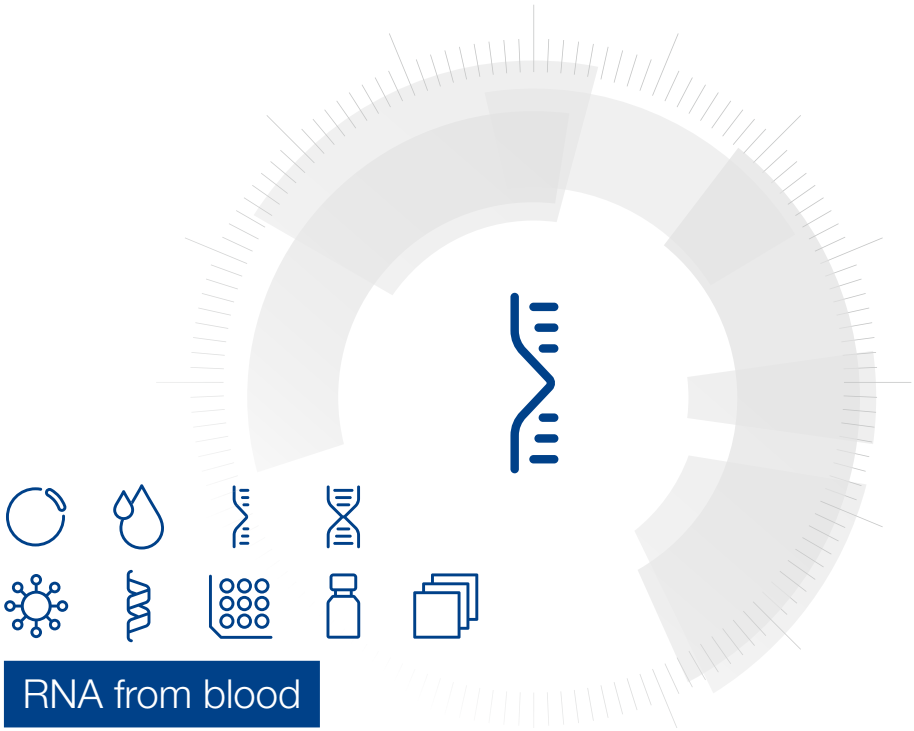
Bioanalysis

MACHEREY-NAGEL

User manual



RNA from blood

- NucleoSpin® 8 RNA Blood

October 2023 / Rev.01

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1 Components

1.1 Kit contents

	NucleoSpin® 8 RNA Blood®	
REF	**12 × 8 preps 740220**	**60 × 8 preps 740220.5**
Lysis Buffer DL	50 mL	2 × 100 mL 1 × 50 mL
Wash Buffer RB2	80 mL	360 mL
Wash Buffer RB3 (Concentrate)[2]	50 mL	2 × 100 mL
Wash Buffer RB4 (Concentrate)[2]	65 mL	65 mL
RNase-free H_2O	30 mL	125 mL
Reaction Buffer for rDNase	30 mL	2 × 60 mL
rDNase, RNase-free (lyophilized)[2]	2 vials (size D)	10 vials (size D)
Liquid Proteinase K	1.5 mL	3 × 2 mL
NucleoSpin® RNA Blood Binding Strips (blue rings)	12	60
MN Wash Plates[3]	2	5
Rack of Tube Strips[4]	3	15
Square-well Blocks (including one self-adhering PE-Foil)	2	5
Elution Plates U-bottom (including one Self-adhering PE Foil)	2	5
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[1] Patent pending

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

[3] Includes six paper sheets. They are not used when following the centrifuge protocol in section 5.2 for the isolation of total RNA.

[4] Set of 1 rack, 12 strips with 8 tubes each, and 12 cap strips

1.2 Reagents to be supplied by user

- 96 – 100 % ethanol

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.

2 Product description

2.1 The basic principle

The **NucleoSpin® 8 RNA Blood** kit offers a direct total blood lysis from up to 400 µL whole blood collected in standard (e.g., EDTA, Na-citrate, or Li-heparin) blood collection tubes. One of the most important aspects in the RNA isolation is to prevent RNA degradation during the isolation procedure. With the **NucleoSpin® 8 RNA Blood** method, leukocytes (the main source of RNA in whole blood) and other blood cells are lysed by incubating the whole blood in a solution containing large amounts of chaotropic ions. This lysis buffer immediately inactivates RNases (which are present in virtually all biological materials) and creates, in combination with Buffer RB4, appropriate binding conditions which favor adsorption of RNA to the silica membrane. A tedious and selective erythrocyte lysis, as well as preparation of a leukocyte pellet, is not necessary. Contaminating DNA, which is also bound to the silica membrane, is removed by a recombinant DNase solution (supplied). The recombinant DNase solution is directly applied onto the silica membrane during the preparation. Simple washing steps with two different buffers remove salts, metabolites, and macromolecular cellular components. Finally, the pure RNA is eluted under low ionic strength conditions with RNase-free H_2O (supplied).

2.2 Kit specifications

- The **NucleoSpin® 8 RNA Blood** kits are recommended for the isolation of RNA from fresh or frozen whole blood (e.g., stabilized with EDTA, Na-citrate, or Li-heparin).
- The **NucleoSpin® 8 RNA Blood** kits can be used on fully automated common laboratory workstations (see section 2.4).
- The **NucleoSpin® 8 RNA Blood** kits can be used manually under vacuum or under centrifugation. For use under centrifugation, additional consumables for waste collection

(e.g., MN Square-well Blocks) have to be ordered separately. Please see section 2.3 for further details.

- The **NucleoSpin® 8 RNA Blood** kits allow the purification of RNA with an A_{260}/A_{280} ratio typically exceeding 1.9.
- The isolated RNA is ready to use for typical downstream applications (e.g., reverse transcriptase-PCR (RT-PCR)).
- RNA isolated with the **NucleoSpin® 8 RNA Blood** kit is typically of high integrity. However, RNA integrity strongly depends on the sample quality.
- The amount of DNA contamination is significantly reduced during on-column digestion with rDNase. However, in very sensitive applications, it may be possible to detect traces of DNA. The probability of DNA detection with PCR increases with:

  1. the number of DNA copies per preparation: single copy target < plastidial / mitochondrial target < plasmid transfected into cells.
  2. decreasing PCR amplicon size.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® 8 RNA Blood
Format	8-well strips
Processing	Manual or automated, vacuum or centrifugation
Sample material	< 400 µL fresh or frozen whole blood (e.g., stabilized with EDTA, Na-citrate, or Li-heparin)
Fragment size	> 200 nt
Typical yield	~ 7 µg (3 – 20 µg) per 1 mL blood sample
A_{260}/A_{280}	1.9 – 2.1
Elution volume	50 – 130 µL
Preparation time	~ 60 min/6 strips
Binding capacity	100 µg
Use	**For research use only**

If smaller volumes than 400 µL blood are used, adjust the volumes of Buffer DL and Buffer RB4 in step 1 and 2 of the corresponding protocol by maintaining the following ratio:

1 : 1 : 1 (sample / Buffer DL / Buffer RB4)
Example: 300 µL blood + 300 µL Buffer DL + 300 µL Buffer RB4

The volume of Liquid Proteinase K can be calculated as follows:
Blood volume µL / 40 = volume Proteinase K µL
Example: 300 µL blood / 40 = 7.5 µL Liquid Proteinase K

2.3 Required hardware

For an efficient lysis of the whole blood samples a suitable shaker is required (e.g., Thermomixer Comfort with adapter plate for microtiterplates or deep-well plate (Eppendorf); VARIOMAG® TELESHAKER (Thermo Scientific)).

Vacuum processing

The **NucleoSpin® 8 RNA Blood** kit can be used manually with the NucleoVac 96 Vacuum Manifold (see ordering information) by using the Starter Set A. This Starter Set contains Column Holders A and NucleoSpin® Dummy Strips (see ordering information).

Starter Set A is also required for automation on laboratory platforms with standard 96-well plate vacuum chambers.

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.

Centrifugation

For centrifugation, a microtiterplate centrifuge is required. This centrifuge must be able to accommodate the NucleoSpin® RNA Blood Binding Strips stacked on a square-well block and reach accelerations of 5,600 – 6,000 x *g* (bucket height: 85 mm). In addition, Starter Set C (see ordering information) is required. Starter Set C contains two Column Holders C, two MN Square-well Blocks, and two Racks of Tube Strips.

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 emptying and reusing the MN-Square-well Blocks, we recommend using six MN Square-well Blocks if working with two Column Holders C (see ordering information). Alternatively, it is possible to empty the MN Square-well Blocks after every centrifugation step, reducing the total amount of MN Square-well Blocks needed.

2.4 Automated processing on robotic platforms

NucleoSpin® 8 RNA Blood can be readily automated on common laboratory robotic workstations. For vacuum processing, the use of the disposable MN Wash Plate inside the vacuum manifold is recommended. The use of the MN Wash Plate reduces the risk of cross-contamination caused by spraying of solutions during vacuum filtration steps.

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.

2.5 Handling, preparation, and storage of starting material

NucleoSpin® 8 RNA Blood kits are designed for isolating total RNA from fresh or frozen whole blood. Whole blood should be collected in the presence of an anticoagulant, preferably EDTA, Na-citrate, or Li-heparin.

To obtain optimal results, it is recommended processing blood samples within a few hours after collection (when EDTA, Na-citrate, or Li-heparin collection tubes are used). Samples should be stored at 4 °C for no longer than 24 hours. mRNAs derived from blood cells have different stabilities. To ensure that the isolated RNA contains a representative distribution of mRNAs, blood samples should not be stored for long periods prior RNA isolation.

If long term storage of stabilized whole blood is necessary, it is recommended to aliquot the blood samples and add the indicated volume of Lysis Buffer DL without adding Proteinase K. Store the lysates at -20 °C. After thawing, add Proteinase K.

3 Storage conditions and preparation of working solutions

Attention:

Buffers DL and RB2 contain guanidinium thiocyanate. Wear gloves and goggles!

- Store lyophilized **rDNase (RNase-free)** at 4 °C on arrival (stable up to 1 year).
- Store **Lysis Buffer DL** in the dark.
- All other kit components should be stored at room temperature (18 – 25 °C) and are stable for up to one year. Storage at lower temperatures may cause salt precipitation. If salt precipitation is observed, incubate the bottle at 30 – 40 °C for several minutes and mix well until all precipitates are redissolved.
- After first use, it is recommended to store Liquid Proteinase K at 4 °C or -20 °C.

Before starting any **NucleoSpin® 8 RNA Blood** procedure, prepare the following:

- **rDNase (RNase-free)**: Add indicated volume of **RNase-free H_2O** (see table below) to the **rDNase vial** and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. Dispense into aliquots and store at -20 °C. The frozen working solution is stable for 6 months. Do not freeze/thaw the aliquots more than three times. (Be careful when opening the vial as some particles of the lyophilisate may be attached to the lid.)
- **rDNase reaction mixture**: For each sample to be processed mix 1**0 µL reconstituted rDNase** with **90 µL Reaction Buffer for rDNase**.
- **Wash Buffer RB3**: Add the indicated volume of **96 – 100 % ethanol** (see table below) to **Buffer RB3 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer RB3 at room temperature (18 – 25 °C) for up to one year.
- **Wash Buffer RB4**: Add the indicated volume of **96 – 100 % ethanol** (see table below) to **Buffer RB4 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer RB4 at room temperature (18 – 25 °C) for up to one year.

	NucleoSpin® 8 RNA Blood	
REF	**12 × 8 preps** **740220**	**60 × 8 preps** **740220.5**
rDNase, RNase-free (lyophilized)	2 vials (size D) Add 540 µL RNase-free H_2O to each vial	10 vial s(size D) Add 540 µL RNase-free H_2O to each vial
Wash Buffer RB3 Concentrate	50 mL Add 200 mL ethanol	2 × 100 mL Add 400 mL ethanol to each bottle
Wash Buffer RB4 Concentrate	65 mL Add 150 mL ethanol	65 mL Add 150 mL ethanol

4 Safety instructions

When working with the **NucleoSpin® 8 RNA Blood** 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***).

The waste generated with the **NucleoSpin® 8 RNA Blood** 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® 8 RNA Blood – vacuum processing

- For hardware requirements refer to section 2.3.
- For detailed information regarding the vacuum manifold set-up see page 12.
- For detailed information on each step see page 13.

Before starting the preparation:

- Check if Buffer RB3, Buffer RB4, and rDNase were prepared according to section 3.

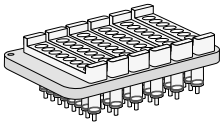
Protocol-at-a-glance

1	Lyse blood	400 µL blood 400 µL DL 10 µL Liquid Proteinase K RT, 15 min (shake 1,000 – 1,200 rpm)
2	Adjust binding conditions	400 µL RB4 Pipette up and down 10 – 15 times to mix
3	Transfer lysates to NucleoSpin® RNA Blood Binding Strips	
4	Bind RNA to silica membrane of the NucleoSpin® RNA Blood Binding Strips	-0.2 bar*, 1 min
5	Desalt silica membrane	500 µL RB3 – 0.2 bar*, 3 min
6	Incubate with rDNase	95 µL rDNase reaction mixture RT, 15 min
7	Wash and dry silica membrane	500 µL RB2 – 0.2 bar*, 1 min 800 µL RB3 – 0.2 bar*, 1 min 500 µL RB4 – 0.2 bar*, 1 min Remove MN Wash Plate Dry silica membrane (Maximum vacuum, 10 min)
8	Elute RNA	75 – 130 µL RNase-free H2O RT, 2 min -0.6 bar*, 1 min

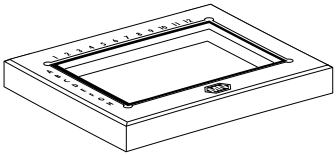
* Reduction of atmospheric pressure

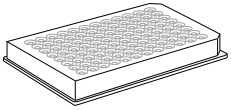
Setup of vacuum manifold:

Binding / Washing / Elution steps

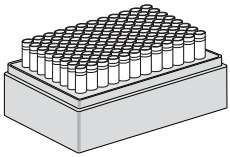


Column Holder A with NucleoSpin® Binding Strips inserted. Ununsed rows have to be filled with NucleoSpin® Dummy Strips

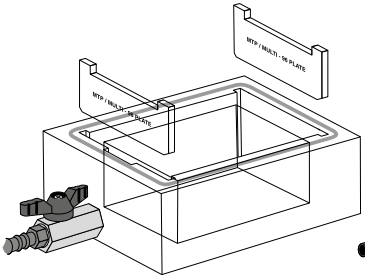




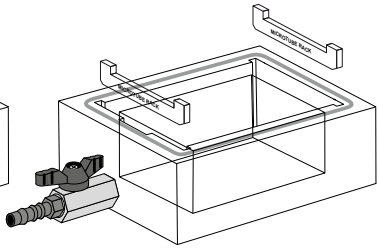
MN Wash Plate



Rack of Tube Strips



Manifold base with spacers ‘MTP/Multi-96 Plate’ inserted



Manifold base with spacers for ‘Microtube Rack’ inserted

Wash step

Elution step

Detailed protocol

For vacuum processing of the **NucleoSpin® 8 RNA Blood** kit all necessary consumables are included if six 8-well Strips are processed at once. If less samples are processed, additional consumables (e.g., Elution Plate, Square-well Blocks) have to be ordered (see ordering information).

When processing a large number of samples under vacuum, cross-contamination is a major concern due to spraying of liquids or aerosol formation. The MN Wash Plate (included) prevents this contamination effected by droplets at the outlets of the individual wells of the NucleoSpin® RNA Blood Binding Strips. This consistent and effective tool is highly recommended for vacuum processing.

When using the **NucleoSpin® 8 RNA Blood** kit under vacuum, the NucleoVac 96 vacuum manifold is required (see ordering information). In addition, the Starter Set A is also required because it contains two Column Holders A and the NucleoSpin® Dummy Strips to close / seal unused rows of the column holder when applying vacuum.

The use of NucleoSpin® RNA Blood Binding Strips in a Column Holder A allows the isolation of up to n x 8 samples (n = 1 – 6). Insert as many NucleoSpin® RNA Blood Binding Strips as required into the reusable column holder. Close unused openings of the column holder with NucleoSpin® Dummy Strips and place column holder containing NucleoSpin® RNA Blood Binding Strips and NucleoSpin® Dummy Strips onto NucleoVac 96 Vacuum Manifold.

If a final elution step by centrifugation is preferred, either a Column Holder C or a Support Frame for Column Holder A (see ordering information) is required.

This standard protocol is recommended for purification of RNA from 400 µL fresh or frozen whole blood stabilized with, for example, EDTA, Na-citrate, or Li-heparin. If smaller volumes than 400 µL blood are used, adjust the volumes of Buffer DL and Buffer RB4 in step 1 and 2 according to section 2.2 (ratio **1 : 1 : 1** (sample / Buffer DL / Buffer RB4)).

Before starting the preparation:

- Check if Buffer RB3, RB4, and rDNase were prepared according to section 3.

1 Lyse blood

Add **400 µL blood** to each well of a Square-well block (included).

Add **400 µL Buffer DL** to each well. Mix by shaking (**1,000 – 1,200 rpm**) for **1 min**.

For each blood sample, add **10 µL Liquid Proteinase K**.

Incubate for **15 min** at room temperature on a shaker (**1,000 – 1,200 rpm**).

2 Adjust binding conditions

Add **400 µL Buffer RB4** to each sample. Mix by pipetting up and down at least 10 – 15 times.

Optional: Mix by shaking (1,000 rpm).

Note: Buffer DL and Buffer RB4 have to be used in the same volume ratio.

Prepare NucleoVac 96 Vacuum Manifold

Insert the appropriate number of NucleoSpin® RNA Blood 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 Blood Binding Strips are tightly inserted into the Column Holder A. If the strips are not tightly inserted, this 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 B lood Binding Strips into the manifold lid and place lid on the manifold base.

3 Transfer lysates to NucleoSpin® RNA Blood Binding Strips

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

4 Bind RNA to silica membrane

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

5 Desalt silica membrane

Desalt the membrane by adding **500 µL Buffer RB3** to each well and apply vacuum (**-0.2 bar*, 3 min**) until all buffer has passed through the wells. Release the vacuum.

6 Incubate with rDNase

Prepare rDNase reaction mixture as described in section 3: Pipette **95 µL rDNase reaction mixture** directly to the bottom of each well in the NucleoSpin® RNA Blood 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 comes into contact with the silica membrane and that the membrane is wet completely.

7 Wash and dry silica membrane

1st wash

Add **500 µL Buffer RB2** to each well of the NucleoSpin® RNA Blood 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 RB3** to each well of the NucleoSpin® RNA Blood 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 RB4** to each well of the NucleoSpin® RNA Blood Binding Strips. Apply vacuum (**-0.2 bar*, 1 min**) until all buffer has passed through the wells. Release the vacuum.

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 Blood Binding Strips from the vacuum manifold. Put it on a clean paper towel to remove residual ethanol-containing wash buffer. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

Dry silica membrane

Remove any residual wash buffer from the NucleoSpin® RNA Blood Binding Strips. If necessary, tap the outlets of the NucleoSpin® RNA Blood Binding Strips onto a clean Paper Sheet (supplied with the MN Wash Plate) or soft tissue until there are no more drops observed.

Insert the Column Holder A with inserted NucleoSpin® RNA Blood 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 RB4 inhibits enzymatic reactions and has to be removed completely before eluting RNA.

Finally, release the vacuum.

8 **Elute RNA**

Place the Elution Plate U-bottom onto the spacers ('MTP / Multi-96 Plate') of the vacuum manifold. Pipette **75 – 130 µL 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 (**-0.6 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 Rack' 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.

5.2 NucleoSpin® 8 RNA Blood – centrifuge processing

- For hardware requirements refer to section 2.3.
- For detailed information on each step see page 23.

Before starting the preparation:

- Check if Buffer RB3, Buffer RB4, and rDNase were prepared according to section 3

Protocol-at-a-glance

1	Lyse blood	400 µL blood 400 µL DL 10 µL Liquid Proteinase K RT, 15 min (shake 1,000 – 1,200 rpm)
2	Adjust binding conditions	Add 400 µL Buffer RB4 to each sample. Mix by pipetting up and down at least 10 – 15 times. Optional: Mix by shaking (1,000 rpm). Note: Buffer DL and Buffer RB4 have to be used in the same volume ratio.
3	Transfer lysates to NucleoSpin® RNA Blood Binding Strips	
4	Bind RNA to silica membrane of the NucleoSpin® RNA Blood Binding Strips	5,600 – 6,000 x *g*, 2 min
5	Desalt silica membrane	500 µL RB3 5,600 – 6,000 x *g*, 2 min
6	Incubate with rDNase	95 µL rDNase reaction mixture RT, 15 min

7	**Wash and dry silica membrane**	**500 µL RB2** **5,600 – 6,000 x *g*, 2 min** **800 µL RB3** **5,600 – 6,000 x *g*, 2 min** **500 µL RB4** **5,600 – 6,000 x *g*, 10 min**
8	**Elute RNA**	**50 – 130 µL RNase-free H2O** **RT, 2 min** **5,600 – 6,000 x *g*, 3 min**

Detailed protocol

This standard protocol is recommended for purification of RNA from fresh or frozen whole blood stabilized with, for example, EDTA, Na-citrate, or Li-heparin. The use of NucleoSpin® RNA Blood Binding Strips in a Column Holder C allows the isolation of up to n x 8 samples (n = 1 – 6) at the same time. Insert as many of the NucleoSpin® RNA Blood Binding Strips as needed into the same positions of each one of the two reusable column holders. Then, place these column holders onto the MN Square-well Blocks (not supplied) and label the column holders or 8-well strips for later identification.

Always use 2 Column Holders C containing the same number of NucleoSpin® RNA Binding Strips for centrifugation. As a result, this allows multiples of 16 samples to be processed in parallel and the centrifuge does not need to be balanced. In addition, we recommend inserting the NucleoSpin® RNA Blood Binding Strips around the center of the column holder.

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

This standard protocol is recommended for purification of RNA from 400 µL fresh or frozen whole blood stabilized with, for example, EDTA, Na-citrate, or Li-heparin. If smaller volumes than 400 µL blood are used, adjust the volumes of Buffer DL and Buffer RB4 in step 1 and 2 according to section 2.2 (ratio 1 : 1 : 1 (sample / Buffer DL / Buffer RB4).

Before starting the preparation:

- Check if Buffer RB3, RB4, and rDNase were prepared according to section 3.

1 Lyse blood

Add **400 µL blood** to each well of a Square-well block (included).

Add **400 µL Buffer DL** to each well. Mix by shaking (**1,000 – 1,200 rpm**) for **1 min**.

For each blood sample, add **10 µL Liquid Proteinase K**.

Incubate for **15 min** at **room temperature** on a shaker (**1,000 – 1,200 rpm**).

2 Adjust binding conditions

Add **400 µL Buffer RB4** to each sample. Mix by pipetting up and down at least 10 – 15 times. Optional: Mix by shaking (1,000 rpm).

Note: Buffer DL and Buffer RB4 have to be used in the same volume ratio.

3 Transfer lysates to NucleoSpin® RNA Blood Binding Strips

Insert desired number of NucleoSpin® RNA Blood Binding Strips into Column Holder C and place it on a MN Square-well Block (not supplied) for collection of flow-through. If using more than one block, label the column holders for future identification. Transfer lysates into the wells of the NucleoSpin® RNA Blood Binding Strips.

4 Bind RNA to silica membrane

Centrifuge for **2 min** at **5,600 – 6,000 x *g***. Discard MN Square-well Block with flow-through and place NucleoSpin® RNA Blood Binding Strips inserted into Column Holder C on a new MN Square-well Block (not supplied).

5 Desalt silica membrane

Desalt the membrane by adding **500 µL Buffer RB3** to each well and centrifuge for **2 min** at **5,600 – 6,000 x *g***.

6 Incubate with rDNase

Prepare rDNase reaction mixture as described in section 3: Leave the NucleoSpin® RNA Blood Binding Strips inserted into Column Holder C on the MN Square-well Block. Pipette **95 µL rDNase reaction mixture** directly to the bottom of each well of the NucleoSpin® RNA Blood 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.

7 Wash silica membrane

1st wash

Add **500 µL Buffer RB2** to each well of the NucleoSpin® RNA Blood Binding Strips. Place the Column Holder C with the NucleoSpin® RNA Blood Binding Strips onto the MN Square-well Block. Then, place it into the rotor bucket and centrifuge for **2 min** at **5,600 – 6,000 x *g***. Discard the MN Square-well Block.

Place NucleoSpin® RNA Blood Binding Strips inserted into the Column Holder C onto a new MN Square-well Block (not supplied).

2nd wash

Add **800 µL Buffer RB3** to each well of the NucleoSpin® RNA Blood Binding Strips and centrifuge for **2 min** at **5,600 – 6,000 x *g***. Empty the MN Square-well Block. Place the NucleoSpin® RNA Blood Binding Strips inserted into Column Holder C back onto the MN Square-well Block.

3rd wash

Add **500 µL Buffer RB4** to each well of the NucleoSpin® RNA Blood Binding Strips and centrifuge for or **10 min** at **5,600 – 6,000 x *g***. Discard MN Square-well Block.

Dry silica membrane

Residual wash buffer from the NucleoSpin® RNA Blood Binding Strips is removed by the extended centrifugation time of 10 min after adding Wash Buffer RB4 (described in the third washing step). This prolonged time is necessary to eliminate any trace amounts of ethanol.

Note: The ethanol in Buffer RB4 inhibits enzymatic reactions and has to be removed completely before eluting RNA.

8 Elute RNA

For elution, place Column Holder C, with the NucleoSpin® RNA Blood Binding Strips, onto the Rack of Tube Strips (included) and pipette **50 – 130 µL RNase-free H_2O** directly to the bottom of each well. Make sure that all of the water comes into contact with the silica membrane and that the membrane is wet completely. 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.

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

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 through 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. *Sample material* • Sample material was not fresh. Whenever possible, use fresh blood samples.
Poor RNA quality or yield	*Reagents not applied or prepared properly* • Reagents were not properly prepared. Add the indicated volume of RNase-free H_2O to the rDNase vial and 96 – 100 % ethanol to Buffer RB3 and Buffer RB4 Concentrate and mix (see section 3). *Kit storage* • Store aliquots of the reconstituted rDNase 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. *Elution* • Be sure that all of the water comes into contact with the silica membrane. There should not be any water droplets on the walls of the columns. The membrane needs to be completely wet. • Elute two times (e.g., 2 × 50 µL).
Clogged wells	*Insufficient vacuum* • Prolong vacuum time to 5 – 10 min at -0.4 to -0.6 bar (reduction of atmospheric pressure) (step 4 'Bind RNA to silica membrane'). • Prolong centrifugation step to 10 min (*at 5,600 – 6,000 x g*).
Colored membrane after last wash step with RB3	*Insufficient washing* • Repeat RB3 wash step (800 µL).

Problem	Possible cause and suggestions
Contamination with genomic DNA	*rDNase not active* • Reconstitute and store lyophilized rDNase according to the instructions in section 3. ***Too much material used*** • Reduce quantity of blood used. • Increase mixing cycles after addition of Buffer RB4 to the lysate. • Do not release vacuum until all buffer has passed through (important after every step).
Suboptimal performance of RNA in downstream experiments	*Carry over of ethanol* • Be sure to remove all ethanolic Buffer RB4 after the final washing step prior elution. Dry the NucleoSpin® RNA Blood Binding Strips for at least 10 min with maximum vacuum or by 10 min centrifugation. • Do not release vacuum until all buffer has passed through (important after every step).
Vacuum pressure is not sufficient	• Check if the vacuum manifold lid fits tightly on the manifold base if vacuum is turned on. • Close unused rows with NucleoSpin® Dummy Strips
Buffer volumes are not sufficient	• 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.
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 Blood Binding Strips* • Be sure that no liquid drops out of the tips while moving the tips above the binding strips.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 8 RNA Blood	740220 740220.5	12 × 8 preps 60 × 8 preps

Product	REF	Pack of
NucleoSpin® 96 RNA Blood	740225.2 740225.4	2 × 96 preps 4 × 96 preps
MN Square-well Block	740476 740476.24	4 24
Round-well Block Low	740482	4
Round-well Block	740671	20
Rack of Tube Strips (1 set consists of 1 rack, 12 strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Rack of Tube Strips	740637	5 racks
Cap Strips	740478 740478.24	48 288
MN Wash Plate	740479 740479.24	4 24
Elution Plate U-bottom (with Self-adhering Foil)	740486.24	24 sets
Self-adhering PE Foil	740676	50
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Starter Set A (for use of 8-well strips on the NucleoVac 96 Vacuum Manifold and automated platforms, contains 2 Column Holders A and 12 NucleoSpin® Dummy Strips)	740682	1
Starter Set C (for use of 8-well strips on the NucleoVac 96 Vacuum Manifold and automated platforms, contains 2 Column Holders C, 2 MN Square-well Blocks and 2 Racks of Tube Strips)	740684	1
MN Frame (for optimized handling of 8-well strips and 96-well plates on other than NucleoVac Vacuum Manifolds)	740680	1
MN Support Frame for Column Holder A	740480	

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

6.3 Product use restriction / warrantys

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-NAGELS 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 costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

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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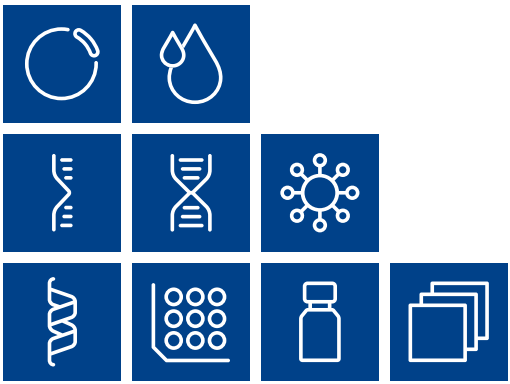
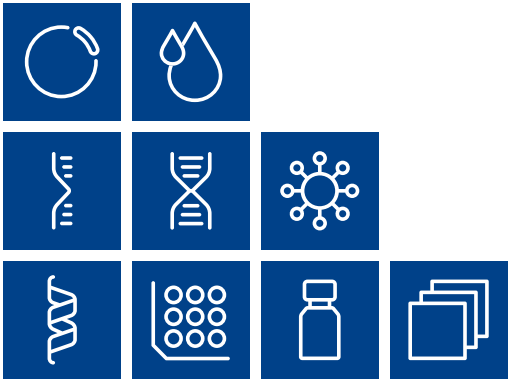
Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 (0) 24 21 969-333
support@mn-net.com



Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany
DE +49 24 21 969-0 info@mn-net.com
FR +33 388 68 22 68 sales-fr@mn-net.com
CH +41 62 388 55 00 sales-ch@mn-net.com
US +1 888 321 62 24 sales-us@mn-net.com