

MACHEREY-NAGEL

User manual



RNA isolation

- NucleoSpin® 96 RNA
- NucleoSpin® 96 RNA Core Kit

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1 Components

1.1 Kit contents

REF	NucleoSpin® 96 RNA		
	2 × 96 preps 740709.2	4 × 96 preps 740709.4	24 × 96 preps ¹ 740709.24
Lysis Buffer RA1	125 mL	2 × 125 mL	12 × 125 mL
Wash Buffer RA2	160 mL	360 mL	6 × 360 mL
Wash Buffer RA3 (Concentrate) ²	100 mL	2 × 100 mL	12 × 100 mL
Wash Buffer RA4 (Concentrate) ²	65 mL	2 × 65 mL	12 × 65 mL
Reaction Buffer for rDNase	30 mL	60 mL	6 × 60 mL
rDNase, RNase-free (lyophilized) ²	4 vials	8 vials	48 vials
RNase-free H ₂ O	125 mL	2 × 125 mL	12 × 125 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, U-bottom (including Self adhering PE Foil)	2	4	24
User manual	1	1	6

¹ The kit for 24 × 96 preparations (REF 740709.24) consists of 6 × REF 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 5.4 for the isolation of RNA.

Kit contents continued

NucleoSpin® 96 RNA Core Kit	
REF	4 × 96 preps 740466.4
Lysis Buffer RA1	2 × 125 mL
Wash Buffer RA2	360 mL
Wash Buffer RA3 (Concentrate) ¹	2 × 100 mL
Wash Buffer RA4 (Concentrate) ¹	2 × 65 mL
Reaction Buffer for rDNase	60 mL
rDNase, RNase-free (lyophilized) ¹	8 vials
RNase-free H ₂ O	2 × 125 mL
NucleoSpin® RNA Binding Plates (blue rings)	4
User manual	1

1.2 Reagents to be supplied by user

- 96–100 % ethanol (for preparation of working solutions; see section 3)
- Reducing agent (β-mercaptoethanol, or DTT (dithiothreitol), or TCEP (BisTris (Bis-(2-hydroxyethyl)-imino-tris(hydroxymethyl)-methane)) as supplement for Lysis Buffer RA1
- NucleoSpin® RNA Filter Plates (optional; see section 2.7)

For more detailed information regarding special hardware required for centrifuge, vacuum or positive pressure processing, please see section 2.4. For ordering information, please see section 6.2.

For recommended accessories for use of the flexible **NucleoSpin® 96 RNA Core Kit** (reduced kit composition; REF 740466.4), please see section 2.5.

1.3 About this user manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the internet at www.mn-net.com.

Please contact Technical Service regarding information about changes of the current user manual compared to previous or updated revisions.

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 while working with RNA is to prevent RNA degradation during the isolation procedure. With the **NucleoSpin® 96 RNA** kits, cells or tissues 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. In combination with Buffer RA4, it furthermore creates the appropriate binding conditions that favor RNA adsorption to the silica membrane. Contaminating DNA, which is also bound to the silica membrane, is removed by directly applying DNase 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® 96 RNA** kits are designed for fast 96-well small-scale purification of RNA from up to 20 mg tissue or cells. Fresh, frozen, or stabilized sample material can be processed.
- The **NucleoSpin® 96 RNA** kits can be used manually under vacuum, positive pressure or under centrifugation.
- The **NucleoSpin® 96 RNA** kits can be used fully automated on common laboratory workstations (see section 2.6).
- The kits provide reagents and consumables for purification of up to 100 µg highly-pure RNA suitable for direct use in standard molecular biology applications, like RT-PCR, TaqMan, Northern Blot, or microarray analysis.
- The **NucleoSpin® 96 RNA** kits yield RNA of highest purity and integrity.
- Using the **NucleoSpin® 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.
- For research use only.

2.3 Yield and quality of RNA

- **NucleoSpin® 96 RNA** can be used under vacuum, positive pressure 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.

- Typical amounts of starting material and anticipated yields are shown in Table 2. Please note that yield of RNA strongly depends on the starting material and on complete lysis / homogenization. Results may vary. For more information about the lysis / homogenization process, see section 2.7.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® 96 RNA			
	Animal tissue		Cell culture	
	Vacuum	Centrifuge	Vacuum	Centrifuge
Technology	Silica membrane technology			
Format	96-well plates			
Processing	Manual or automated, vacuum, positive pressure or centrifugation			
Max. sample size	10–30 mg	30 mg	2×10^6 cells	1×10^7 cells
Typical yield	Up to 40 µg	Up to 100 µg	Up to 20 µg	Up to 100 µg
Fragment size	> 200 nt			
A_{260}/A_{280}	1.9–2.1			
Typical RIN (RNA integrity number)	Sharp rRNA bands with no substantial degradative bands visible; 28S:18S ~ 2.1 Excellent RNA Integrity Number (RIN) values typically: > 9 (cells), ≥ 7 (tissue)			
Typical concentration	50–200 ng/µL			
Elution volume	50–130 µL			
Preparation time	70 min/plate			
Binding capacity	100 µg			

- 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.
- Isolated RNA is of highest quality and integrity indicating a highly efficient inactivation of RNases and gentle purification.

2.4 Required hardware

NucleoSpin® 96 RNA can be processed under vacuum, positive pressure or centrifugation. Certain hardware for processing is required.

Centrifugation

For centrifugation, a microtiterplate centrifuge is required. This centrifuge must be able to accommodate the NucleoSpin® RNA Binding Plate stacked on a Round-or Square-well Block and reach accelerations of 5,600–6,000 x g (bucket height: 85 mm).

Regarding waste collection, suitable consumables (e.g., MN Square-well Blocks) are necessary and they are not included in the kit. For the most convenient handling, without the need of emptying and reusing the MN Square-well Blocks, we recommend using six MN Square-well Blocks if two 96-well plates are processed at once (see ordering information). Alternatively, it is possible to empty the MN Square-well Blocks after every centrifugation step, reducing the amount of MN Square-well Blocks needed.

Vacuum processing

The **NucleoSpin® 96 RNA** kit can be used with the NucleoVac 96 Vacuum Manifold (see ordering information). 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® RNA Binding Plate and thus guarantee proper vacuum.

Establish a reliable vacuum source for the NucleoVac 96 Vacuum Manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of -0.2 to -0.4 bar (reduction of atmospheric pressure). The use of the NucleoVac Vacuum Regulator (see ordering information) is recommended. Alternatively, adjust the 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 being used, the vacuum times may need to be increased for complete filtration. Additionally, a suitable centrifuge for sample preparation steps may be required.

Positive pressure processing

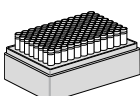
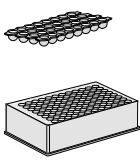

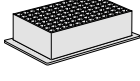
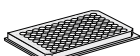
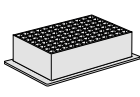
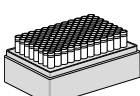

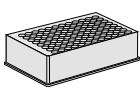
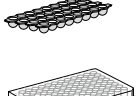
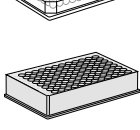
Please contact Technical service regarding information for positive pressure processing.

2.5 Recommended accessories for use of the NucleoSpin® 96 RNA Core Kit

The **NucleoSpin® 96 RNA Core Kit** provides buffers, rDNase, and NucleoSpin® RNA Binding Plates. Accessory plates (e.g., lysis plates, elution plates) are not provided with the core kit. The reduced kit composition along with a variety of separately available accessories, allow optimal adjustment of the kit to individual user needs. The user can select additional consumables according to his / her requirements for highest flexibility.

For use of **NucleoSpin® 96 RNA Core Kit**, follow the standard protocols (see section 5.1 and 5.2).

Recommended accessories for use of the **NucleoSpin® 96 RNA Core Kit** (4 × 96 preps) are available from MACHERY-NAGEL (see ordering information).

Protocol step	Suitable consumables, not supplied with the core kits	Remarks
Lyse samples	4 x Rack of Tubes Strips with Cap Strips per 4 × 96 preps or	
	4 x Round-well Block with Cap Strips per 4 × 96 preps or	
	4 x MN Square-well Block	
	4 x MN Square-well Block	
Bind RNA to the membrane	4 x MN Wash Plate per 4 × 96 preps	 MN Wash Plate minimizes the risk of cross contamination (vacuum processing only).
	2 x MN Square-well Block	 For waste collection during centrifugation (reusable)
Elute RNA	4 x Rack of Tubes Strips with Cap Strips per 4 × 96 preps or	
	4 x Round-well Block with Cap Strips per 4 × 96 preps or	
	4 x Elution Plate U-bottom or	 For vacuum processing only
	4 x Round-well Block Low, U-bottom	 For processing under centrifugation
	4 x Round-well Block Low, U-bottom	

2.6 Automated processing on robotic platforms

NucleoSpin® 96 RNA can be used fully automated on many common laboratory workstations. Please contact MN for the availability of scripts and general considerations about adapting **NucleoSpin® 96 RNA** on a certain workstation.

Visit MN online at www.mn-net.com or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions, and selection of the protocol. Several application notes of the **NucleoSpin® 96 RNA** kit on various liquid handling instruments can also be found at www.mn-net.com at Bioanalysis/Literature.

Full processing under vacuum enables complete automation without the need of centrifugation steps for drying or elution. However, a final elution step by centrifugation is recommended in order to achieve higher concentrated DNA eluates.

The risk of cross-contamination is reduced by optimized vacuum settings during the elution step and by the improved shape of the outlets of the NucleoSpin® RNA Binding Plate.

Drying of the NucleoSpin® RNA Binding Plates only under vacuum is sufficient, as the bottom of the plate is protected by the MN Wash Plate during the washing steps. As a result, it is recommended integrating the MN Wash Plate into the automated procedure. The MN Frame (see ordering information) can be used to place the disposable MN Wash Plate inside the vacuum chamber. This also reduces the risk of cross-contamination, as common metal adaptors tend to get contaminated by gDNA. Thorough cleaning of the vacuum chamber is recommended after each run to prevent DNA-containing aerosols from forming.

2.7 Sample storage and homogenization

RNA 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 samples are either immediately frozen and stored at -70 °C, placed 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 RNA.

If larger cell numbers or large amounts of tissue are used, a filtration step of the RA1 Lysate through the NucleoSpin® RNA Filter Plate (see ordering information) is recommended for optimal homogenization and removal of particles.

Cell culture

- Up to 2×10^6 cells can be processed under vacuum. Using a centrifuge, up to 1×10^7 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 $500 \times g$. The supernatant has to be removed completely. Lyse cells by addition of 300 μ L Buffer RA1. Follow the standard protocol for cell cultures.
- For adherent cell cultures in 96-well format, make sure that the culture medium is completely removed. Lyse cells by addition of 130 μ L Buffer RA1. Follow the standard protocol for cell cultures grown in 96-well plates.
- If using more than 1×10^6 cells, it is recommended using a commercial homogenizer for lysis with Buffer RA1 in order to reduce viscosity. To prevent the NucleoSpin® RNA Binding Plate from clogging, it is also recommended filtrating the

lysates through the NucleoSpin® RNA Filter Plate (not included in the kit) before applying them to the NucleoSpin® RNA Binding 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 tissue homogenization. Follow the standard protocol for tissue samples.
- 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 % β-mercaptoethanol to Buffer RA1. β-mercaptoethanol supports the inhibition of RNases.
- For sample homogenization and removal of cell debris, it is recommended filtering the lysates through the NucleoSpin® RNA Filter Plate (not included in the kit) before applying them to the NucleoSpin® RNA Binding 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. Some typical results are depicted in the following table:

Table 2: Yields of RNA with NucleoSpin® 96 RNA

Sample source	Max. starting material	Max. yield of RNA
Spleen	20 mg	50 µg
Kidney	30 mg	45 µg
Brain	30 mg	20 µg
Liver	30 mg	80 µg

Yield of RNA depends strongly on the tissue and the effectiveness of lysis/homogenization. Therefore, results may strongly vary.

Methods for sample homogenization

- Commercial homogenizers, for example, 2010 Geno/Grinder (COPS Diagnostics) can be used for sample homogenization.

Use of NucleoSpin® RNA Filter Plate

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

Centrifugation

Place the NucleoSpin® RNA Filter Plate (see ordering information) on a MN Square-well Block and apply the sample lysates in Buffer RA1 to 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 a MN Square-well Block in the manifold and close the manifold with the manifold lid.

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 flowthrough collected in the MN Square-well Block. Please note that the dead volume of the NucleoSpin® RNA Filter Plate is rather large compared to the processing under centrifugation, thus processing under vacuum is only recommended when complete automation is desired.

2.8 Elution of pure RNA

Due to dead volume of the silica membrane by using vacuum, please notice that the difference between the dispensed elution buffer and the recovered elution buffer containing RNA is approximately 20 μL . For RNA elution, a volume of 50–130 μL nuclease free water is recommended. Higher RNA concentrations are obtained when using a dispense volume of 50 μL , however, 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 1). To achieve this, vacuum settings during the elution have to be adjusted carefully (smooth elution) to avoid splattering of liquid.

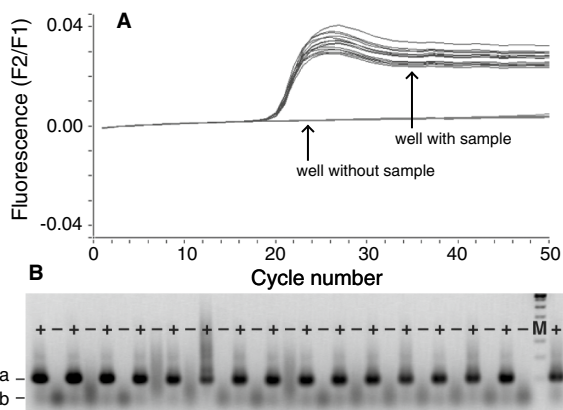


Figure 1 HeLa cells (5×10^5 each) were pelleted in a 96-well culture plate in a chess board pattern. RNA was prepared using NucleoSpin[®] 96 RNA.
 (a) RT-PCR detection of RNA was performed with 1.5 μL of the eluate (total eluate 80 μL) in a LightCycler[®] (0.5 μM GAPDH primer, LightCycler[®]-RNA Amplification Kit Hybridization Probes).
 (b) 20 μL of the LightCycler[®] assay were loaded on a 2% agarose gel.
 +: Total RNA from 5×10^5 HeLa cells
 -: Empty control well (next to a well containing cells)
 a: 200 bp product of the GAPDH gene
 b: Primers

3 Storage conditions and preparation of working solutions

Attention: Buffers RA1 and RA2 contain chaotropic salts. Wear gloves and goggles!

CAUTION: Buffers RA1 and RA2 contain guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Storage conditions:

- Store lyophilized **RNase-free rDNase** at 4 °C on arrival (stable for at least one year).
- All other components of the NucleoSpin® 96 RNA kit should be stored at room temperature and are stable until: see package label. 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® 96 RNA** protocol, prepare the following:

- **Reconstitute RNase-free rDNase:** Add 540 µL RNase-free H₂O to the rDNase vial and incubate for 1 min at room temperature. Gently swirl the vial to completely dissolve the rDNase. In case of not processing a whole 96-well plate, dispense the reconstituted rDNase solution into aliquots and store at -20 °C. This rDNase solution is stable for at least 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. Indicate that ethanol is added by marking the bottle label. Store Wash Buffer RA3 at 15–25 °C for at least one year.
- **Wash Buffer RA4:** Add the indicated volume of 96–100 % ethanol to the Buffer RA4 Concentrate. Mark the bottle label to indicate that ethanol has been added. Store Wash Buffer RA4 at 15–25 °C for at least one year.

NucleoSpin® 96 RNA			
REF	2 × 96 preps 740709.2	4 × 96 preps 740709.4	24 × 96 preps 740709.24
Wash Buffer RA3 (Concentrate)	1 × 100 mL Add 400 mL ethanol	2 × 100 mL Add 400 mL ethanol to each bottle	12 × 100 mL Add 400 mL ethanol to each bottle
Wash Buffer RA4 (Concentrate)	1 × 65 mL Add 150 mL ethanol	2 × 65 mL Add 150 mL ethanol to each bottle	12 × 65 mL Add 150 mL ethanol to each bottle

NucleoSpin® 96 RNA Core Kit	
REF	4 × 96 preps 740466.4
Wash Buffer RA3 (Concentrate)	2 × 100 mL Add 400 mL ethanol to each bottle
Wash Buffer RA4 (Concentrate)	2 × 65 mL Add 150 mL ethanol to each bottle

4 Safety instructions

When working with the **NucleoSpin® 96 RNA** and **NucleoSpin® 96 RNA Core** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at <http://www.mn-net.com/msds>).



Caution: Guanidinium thiocyanate in buffer RA1 and buffer RA2 can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® 96 RNA** and **NucleoSpin® 96 RNA Core** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocols

5.1 NucleoSpin® 96 RNA – vacuum processing

- For hardware requirements, refer to section 2.4.
- For detailed information regarding the vacuum manifold setup, see page 19.
- For detailed information about each step, see page 20.
- For use of the NucleoSpin® 96 RNA Core Kit (REF 740466.4), refer to section 2.5 regarding recommended accessories.

Before starting the preparation:

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

Protocol at a-glance

1	Harvest cells	500 x g, 5 min
2	Lyse cells or tissue <i>Optional: If using tissue samples or large number of cells, clearing of lysate with the NucleoSpin® RNA Filter Plate is recommended</i> Transfer cleared lysate to MN Square-well Block	300 µL RA1 (cells, tissue) (+3 µL β-ME) or 130 µL RA1 (cells)1 (+1.3 µL β-ME)
3	Prepare binding Mix by pipetting up and down at least 10–15 times	300 µL RA4 (cells, tissue) or 130 µL RA4 (cells)1
Prepare vacuum manifold		
4	Transfer crude lysates to NucleoSpin® RNA Binding Plate	
5	Bind RNA to silica membrane of the NucleoSpin® RNA Binding Plate	-0.2 bar², 1 min
6	Desalt silica membrane by washing	500 µL RA3–0.2 bar¹, 3 min

¹ Cells grown in 96-well plates only

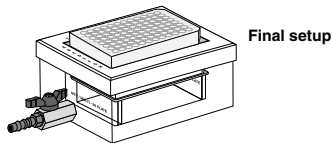
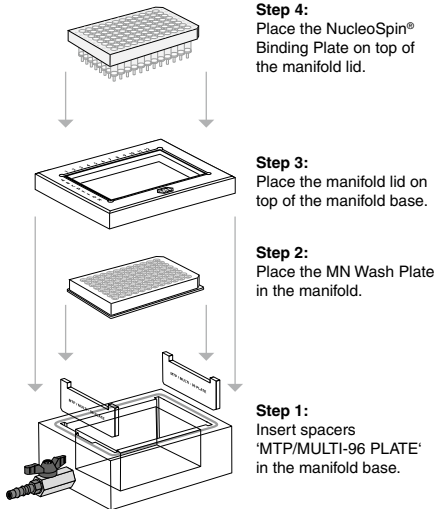
² Reduction of atmospheric pressure

7	Digest DNA	95 µL rDNase reaction mixture
		Room temperature, 15 min
8	Wash silica membrane	500 µL RA2
		800 µL RA3
		500 µL RA4 – 0.2 bar ¹ , 1 min each step
		Remove MN Wash Plate
9	Dry NucleoSpin® RNA Binding Plate by applying vacuum	Maximum vacuum (-0.6 bar ¹), 10 min
<i>Optional: Dry the outlets of the NucleoSpin® RNA Binding Plate by placing it on a Paper Sheet before applying vacuum</i>		
10	Elute RNA	75 µL RNase-free H ₂ O
		Incubate 2 min
		-0.5 bar ¹ , 1 min

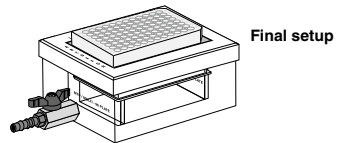
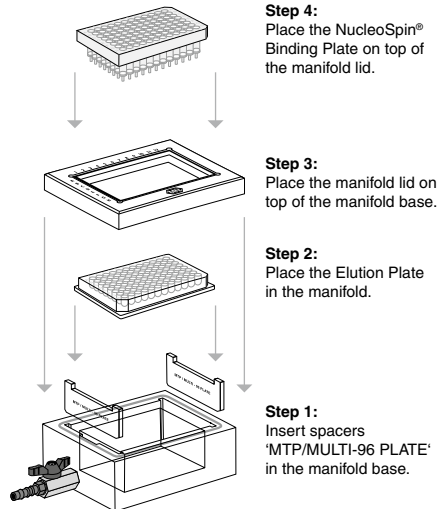
¹ Reduction of atmospheric pressure

Setup of vacuum manifold:

Binding / Washing steps



Elution step



Detailed protocol

- For hardware requirements, refer to section 2.4.
- For detailed information regarding the vacuum manifold setup, see page 23.
- For use of the NucleoSpin® 96 RNA Core Kit (REF 740466.4), refer to section 2.5 regarding recommended accessories.

Before starting the preparation:

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

1 Harvest cells

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

2 Lyse cells or tissue

Cells, tissue samples

Add **300 µL Buffer RA1 / 1 % β-mercaptoethanol [vol/vol]** to each sample. Cells can be lysed by repeated pipetting up and down or vigorous shaking of the sealed/closed plate or reaction tube. For homogenization of tissue samples please refer to section 2.7.

Note: As alternative to β-ME, the reducing agent DTT or TCEP may be used. Use a final concentration of 10–20 mM DTT or TCEP within the Lysis Buffer RA1.

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 % β-mercaptoethanol [vol/vol]** to the cells in each well of the cell-culture plate. Cells can be lysed by repeated pipetting up and down or vigorous shaking of the sealed plate.

Note: Use of β-mercaptoethanol is recommended, but not essential for most cell types (also see section 2.7).

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 container into the center of the manifold. Place the MN Wash Plate on top of 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 bar¹; 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 bar¹; 3 min) until all buffer has passed through the wells. Release the vacuum.

7 Digest DNA

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

1 st wash

Add **500 µL Buffer RA2** to each well of the NucleoSpin® RNA Binding Plate. Apply vacuum (-0.2 bar¹; 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 bar¹; 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 bar¹; 1 min) until all buffer has passed through the wells. Release the vacuum.

¹ Reduction of atmospheric pressure

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.

9 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 bar¹)** 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 RNA.

Finally, release the vacuum.

10 Elute RNA

Place the Elution Plate U-bottom onto the spacers 'MTP/MULTI-96 PLATE' of the vacuum manifold. Pipette **75 µL RNase-free H₂O** directly to the bottom of each well (75 µL are recommended, 50–130 µL are possible, see section 2.8). Incubate for **2 min** at **room temperature**. Build up the vacuum with the valve closed. Once the **maximum vacuum (-0.5 bar¹)** is achieved, open the valve and apply vacuum for 1 min.

¹ Reduction of atmospheric pressure

5.2 NucleoSpin® 96 RNA – centrifuge processing

- For hardware requirements, refer to section 2.4.
- For detailed information on each step, see page 30.
- For use of the NucleoSpin® 96 RNA Core Kit (REF 740466.4), refer to section 2.5 regarding recommended accessories.

Before starting the preparation:

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

Protocol at a glance

1	Harvest cells	500 x g, 5 min,
2	Lyse cells or tissue <i>Optional: If using tissue samples or large number of cells, clearing of lysate with the NucleoSpin® RNA Filter Plate is recommended</i>	300 µL RA1 (cells, tissue) (+3 µL β-ME) or 130 µL RA1 (cells) ¹ (+1.3 µL β-ME)
3	Prepare binding Mix by pipetting up and down at least 10–15 times	300 µL RA4 (cells, tissue) or 130 µL RA4 (cells) ¹
4	Transfer crude lysates to NucleoSpin® RNA Binding Plate	
5	Bind RNA to silica membrane of the NucleoSpin® RNA Binding Plate	5,600–6,000 x g, 2 min
6	Desalt silica membrane by washing	500 µL RA3 5,600–6,000 x g, 2 min
7	Digest DNA	95 µL rDNase reaction mixture Room temperature, 15 min

¹ Cells grown in 96-well plates only

8	Wash silica membrane	500 µL RA2
		5,600 – 6,000 x g, 2 min
		800 µL RA3
		5,600 – 6,000 x g, 2 min
		500 µL RA4
		5,600 – 6,000 x g, 10 min ¹
9	Dry NucleoSpin® RNA Binding Plate	Not necessary
10	Elute RNA	75 µL RNase-free H ₂ O
		Incubate 2 min
		5,600 – 6,000 x g, 2 min

¹ Prolonged centrifugation time is required to evaporate ethanol from last wash step.

Detailed protocol

- For hardware requirements, refer to section 2.4.
- For use of the NucleoSpin® 96 RNA Core Kit (REF 740466.4), refer to section 2.5 regarding recommended accessories.

Before starting the preparation:

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

1 Harvest cells

If cells have been grown in suspension, aliquots of up to 1×10^7 cells can be transferred into the wells of a 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), and remove the supernatant by pipetting.

2 Lyse cells of tissue

Cells, tissue samples

Add 300 µL Buffer RA1 / 1 % β-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 2.7.

Note: As alternative to β-ME the reducing agent DTT or TCEP may be used. Use a final concentration of 10–20 mM DTT or TCEP within the Lysis Buffer RA1.

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 % β-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 β-mercaptoethanol is recommended, but not essential for most cell types (also see section 2.7).

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 a 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 Digest DNA

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

1 st 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 RNA

For elution, place the NucleoSpin® RNA Binding Plate onto a Round-well Block (included in the kit) and pipette **75 µL RNase-free H₂O** directly to the bottom of each well (75 µL are recommended, 50–30 µL are possible, see section 2.8). 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 centrifuge for **3 min** at **5,600–6,000 x g**.

Alternatively, elution in a MN Square-well Block (see ordering information) is possible. For elution, place the NucleoSpin® RNA Binding Plate on top of a MN Square-well Block and centrifuge.

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

5.3 Automated purification using common laboratory automation workstations

Before starting the preparation:

- Check if Wash Buffer RA3, Wash Buffer RA4, and rDNase reaction mixture were 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.

1 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.

2 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.

3 Harvest cells: If cells have been grown in suspension, aliquots of up to 2×10^6 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 2.7.

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

5 Select method for 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 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.

6 For increased RNA concentration, dispense at least 75 µL of RNase-free H₂O 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 Plate and tap it on a sheet of filter paper to remove residual wash buffer.

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

5.4 Clean up of RNA

This support protocol is designed for clean up of prepurified RNA samples (e.g., from extractions using phenol/chloroform based purification procedures, precipitation protocols or following enzymatic reactions). The NucleoSpin® 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 were prepared according to section 3.

-
- 1 For each **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 1.4 mL as this is the maximum capacity of the individual wells.

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

Proceed with step 5 of the standard procedure (Bind RNA to the silica membrane, section 5.1 or 5.2).

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

5.5 NucleoSpin® 96 RNA with NucleoZOL

For performing the support protocol NucleoSpin® 96 RNA with NucleoZOL the use of NucleoZOL, Buffer MX, Square-well Blocks, and Elution Plates is required (see ordering information).

1 Harvest cells

To collect up to 5×10^6 cells grown in suspension, aliquot the cell culture to the wells of a Square-well Block and centrifuge the culture to sediment the cells. Discard the supernatant.

Note: Do not fill more than 2 mL to each well of the Square-well Block at once. Cells can be collected by repeating the step multiple times.

2 Add NucleoZOL

Add at least **500 µL NucleoZOL** to the cell pellet.

Disrupt the sample material mechanically by pipetting up and down or vortexing.

Note: Insufficient volume of the reagent will lead to DNA contamination of the isolated RNA. Do not wash the cells before addition of NucleoZOL. Washing of cells might result in RNA degradation.

3 Precipitate contaminants

Add **200 µL RNase-free water** to the lysate.

Shake the sample vigorously for **15 s**. **Incubate** at room temperature for **5 min**.

Centrifuge sample for **15 min** at **12,000 x g** at room temperature.

Note: DNA, Proteins and polysaccharides will form a semi-solid pellet at the bottom of the wells. The RNA is still solubilized in the supernatant.

4 Transfer cleared lysate

Transfer **500 µL supernatant** to a new Square-well Block

5 Adjust binding conditions

Add **500 µL Buffer MX** to each well of the Square-well Block and **mix** by shaking or pipetting up and down.

6 Load sample

Load **1000 µL lysate** into each well of the NucleoSpin® 96 RNABinding Plate.

7 Bind RNA

Apply vacuum until all lysates have passed through the wells (-0.2 bar; 2 min).

8 Wash RNA

Proceed with step 8 of the NucleoSpin® 96 RNA user manual.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded/ no RNA obtained	<p data-bbox="309 309 508 331"><i>RNase contamination</i></p> <ul data-bbox="309 347 972 596" style="list-style-type: none"> • 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 % β-mercaptoethanol to Buffer RA1.
	<p data-bbox="309 612 460 635"><i>Sample material</i></p> <p data-bbox="309 651 904 676">Sample material not fresh. Whenever possible, use fresh material</p>
Poor RNA quality or yield	<p data-bbox="309 703 701 726"><i>Reagents not applied or prepared properly</i></p> <ul data-bbox="309 742 964 858" style="list-style-type: none"> • Reagents not properly prepared. Add the indicated volume of RNase-free H₂O to the DNase vial and 96 – 100 % ethanol to Buffer RA3 and Buffer RA4 Concentrate and mix. • Add 1 % β-mercaptoethanol to Buffer RA1.
	<p data-bbox="309 874 409 896"><i>Kit storage</i></p> <ul data-bbox="309 912 938 1072" style="list-style-type: none"> • Store aliquots of the reconstituted DNase at -20 °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. <p data-bbox="309 1088 460 1110"><i>Sample material</i></p> <ul data-bbox="309 1126 938 1177" style="list-style-type: none"> • If using more than 10⁶ cells, use a shaker or a commercial homogenizer for optimal homogenization of the starting material.

Problem	Possible cause and suggestions
Poor RNA quality or yield <i>(continued)</i>	<p data-bbox="309 209 370 228"><i>Elution</i></p> <ul data-bbox="309 252 975 323" style="list-style-type: none"> • 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.
	<p data-bbox="309 344 748 363"><i>Clogging of the NucleoSpin® RNA Binding Plate</i></p> <ul data-bbox="309 387 975 584" style="list-style-type: none"> • If using too much sample or if tissue lysate has not been successfully cleared, clogging of the NucleoSpin® RNA Binding Plate may appear. Reduce sample amount and raise time for vacuum filtration or centrifugation steps to prevent this. If clogging happens during the run, take the remaining lysate off the NucleoSpin® RNA Binding Plate, discard it, and proceed with the desalting step (Buffer RA3). The use of the NucleoSpin® RNA Filter Plate is recommended to clear the lysate.
Contamination of RNA with genomic DNA	<p data-bbox="309 611 473 630"><i>rDNase not active</i></p> <ul data-bbox="309 654 975 699" style="list-style-type: none"> • Reconstitute and store lyophilized rDNase according to instructions in section 3.
	<p data-bbox="309 719 530 738"><i>Too much material used</i></p> <ul data-bbox="309 762 975 823" style="list-style-type: none"> • Reduce quantity of tissue. • Increase mixing cycles after adding Buffer RA4 to the lysate.
Suboptimal performance of RNA in downstream experiments	<p data-bbox="309 847 501 866"><i>Carry over of ethanol</i></p> <ul data-bbox="309 890 975 967" style="list-style-type: none"> • Be sure to remove all of ethanolic Buffer RA4 after the final wash step. Dry the NucleoSpin® RNA Binding Plate for at least 10 min with maximum vacuum or by 10 min centrifugation.
Insufficient vacuum pressure	<p data-bbox="309 999 613 1018"><i>Vacuum pressure is not sufficient</i></p> <ul data-bbox="309 1042 975 1078" style="list-style-type: none"> • Check if the vacuum manifold lid fits tightly on the manifold base while vacuum is applied.
Insufficient buffer volumes	<p data-bbox="309 1110 591 1129"><i>Buffer volumes are not enough</i></p> <ul data-bbox="309 1153 975 1286" style="list-style-type: none"> • Buffers are delivered in sufficient, but limited amounts. Calculate the required buffer volumes and pour an additional amount of 10 % into the reservoirs if using a robotic platform. • 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	<p><i>Splattering of eluate</i></p> <ul style="list-style-type: none"> 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.
	<p><i>Transfer of sample solution to the NucleoSpin® RNA Trace Binding Plate</i></p> <ul style="list-style-type: none"> Be sure that no liquid drops out of the tips while moving the tips above the binding plate.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 96 RNA	740709.2	2 × 96 preps
	740709.4	4 × 96 preps
	740709.24	24 × 96 preps
NucleoSpin® 96 RNA Core Kit	740466.4	4 × 96 preps
NucleoSpin® 8 RNA	740698	12 × 8 preps
	740698.5	60 × 8 preps
NucleoSpin® 8 RNA Core Kit	740465.4	48 × 8 preps
NucleoSpin® RNA Filter Plate	740711	4
Buffer RA1	740961.55	500 mL
NucleoZOL (400 mL NucleoZOL required for 4 × 96 samples)	740404.200	200 mL
Buffer MX (240 mL Buffer MX required for 4 × 96 samples)	740405.60	60 mL
Buffer RA4 Concentrate (for 1 L Buffer RA4)	740960	300 mL
TCEP	740395.107	107 g
MN Square-well Block	740476	4
	740476.24	24
Square-well Block (8 × Square-well Block required for 4 × 96 samples)	740481	4
	740481.24	24
Round-well Block Low, U-bottom (set consists of 1 Round-well Block Low, U-bottom and Self adhering PE Foil)	740487	4 sets
	740487.24	24 sets

Product	REF	Pack of
Round-well Block with Cap Strips (set consists of 1 Round-well Block 12 Cap Strips)	740475 740475.24	4 sets 24 sets
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
Elution Plate U-bottom (set consists of Elution Plate U-bottom and Self adhering PE Foil)	740486.24	24 sets
Cap Strips	740478 740478.24	48 288
MN Wash Plate	740479 740479.24	4 24
Self adhering PE Foil	740676	50
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
MN Frame	740680	1

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

6.3 Product use restriction/warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

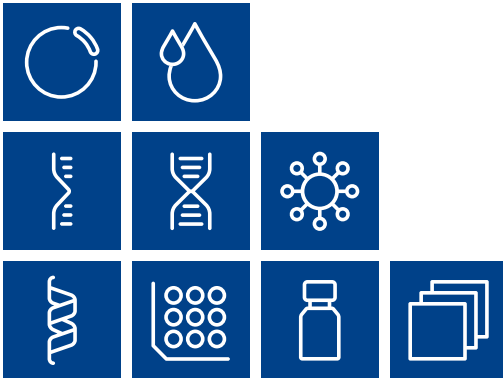
This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents 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 a contract of sale or of this warranty.

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Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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