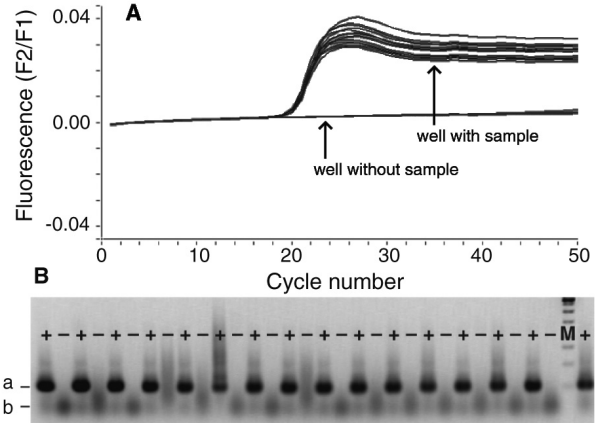


Figure 4: HeLa cells (5 x 10^5 each) were pelleted in 6 wells of a 96-well cultue plate in a checker board pattern. Total RNA was prepared using NucleoSpin® 96 RNA. (A) RT-PCR detection of total RNA was performed with 1.5 µl of the eluate (total eluate 80 µl) in a LightCycler™ (0.5 µM GAPDH primer, LightCycler™-RNA Amplification Kit Hybridization Probes). (B) 20 µl of the LightCycler™ assay were loaded on a 2% agarose gel.
+: Total RNA from 5 x 10^5 HeLa cells
–: Empty control well (next to a well containing cells)
A: 200 bp product of the GAPDH gene
B: Primers

2.6 Automated processing on robotic platforms

NucleoSpin® 8/96 RNA can readily be automated on common laboratory robotic workstations.

Visit MN on the internet at *www.mn-net.com* or contact your local MACHEREY-NAGEL distributor for availability of ready-to-run scripts and for technical support regarding hardware, software, setup instructions, and selection of the protocol. Several application notes are available on request or can be downloaded from our web-page.

3 Storage conditions and preparation of working solutions

Attention:

Buffers RA1 and RA2 contain guanidinium salt which is an irritant! Wear gloves and goggles when handling them!

- Store lyophilized **RNase-free rDNase** at +4°C on arrival (stable up to 1 year).
- All other components of the NucleoSpin® 8/96 RNA kit should be stored at room temperature for a maximum of 1 year. Storage at lower temperatures may cause precipitation of salts. If a salt precipitate is observed, incubate the bottle at 30-40°C for some minutes and mix well until all of the precipitate is redissolved.

Before starting with any **NucleoSpin® 8/96 RNA** procedure prepare the following:

- Reconstitute **RNase-free rDNase**: Add 540 µl RNase-free H_2O to the rDNase vial and incubate for 1 min at room temperature. Gently swirl the vial to completely dissolve the rDNase. If not processing a whole 96-well plate, dispense the reconstituted rDNase solution into aliquots and store at -18°C. This solution of rDNase is stable for up to six months. Do not freeze/thaw the aliquots more than three times. Prepare **rDNase reaction mixture**: For each sample to be processed mix 10 µl reconstituted rDNase with 90 µl Reaction Buffer for rDNase.
- **Wash Buffer RA3:** Add the indicated volume of 96-100% ethanol to the Buffer RA3 Concentrate. Mark the label of the bottle to indicate that ethanol is added. Store Wash Buffer RA3 at room temperature (20-25°C) for up to one year.
- **Wash Buffer RA4:** Add the indicated volume of 96-100% ethanol to the Buffer RA4 Concentrate. Mark the label of the bottle to indicate that ethanol is added. Store Wash Buffer RA4 at room temperature (20-25°C) for up to one year.
- Pocessing under vacuum: Establish a reliable vacuum source for the NucleoVac 96 Vacuum Manifold. The manifold may be used with vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of 0.2-0.4 bar (pressure difference). The use of the NucleoVac Vacuum Regulator (see ordering information) is recommended. Alternatively, adjust vacuum so that during the purification the sample flows through the column with a rate of 1-2 drops per second. Depending on the amount of sample used the vacuum times may have to be increased for complete filtration.

	NucleoSpin® 8 RNA	
	12 x 8 preps	**60 x 8 preps**
Cat. No.	**740698**	**740698.5**
Wash Buffer RA3 (Concentrate)	1 x 40 ml Add 160 ml ethanol	2 x 90 ml Add 360 ml ethanol to each bottle
Wash Buffer RA4 (Concentrate)	1 x 30 ml Add 70 ml ethanol	2 x 65 ml Add 150 ml ethanol to each bottle

	NucleoSpin® 96 RNA		
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps**[1]
Cat. No.	**740709.2**	**740709.4**	**740709.24**
Wash Buffer RA3 (Concentrate)	1 x 90 ml Add 360 ml ethanol	2 x 90 ml Add 360 ml ethanol to each bottle	12 x 90 ml Add 360 ml ethanol to each bottle
Wash Buffer RA4 (Concentrate)	1 x 65 ml Add 150 ml ethanol	2 x 65 ml Add 150 ml ethanol to each bottle	12 x 65 ml Add 150 ml ethanol to each bottle

[1] The kit for 24 x 96 preparations (Cat. No. 740709.24) consists of 6 x Cat. No. 740709.4.

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® 8/96 RNA** 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
rDNase	rDNase, lyophilized	✖ Xi*	May cause sensitization by inhalation and skin contact	R 42/43	S 22-24
RA1	Guanidine thiocyanate	✖ Xn*	Harmful by inhalation, in contact with skin, and if swallowed	R 20/21/22	S 13
RA2	Guanidine thiocyanate + ethanol < 24%	✖ Xn*	Flammable - Harmful by inhalation, in contact with skin, and if swallowed	R 10-20/21/22	S 7-13-16

Risk phrases

R 10	Flammable
R 20/21/22	Harmful by inhalation, in contact with the skin, and if swallowed
R 42/43	May cause sensitization by inhalation and skin contact

Safety phrases

S 7	Keep container tightly closed
S 13	Keep away from food, drink, and animal feedstuffs
S 16	Keep away from sources of ignition - No smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin

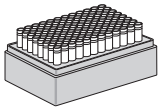
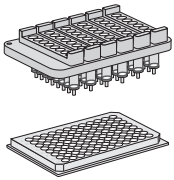
* Hazard labeling not neccessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

5 General procedure

NucleoSpin® 8 RNA, vacuum processing
(For details on each step see section 5.1.)

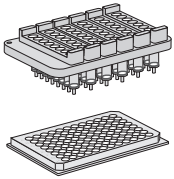
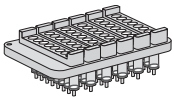
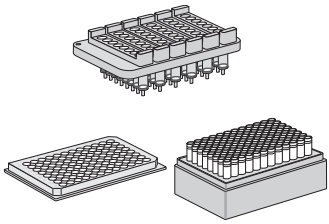
Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture was prepared according to section 3.

1	**Harvest** cells	**500 x *g* 5 min**	
2	**Lyse** cells or tissue *Optional: If using tissue samples or large number of cells, clearing of lysate with the NucleoSpin® RNA Filter Strips is recommended.*	**300 µl RA1 (cells, tissue) (+ 3 µl ß-ME)** or **130 µl RA1 (cells)[1] (+ 1.3 µl ß-ME)**	 Rack of Tube Strips
3	**Prepare binding** Pipette up and down 10 - 15 times to mix and resuspend genomic DNA that might have precipitated during the addition of Buffer RA4.	**300 µl RA4 (cells, tissue)** **or** **130 µl RA4 (cells)[1]**	
		Prepare vacuum manifold	
4	**Transfer of crude** lysates to NucleoSpin® RNA Binding Strips		 Column Holder A with NucleoSpin® RNA Binding Strips and MN Wash Plate
5	**Bind** RNA to silica membrane of the NucleoSpin® RNA Binding Strips	**- 0.2 bar[2] 1 min**	

[1] Cells grown in 96-well plates only

[2] Reduction of atmospheric pressure

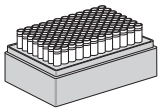
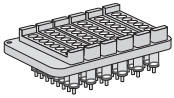
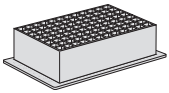
6	**Desalt** silica membrane by washing	**500 µl RA3** **- 0.2 bar[2]** **3 min**	
7	**DNase incubation**	**95 µl rDNase reaction mixture** **Room temperature** **15 min**	 Column Holder A with NucleoSpin® RNA Binding Strips and MN Wash Plate
8	**Wash** silica membrane	**500 µl RA2** **800 µl RA3** **500 µl RA4** **- 0.2 bar[2]** **1 min each step**	
9	**Dry** NucleoSpin® RNA Binding Strips by applying vacuum *<u>Optional:</u> Dry the outlets of the NucleoSpin® RNA Binding Strips by placing it on a Paper Sheet before applying vacuum.*	**Remove MN Wash Plate** **Maximum vacuum[2]** **10 min**	 Column Holder A with NucleoSpin® RNA Binding Strips
10	**Elute** highly pure total RNA	**75 µl RNase-free H_2O** **Incubate 2 min** **- 0.5 bar[2]** **1 min**	 Column Holder A with NucleoSpin® RNA Binding Strips on Elution Plate or Rack of Tube Strips

[2] Reduction of atmospheric pressure

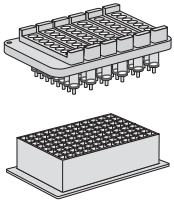
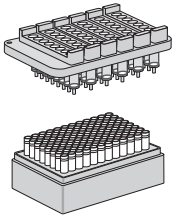
NucleoSpin® 8 RNA under centrifugation
(For details on each step see section 5.2.)

Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture was prepared according to section 3.

1	**Harvest** cells	**500 x *g* 5 min**	
2	**Lyse** cells or tissue *Optional: If using tissue samples or large number of cells, clearing of lysate with the NucleoSpin® RNA Filter Strips is recommended.*	**300 µl RA1 (cells, tissue) (+ 3 µl ß-ME) or 130 µl RA1 (cells)[1] (+ 1.3 µl ß-ME)**	 Rack of Tube Strips
3	**Prepare binding** Pipette up and down 10 - 15 times to mix and resuspend genomic DNA that might have precipitated during the addition of Buffer RA4.	**300 µl RA4 (cells, tissue) or 130 µl RA4 (cells)[1]**	
4	**Transfer of crude** lysates to NucleoSpin® RNA Binding Strips		  Column Holder C with NucleoSpin® RNA Binding Strips and MN Square-well Block
5	**Bind** RNA to silica membrane of the NucleoSpin® RNA Binding Strips	**5,000 - 6,000 x *g* 2 min**	
6	**Desalt** silica membrane by washing	**500 µl RA3 -5,000 - 6,000 x *g* 2 min**	

[1] Cells grown in 96-well plates only

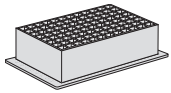
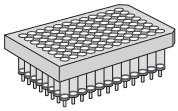
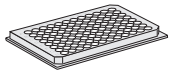
7	**DNase incubation**	**95 µl rDNase reaction mixture** **Room temperature 15 min**	
8	**Wash** silica membrane	**500 µl RA2** **5,000 - 6,000 x *g* 2 min** **800 µl RA3** **5,000 - 6,000 x *g* 2 min** **500 µl RA4** **5,000 - 6,000 x *g* 10 min[2]**	 Column Holder C with NucleoSpin® RNA Binding Strips and MN Square-well Block
9	**Dry** NucleoSpin® RNA Binding Strips	**Not necessary**	
10	**Elute** highly pure total RNA	**75 µl RNase-free H_2O** **Incubate 2 min** **5,000 - 6,000 x *g* 2 min**	 Column Holder C with NucleoSpin® RNA Binding Strips on Rack of Tube Strips

[2] Prolonged centrifugation time is required to evaporate ethanol from last wash step.

NucleoSpin® 96 RNA, vacuum processing
(For details on each step see section 5.3.)

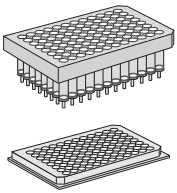
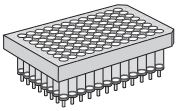
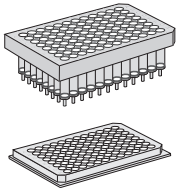
Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture was prepared according to section 3.

1	**Harvest** cells	**500 x *g* 5 min**	
2	**Lyse** cells or tissue *<u>Optional</u>: If using tissue samples or large number of cells, clearing of lysate with the NucleoSpin® RNA Filter Plate is recommended.* Transfer cleared lysate to MN Square-well Block	**300 µl RA1 (cells, tissue) (+ 3 µl ß-ME) or 130 µl RA1 (cells)[1] (+ 1.3 µl ß-ME)**	 MN Square-well Block
3	**Prepare binding** Pipette up and down 10 - 15 times to mix and resuspend genomic DNA that might have precipitated during the addition of Buffer RA4.	**300 µl RA4 (cells, tissue) or 130 µl RA4 (cells)[1]**	
		Prepare vacuum manifold	
4	**Transfer of crude** lysates to NucleoSpin® RNA Binding Plate		
5	**Bind** RNA to silica membrane of the NucleoSpin® RNA Binding Plate	**- 0.2 bar[2] 1 min**	 NucleoSpin® RNA Binding Plate and MN Wash Plate

[1] Cells grown in 96-well plates only

[2] Reduction of atmospheric pressure

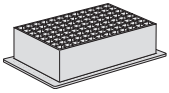
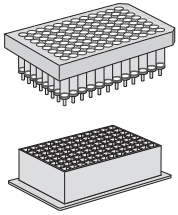
6	**Desalt** silica membrane by washing	**500 µl RA3** **- 0.2 bar[2]** **3 min**	
7	**DNase incubation**	**95 µl rDNase reaction mixture** **Room temperature** **15 min**	 NucleoSpin® RNA Binding Plate and MN Wash Plate
8	**Wash** silica membrane	**500 µl RA2** **800 µl RA3** **500 µl RA4** **- 0.2 bar[2]** **1 min each step**	
9	**Dry** NucleoSpin® RNA Binding Plate by applying vacuum *Optional: Dry the outlets of the NucleoSpin® RNA Binding Plate by placing it on a paper sheet before applying vacuum.*	**Remove MN Wash Plate** **Maximum vacuum[2]** **10 min**	 NucleoSpin® RNA Binding Plate
10	**Elute** highly pure total RNA	**75 µl RNase-free H_2O** **Incubate 2 min** **- 0.4 bar[2]** **1 min**	 NucleoSpin® RNA Binding Plate and Elution Plate U-bottom

[2] Reduction of atmospheric pressure

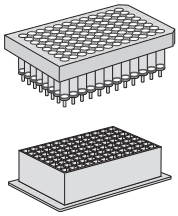
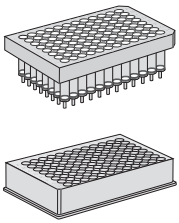
NucleoSpin® 96 RNA under centrifugation
(For details on each step see section 5.4.)

Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture was prepared according to section 3.

1	**Harvest** cells	**500 x *g* 5 min**	
2	**Lyse** cells or tissue *Optional: If using tissue samples or large number of cells, clearing of lysate with the NucleoSpin® RNA Filter Plate is recommended.*	**300 µl RA1 (cells, tissue) (+ 3 µl ß-ME) or 130 µl RA1 (cells)[1] (+ 1.3 µl ß-ME)**	 MN Square-well Block
3	**Prepare binding** Pipette up and down 10 - 15 times to mix and resuspend genomic DNA that might have precipitated during the addition of Buffer RA4.	**300 µl RA4 (cells, tissue) or 130 µl RA4 (cells)[1]**	
4	**Transfer of crude** lysates to NucleoSpin® RNA Binding Plate		 NucleoSpin® RNA Binding Plate and MN Square-well Block
5	**Bind** RNA to silica membrane of the NucleoSpin® RNA Binding Plate	**5,000 - 6,000 x *g* 2 min**	
6	**Desalt** silica membrane by washing	**500 µl RA3 5,000 - 6,000 x *g* 2 min**	

[1] Cells grown in 96-well plates only

7	**DNase incubation**	**95 µl rDNase reaction mixture** **Room temperature** **15 min**	
8	**Wash** silica membrane	**500 µl RA2** **5,000 - 6,000 x *g*** **2 min** **800 µl RA3** **5,000 - 6,000 x *g*** **2 min** **500 µl RA4** **5,000 - 6,000 x *g*** **10 min**[1]	 NucleoSpin® RNA Binding Plate and MN Square-well Block
9	**Dry** NucleoSpin® RNA Binding Plate	**Not necessary**	
10	**Elute** highly pure total RNA	**75 µl RNase-free H_2O** **Incubate 2 min** **5,000 - 6,000 x *g*** **2 min**	 NucleoSpin® RNA Binding Plate and Round-well Block Low

[2] Prolonged centrifugation time is required to evaporate ethanol from last wash step.

5.1 NucleoSpin® 8 RNA - Standard protocol for the manual purification of total RNA under vacuum

For manual processing under vacuum the NucleoVac 96 Vacuum Manifold or another suitable vacuum manifold is required (see ordering information).

Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture was prepared according to section 3.

1 Harvest cells

If cells have been grown in suspension, aliquots of up to 2 x 10^6 cells can be transferred into Tube Strips (included in the kit) or the wells of another suitable deep-well plate (e.g. MN Square-well Block, Round-well Block; see ordering information). Pellet cells by centrifugation (5 min, 500 x *g*), remove the supernatant by pipetting.

2 Lyse cells ot tissue

Cells, tissue samples

Add **300 µl Buffer RA1/1% ß-mercaptoethanol [vol/vol]** to each sample. Cells can be lysed by pipetting up and down repeatedly or vigorous shaking of the sealed/closed plate or reaction tube. For homogenization of tissue samples please refer to section 2.4.

*Optional: It is recommended using the **NucleoSpin® RNA Filter Strips** (see ordering information) for the clarification of tissue lysates.*

Cells cultures grown in 96-well plates

Discard culture medium and if necessary wash cells with PBS buffer once. Add **130 µl Buffer RA1/1% ß-mercaptoethanol [vol/vol]** to the cells in each well of the cell-culture plate. Cells can be lysed by pipetting up and down repeatedly or vigorous shaking of the sealed plate.

Note: Use of ß-mercaptoethanol is recommended, but not essential for most cell types (also see section 2.4).

3 Prepare binding

Depending on the volume of Buffer RA1 used in the lysis step add **300 µl RA4** or **130 µl Buffer RA4** (for lysates from cells grown in 96-well plates) to each well of the lysis plate or tube. Mix by pipetting up and down at least 10 - 15 times.

Note: Buffer RA1 and Buffer RA4 have to be used in the same volume ratio.

Prepare NucleoVac 96 Vacuum Manifold

Insert appropriate number of NucleoSpin® RNA Binding Strips into a Column Holder A. Close any unused openings of the Column Holder A with NucleoSpin® Dummy Strips.

Note: Make sure that the NucleoSpin® RNA Binding Strips are inserted tightly into the Column Holder A. Not properly inserted strips may prevent sealing when vacuum is applied to the manifold.

Insert spacers (MTP/Multi-96 Plate), notched side up, into the grooves located on the short sides of the manifold. Insert the waste reservoir into the center of the manifold. Put the MN Wash Plate on the spacers in the manifold base. Insert Column Holder A with inserted NucleoSpin® RNA Binding Strips into the manifold lid and place lid on the manifold base.

4 Transfer crude lysates to NucleoSpin® RNA Binding Strips

Apply the samples to the wells of the NucleoSpin® RNA Binding Strips.

5 Bind RNA to silica membrane

Apply vacuum until all lysates have passed through the wells **(- 0.2 bar; 1 min)**. Release the vacuum.

6 Desalt silica membrane

Desalt the membrane by adding **500 µl Buffer RA3** to each well and apply vacuum (- 0.2 bar; 3 min) until all buffer has passed through the wells. Release the vacuum.

7 DNase incubation

Prepare rDNase reaction mixture as described in section 3:

Pipette **95 µl rDNase reaction mixture** directly to the bottom of each well of the NucleoSpin® RNA Binding Strips. Do not touch the silica membrane with the pipette tips. Incubate at **room temperature** for **15 min**.

Be sure that all of the rDNase reaction mixture gets into contact with the silica membrane, and that the membrane is completely wetted.

8 Wash silica membrane

1st wash

Add **500 µl Buffer RA2** to each well of the NucleoSpin® RNA Binding Strips. Apply vacuum **(-0.2 bar; 1 min)** until all buffer has passed through the wells. Release the vacuum.

2nd wash

Add **800 µl Buffer RA3** to each well of the NucleoSpin® RNA Binding Strips. Apply vacuum **(-0.2 bar; 1 min)** until all buffer has passed through the wells. Release the vacuum.

3rd wash

Add **500 µl Buffer RA4** to each well of the NucleoSpin® RNA Binding Strips. Apply vacuum **(-0.2 bar; 1 min)** until all buffer has passed through the wells. Release the vacuum.

9 Remove MN Wash Plate

After the final wash step close the valve, release the vacuum and remove the Column Holder A with inserted NucleoSpin® RNA Binding Strips from the vacuum manifold. Put it on a clean paper towel to remove residual EtOH-containing wash buffer. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

Dry NucleoSpin® RNA Binding Strips

Remove any residual wash buffer from the NucleoSpin® RNA Binding Strips. If necessary, tap the outlets of the NucleoSpin® RNA Binding Strips onto a clean Paper Sheet (supplied with the MN Wash Plate) or soft tissue until no further drops come out. Insert the Column Holder A with inserted NucleoSpin® RNA Binding Strips into the manifold lid and close the manifold. Build up the vacuum with the valve closed. Once the **maximum vacuum (-0.6 bar)** is achieved, open the valve and apply vacuum for at least **10 min** to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer RA4 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

Finally, release the vacuum.

10 Elute highly pure total RNA

Place the Elution Plate U-bottom onto the spacers “MTP/Multi-96 Plate“ of the vacuum manifold. Pipette **75 µl**[1] **RNase-free H_2O** directly to the bottom of each well. Incubate for **2 min** at **room temperature**. Build up the vacuum with the valve closed. Once the maximum vacuum (- 0.5 bar) is achieved, open the valve and apply vacuum for 1 min.

Alternatively, elution in Tube Strips (included in the kit) or standard PCR plates is possible. For elution in Tube Strips, place the Rack of Tube Strips on the spacers “Microtube Strips“ inside the manifold. Elution into PCR plates can be performed by placing a PCR plate onto an MN Square-well Block resting on the spacers “Square-well Block“ in the manifold.

[1] 75 µl is recommended. 50 - 130 µl is possible, see section 2.5.

5.2 NucleoSpin® 8 RNA - Standard protocol for the manual purification of total RNA using a centrifuge

For manual processing under centrifugation the Starter Set C is required (see ordering information).

Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture was prepared according to section 3.

1 Harvest cells

If cells have been grown in suspension, aliquots of up to 2 x 10^7 cells can be transferred into Tube Strips (included in the kit) or the wells of another suitable deep-well plate (e.g. MN Square-well Block, Round-well Block; see ordering information). Pellet cells by centrifugation (5 min, 500 x *g*), remove the supernatant by pipetting by pipetting.

2 Lyse cells ot tissue

Cells, tissue samples

Add **300 µl Buffer RA1/1% ß-mercaptoethanol [vol/vol]** to each sample. Cells can be lysed by pipetting up and down repeatedly or vigorous shaking of the sealed/closed plate or reaction tube. For homogenization of tissue samples please refer to section 2.4.

Optional: It is recommended using the ***NucleoSpin® RNA Filter Strips*** *(see ordering information) for the clarification of tissue lysates.*

Cells cultures grown in 96-well plates

Discard culture medium and if necessary wash cells with PBS buffer once. Add **130 µl Buffer RA1/1% ß-mercaptoethanol [vol/vol]** to the cells in each well of the cell-culture plate. Cells can be lysed by pipetting up and down repeatedly or vigorous shaking of the sealed plate.

Note: Use of ß-mercaptoethanol is recommended, but not essential for most cell types (also see section 2.4).

3 Prepare binding

Depending on the volume of Buffer RA1 used in the lysis step add **300 µl RA4** or **130 µl Buffer RA4** (for lysates from cells grown in 96-well plates), to each well of the lysis plate or tube. Mix by pipetting up and down at least 10 - 15 times.

Note: Buffer RA1 and Buffer RA4 have to be used in the same volume ratio.

4 Transfer crude lysates to NucleoSpin® RNA Binding Strips

Insert desired number of NucleoSpin® RNA Binding Strips into the Column Holder C and place on it an MN Square-well Block for collection of flow-through. If using more than one block, label the column holders for later identification. Transfer lysates to the wells of the NucleoSpin® RNA Binding Strips.

5 Bind RNA to silica membrane

Centrifuge for **2 min** at **5,600 - 6,000 x *g***.

6 Desalt silica membrane

Desalt the membrane by adding **500 µl Buffer RA3** to each well and centrifuge for **2 min** at **5,600 - 6,000 x *g*.** Empty MN Square-well Block.

7 DNase incubation

Prepare rDNase reaction mixture as described in section 3:

Place Column Holder C with the NucleoSpin® RNA Binding Strips on the MN Square-well Block. Pipette **95 µl rDNase reaction mixture** directly to the bottom of each well of the NucleoSpin® RNA Binding Strips. Do not touch the silica membrane with the pipette tips. Incubate at **room temperature** for **15 min**.

Be sure that all of the rDNase reaction mixture gets into contact with the silica membrane, and that the membrane is completely wetted.

8 Wash silica membrane

1st wash

Add **500 µl Buffer RA2** to each well of the NucleoSpin® RNA Binding Strips. Place the Column Holder C with the NucleoSpin® RNA Binding Strips on the MN Square-well Block into the rotor bucket and centrifuge for **2 min** at **5,600 - 6,000 x *g*.** Empty MN Square-well Block.

2nd wash

Add **800 µl Buffer RA3** to each well of the NucleoSpin® RNA Binding Strips and centrifuge for or **2 min** at **5,600 - 6,000 x *g*.** Empty MN Square-well Block.

3rd wash

Add **500 µl Buffer RA4** to each well of the NucleoSpin® RNA Binding Strips and centrifuge for or **10 min** at **5,600 - 6,000 x *g.*** Empty MN Square-well Block.

9 Dry NucleoSpin® RNA Binding Strips

Residual wash buffer from the NucleoSpin® RNA Binding Strips is removed by the prolonged centrifugation time of **10 min** after adding Wash Buffer RA4 as described in step 8. This prolonged time is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer RA4 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

10 Elute highly pure total RNA

For elution place Column Holder C with the NucleoSpin® RNA Binding Strips onto the Rack of Tube Strips and pipette **75 µl[1] RNase-free H_2O** directly to the bottom of each well. Make sure that all of the water gets into contact with the silica membrane and that the membrane is completely wetted. Incubate for **2 min** at **room temperature** and for **3 min** at **5,600 - 6,000 x *g.***

Alternatively, elution in an MN Square-well Block or Round-well Block (see ordering information) is possible.

Note: The Elution Plate U-bottom is not suitable for use in a centrifuge.

[1] 75 µl is recommended. 50 - 130 µl is possible, see section 2.5.

5.3 NucleoSpin® 96 RNA - Standard protocol for the manual purification of total RNA under vacuum

For manual processing under vacuum the NucleoVac 96 Vacuum Manifold or another suitable vacuum manifold is required (see ordering information).

Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture was prepared according to section 3.

1 Harvest cells

If cells have been grown in suspension, aliquots of up to 2 x 10^6 cells can be transferred into wells of an MN Square-well Block (included in the kit) or another suitable deep-well plate or reaction tube (e.g. Round-well Block, Tube Strips; see ordering information). Pellet cells by centrifugation (5 min, 500 x *g*), remove the supernatant by pipetting.

2 Lyse cells ot tissue

Cells, tissue samples

Add **300 µl Buffer RA1/1% ß-mercaptoethanol [vol/vol]** to each sample. Cells can be lysed by pipetting up and down repeatedly or vigorous shaking of the sealed/closed plate or reaction tube. For homogenization of tissue samples please refer to section 2.4.

*Optional: It is recommended using the **NucleoSpin® RNA Filter Plate** (see ordering information) for the clarification of tissue lysates.*

Cells cultures grown in 96-well plates

Discard culture medium and if necessary wash cells with PBS buffer once. Add **130 µl Buffer RA1/1% ß-mercaptoethanol [vol/vol]** to the cells in each well of the cell-culture plate. Cells can be lysed by pipetting up and down repeatedly or vigorous shaking of the sealed plate.

Note: Use of ß-mercaptoethanol is recommended, but not essential for most cell types (also see section 2.4).

3 Prepare binding

Depending on the volume of Buffer RA1 used in the lysis step add **300 µl** or **130 µl Buffer RA4** (for lysates from cells grown in 96-well plates), to each well of the lysis plate or tube. Mix by pipetting up and down at least 10 - 15 times.

Note: Buffer RA1 and Buffer RA4 have to be used in the same volume ratio.

Prepare NucleoVac 96 Vacuum Manifold

Insert spacers (MTP/Multi-96 Plate), notched side up, into the grooves located on the short sides of the manifold. Insert the waste reservoir into the center of the manifold. Put the MN Wash Plate on the spacers in the manifold base.

4 Transfer crude lysates to NucleoSpin® RNA Binding Plate

Place a NucleoSpin® RNA Binding Plate into vacuum manifold's lid and apply the samples to the wells.

5 Bind RNA to silica membrane

Apply vacuum until all lysates have passed through the wells **(-0.2 bar; 1 min)**. Release the vacuum.

6 Desalt silica membrane

Desalt the membrane by adding **500 µl Buffer RA3** to each well and apply vacuum (-0.2 bar; 3 min) until all buffer has passed through the wells. Release the vacuum.

7 DNase incubation

Prepare rDNase reaction mixture as described in section 3:

Pipette **95 µl rDNase reaction mixture** directly to the bottom of each well of the NucleoSpin® RNA Binding Plate. Do not touch the silica membrane with the pipette tips. Incubate at **room temperature** for **15 min**.

Be sure that all of the rDNase reaction mixture gets into contact with the silica membrane, and that the membrane is completely wetted.

8 Wash silica membrane

1st wash

Add **500 µl Buffer RA2** to each well of the NucleoSpin® RNA Binding Plate. Apply vacuum **(-0.2 bar; 1 min)** until all buffer has passed through the wells. Release the vacuum.

2nd wash

Add **800 µl Buffer RA3** to each well of the NucleoSpin® RNA Binding Plate. Apply vacuum **(-0.2 bar; 1 min)** until all buffer has passed through the wells. Release the vacuum.

3rd wash

Add **500 µl Buffer RA4** to each well of the NucleoSpin® RNA Binding Plate. Apply vacuum **(-0.2 bar; 1 min)** until all buffer has passed through the wells. Release the vacuum.

9 Remove MN Wash Plate

After the final wash step close the valve, release the vacuum and remove the NucleoSpin® RNA Binding Plate from the vacuum manifold. Put it on a clean paper towel to remove residual EtOH-containing wash buffer. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

Dry NucleoSpin® RNA Binding Plate

Remove any residual wash buffer from the NucleoSpin® RNA Binding Plate. If necessary, tap the outlets of the NucleoSpin® RNA Binding Plate onto a clean Paper Sheet (supplied with the MN Wash Plate) or soft tissue until no further drops come out. Insert the NucleoSpin® RNA Binding Plate into the manifold lid and close the manifold. Build up the vacuum with the valve closed. Once the **maximum vacuum (-0.6 bar)** is achieved, open the valve and apply vacuum for at least **10 min** to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer RA4 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

Finally, release the vacuum.

10 Elute highly pure total RNA

Place the Elution Plate U-bottom onto the spacers “MTP/Multi-96 Plate“ of the vacuum manifold. Pipette **75 µl**[1] **RNase-free H_2O** directly to the bottom of each well. Incubate for **2 min** at **room temperature**. Build up the vacuum with the valve closed. Once the **maximum vacuum (-0.5 bar)** is achieved, open the valve and apply vacuum for **1 min**.

Alternatively, elution in standard PCR plates is possible. Elution into PCR plates can be performed by placing a PCR plate onto an MN Square-well Block resting on the spacers “Square-well Block“ in the manifold.

[1] 75 µl is recommended. 50-130 µl is possible, see section 2.5.

5.4 NucleoSpin® 96 RNA - Standard protocol for the manual purification of total RNA using a centrifuge

Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture was prepared according to section 3.

1 Harvest cells

If cells have been grown in suspension, aliquots of up to 2 x 10^7 cells can be transferred into the wells of an MN Square-well Block (included in the kit) or into the wells of another suitable deep-well plate or reaction tube (e.g. Round-well Block, Tube Strips; see ordering information). Pellet cells by centrifugation (5 min, 500 x *g*), remove the supernatant by pipetting by pipetting.

2 Lyse cells ot tissue

Cells, tissue samples

Add **300 µl Buffer RA1/1% ß-mercaptoethanol [vol/vol]** to each sample. Cells can be lysed by pipetting up and down repeatedly or vigorous shaking of the sealed/closed plate or reaction tube. For homogenization of tissue samples please refer to section 2.4.

*Optional: It is recommended using the **NucleoSpin® RNA Filter Plate** (see ordering information) for the clarification of tissue lysates.*

Cells cultures grown in 96-well plates

Discard culture medium and if necessary wash cells with PBS buffer once. Add **130 µl Buffer RA1/1% ß-mercaptoethanol [vol/vol]** to the cells in each well of the cell-culture plate. Cells can be lysed by pipetting up and down repeatedly or vigorous shaking of the sealed plate.

Note: Use of ß-mercaptoethanol is recommended, but not essential for most cell types (also see section 2.4).

3 Prepare binding

Depending on the volume of Buffer RA1 used in the lysis step add **300 µl Buffer RA4** or **130 µl Buffer RA4** (for lysates from cells grown in 96-well plates), to each well of the lysis plate or tube. Mix by pipetting up and down at least 10 - 15 times.

Note: Buffer RA1 and Buffer RA4 have to be used in the same volume ratio.

4 Transfer crude lysates to NucleoSpin® RNA Binding Plate

Place the NucleoSpin® RNA Binding Plate on an MN Square-well Block and transfer lysates to the wells of the NucleoSpin® RNA Binding Plate.

5 Bind RNA to silica membrane

Centrifuge for **2 min** at **5,600 - 6,000 x *g***.

6 Desalt silica membrane

Desalt the membrane by adding **500 µl Buffer RA3** to each well and centrifuge for **2 min** at **5,600 - 6,000 x *g*.** Empty MN Square-well Block.

7 DNase incubation

Prepare rDNase reaction mixture as described in section 3:

Place the NucleoSpin® RNA Binding Plate on the MN Square-well Block. Pipette **95 µl rDNase reaction mixture** directly to the bottom of each well of the NucleoSpin® RNA Binding Plate. Do not touch the silica membrane with the pipette tips. Incubate at **room temperature** for **15 min**.

Be sure that all of the rDNase reaction mixture gets into contact with the silica membrane, and that the membrane is completely wetted.

8 Wash silica membrane

1st wash

Add **500 µl Buffer RA2** to each well of the NucleoSpin® RNA Binding Plate. Place the NucleoSpin® RNA Binding Plate on the MN Square-well Block into the rotor bucket and centrifuge for **2 min** at **5,600 - 6,000 x *g*.** Empty MN Square-well Block.

2nd wash

Add **800 µl Buffer RA3** to each well of the NucleoSpin® RNA Binding Plate and centrifuge for or **2 min** at **5,600 - 6,000 x *g*.** Empty MN Square-well Block.

3rd wash

Add **500 µl Buffer RA4** to each well of the NucleoSpin® RNA Binding Plate and centrifuge for or **10 min** at **5,600 - 6,000 x *g*.** Empty MN Square-well Block.

9 Dry NucleoSpin® RNA Binding Plate

Residual wash buffer from the NucleoSpin® RNA Binding Plate is removed by the prolonged centrifugation time of **10 min** after adding Wash Buffer RA4 as described in step 8. This prolonged time is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer RA4 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

10 Elute highly pure total RNA

For elution place the NucleoSpin® RNA Binding Plate onto a Round-well Block (included in the kit) and pipette **75 µl[1] RNase-free H_2O** directly to the bottom of each well. Make sure that all of the water gets into contact with the silica membrane and that the membrane is completely wetted. Incubate for **2 min** at **room temperature** and for **3 min** at **5,600 - 6,000 x *g.***

Alternatively, elution in an MN Square-well Block (see ordering information) or standard PCR plates is possible. For elution, place the NucleoSpin® RNA Binding Plate on top of an MN Square-well Block and centrifuge. Elution into PCR plates can be performed by placing a PCR plate between the NucleoSpin® RNA Binding Plate and the MN Square-well Block before centrifugation.

Note: The Elution Plate U-bottom is not suitable for use in a centrifuge.

[1] 75 µl is recommended. 50 - 130 µl is possible, see section 2.5.

5.5 Standard protocol for automated purification of total RNA using common laboratory automation workstations

Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture was prepared according to section 3.

Note: For ready-to-run robot scripts and general information about automation please contact your local distributor or MN directly.

A Place the plastic equipment like plates and the assembled vacuum manifold at the locations of the robotic platforms as specified in the individual robotic programs.

B Add sufficient buffer to the reservoirs or place the buffer bottles at the corresponding positions on the robot worktable.

Calculate the required buffer volumes and pour an additional amount of 10% into the reservoirs. Buffers are delivered in sufficient, but limited amounts and should not be wasted. Do not fill back unused buffer into the bottle.

C **Harvest cells**

If cells have been grown in suspension, aliquots of up to 2 x 10^6 cells can be transferred into the wells of a deep-well plate. Pellet cells by centrifugation 5 min, 500 x *g*).

Tissue samples

For harvesting and homogenization of tissue samples please refer to section 2.4.

D Place the samples at the appropriate position of the robot worktable.

E Select method for total RNA purification and start the run. Seal unused wells with Self-adhering PE Foil (see ordering information).

Use disposable tips with filter for the transfer of sample to the NucleoSpin® RNA Binding Strips or Plate. All other steps may be processed with needles. Adjust vacuum times and strength, if necessary. Take care that the volume of the rDNase reaction mixture is pipetted to the middle of the well.

F Elution of purified total RNA

For increased RNA concentration, dispense at least 75 µl of RNase-free H_2O to the membrane. Lower volumes of elution buffer will cause inhomogeneous results. By using higher volumes of dispensed water, the concentration of eluted RNA will decrease, but the efficiency of elution will increase.

Alternatively, the elution can be performed in a centrifuge to reduce the volume of water needed for elution thus increasing the concentration of the RNA:

Stop the protocol after the vacuum drying step. Remove the NucleoSpin® RNA Binding Strips/Plate and tap it on a sheet of filter paper to remove residual wash buffer.

NucleoSpin® 8 RNA: Insert the NucleoSpin® RNA Binding Strips into Column Holder C (see ordering infornation) and place it on top of a Rack of Tube Strips.

NucleoSpin® 96 RNA: Place the NucleoSpin® RNA Binding Plate on top of a Rack of Tube Strips.

Continue with step 9 from the "Standard protocol for the manual purification of total RNA using a centrifuge".

5.6 Support protocol for clean-up of total RNA

This support protocol is designed for clean-up of pre-purified RNA samples (e.g. from extractions using phenol/chloroform based purification procedures, precipitation protocols or following enzymatic reactions). The NucleoSpin® 8/96 RNA clean-up procedure will eliminate traces of organic solvents, salts, or enzymes.

Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture was prepared according to section 3.

A Per **50 µl (1 volume) sample** add **160 µl (3.2 volumes) Buffer RA1** and **110 µl (2.2 volumes) ethanol (96 - 100%)** to adjust conditions under which the RNA binds to the silica membrane.

It is possible to scale up the volumes. The total volume of Buffer RA1 supplied in the kit is sufficient for a maximum of 300 µl Buffer RA1 per well. See ordering information if additional buffer is required.

Note: Do not exceed a total volume of 1400 µl as this is the maximum capacity of the individual wells.

B Mix by pipetting up and down at least 15 times and transfer samples to the wells of the NucleoSpin® RNA Binding Plate/Strips.

Proceed with step 5 of the standard procedure (Bind RNA to the silica membrane).

Note: rDNase treatment may not be necessary, depending on starting material and upstream application.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded/ no RNA obtained	*RNase contamination* • Create an RNase-free environment on the worktable. Clean trough reservoirs with appropriate solutions. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. • Do not fill back unused buffer from the trough reservoir into the bottle. • Use sterile tips with filter. • Add 1% ß-mercaptoethanol to Buffer RA1. *Sample material* • Sample material not fresh. Whenever possible, use fresh material
Poor RNA quality or yield	*Reagents not applied or prepared properly* • Reagents not properly prepared. Add the indicated volume of RNase-free H_2O to the DNase vial and 96 - 100% ethanol to Buffer RA3 and Buffer RA4 Concentrate and mix. • Add 1% ß-mercaptoethanol to Buffer RA1. *Kit storage* • Store aliquots of the reconstituted DNase at - 18°C. • Store other 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* • If using more than 10^6 cells, use a shaker or a commercial homogenizer for optimal homogenization of the starting material.

Problem	Possible cause and suggestions
	Elution • Be sure that all of the water gets into contact with the silica membrane. No water drops should stick to the walls of the columns. The membrane has to be wetted completely.
Poor RNA quality or yield (continued)	*Clogging of the NucleoSpin® RNA Binding Strips/Plate* • If using too much sample or if tissue lysate has not been successfully cleared using the NucleoSpin® RNA Filter Strips/ Plate, clogging of the NucleoSpin® RNA Binding Strips/Plate may appear. To prevent this reduce sample amount and raise time for vacuum filtration or centrifugation steps. If clogging happens during the run, take the remaining lysate off the NucleoSpin® RNA Binding Strips/Plate, discard it, and proceed with the desalting step (Buffer MDB).
Contamination of RNA with genomic DNA	*DNase not active* • Reconstitute and store lyophilized DNase according to instructions in section 3. *Too much material used* • Reduce quantity of tissue used. • Increase mixing cycles after adding Buffer RA4 to the lysate.
Suboptimal performance of RNA in downstream experiments	*Carryover of ethanol* • Be sure to remove all of ethanolic Buffer RA4 after the final washing step. Dry the NucleoSpin® RNA Binding Strips/Plate for at least 10 min with maximum vacuum or by 10 min centrifugation.
Vacuum manifold	*Vacuum pressure is not sufficient* • Check if the vacuum manifold lid fits tightly on the manifold base if vacuum is turned on.
Buffers	*Buffer volumes are not enough* • Buffers are delivered in sufficient, but limited amounts. Calculate the required buffer volumes and pour an additional amount of 10% into the reservoirs. • Do not fill back unused buffer from reservoir into the bottle to avoid contaminations. Ask technical service for extended buffer volumes.

Problem	Possible cause and suggestions
Cross contamination	*Splattering of eluate* • Reduce the vacuum strength during the elution step. Alternatively a Round-well Block or Rack of Tube Strips (see ordering information) can be used for collecting the eluate if a higher vacuum strength is required during the elution. *Transfer of sample solution to the NucleoSpin® RNA Binding Strips/Plate* • Be sure that no liquid drops out of the tips while moving the tips above the binding strips/plate.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 8 RNA	740698 740698.5	12 x 8 preps 60 x 8 preps
NucleoSpin® 96 RNA	740709.2 740709.4 740709.24	2 x 96 preps 4 x 96 preps 24 x 96 preps
NucleoSpin® RNA Filter Strips	740699.12F 740699.60F	12 60
NucleoSpin® RNA Filter Plate	740711	4
Buffer RA1	740961.55	500 ml
Buffer RA4 Concentrate (for 1 l Buffer RA4)	740960	200 ml
MN Square-well Block	740476 740476.24	4 24
Round-well Block, Low	740482	4
Round-well Block	740671	20

Product	Cat. No.	Pack of
Round-well Block with Cap Strips	740475 740475.24	4 sets 24 sets
Rack of Tube Strips	740637	5 racks
Rack of Tube Strips with Cap Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Cap Strips	740478 740478.24	48 288
MN Wash Plate	740479 740479.24	4 24
Self-adhering PE Foil	740676	50
Starter Set A (for use of 8-well strips on the NucleoVac 96 and automation platforms)	740682	1 set
Starter Set C (for use of 8-well strips under centrifugation)	740684	1 set
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
MN Frame (for optimized handling of 96-well plates with vacuum manifold on BioRobot® 9600, 9604, and 3000 (Qiagen), MultiPROBE II (PerkinElmer), Biomek 2000, and FX (Beckmann Coulter))	740680	1

6.3 Product use restriction / warranty

NucleoSpin® 8/96 RNA 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® 8/96 RNA** 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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