RNA from blood

User manual

NucleoSpin® 96 RNA Blood

This product distributed by

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May 2014 / Rev. 02

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1 Components

1.1 Kit contents

	NucleoSpin® 96 RNA Blood[1]	
	2 x 96 preps	**4 x 96 preps**
REF	**740225.2**	**740225.4**
Lysis Buffer DL	100 mL	2 x 100 mL
Wash Buffer RB2	160 mL	360 mL
Wash Buffer RB3 (Concentrate)[2]	100 mL	2 x 100 mL
Wash Buffer RB4 (Concentrate)[2]	65 mL	2 x 65 mL
RNase-free H_2O	125 mL	125 mL
Reaction Buffer for rDNase	60 mL	2 x 60 mL
rDNase, RNase-free (lyophilized)[2]	4 vials (size D)	8 vials (size D)
Liquid Proteinase K	2 x 1.25 mL	4 x 1.25 mL
NucleoSpin® RNA Blood Binding Plates (blue rings)	2	4
MN Wash Plates[3]	2	4
Square-well Blocks (including one self-adhering PE-Foil)	2	4
Elution Plates U-bottom (including one Self-adhering PE Foil)	2	4
Round-well Block Low (including one Self-adhering PE Foil)	2	4
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1.2 Reagents to be supplied by user

- 96–100 % ethanol

[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.

2 Product description

2.1 The basic principle

The **NucleoSpin® 96 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 RNA isolation is to prevent RNA degradation during the isolation procedure. With the **NucleoSpin® 96 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® 96 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® 96 RNA Blood** kits can be used on fully automated common laboratory workstations (see section 2.4).
- The **NucleoSpin® 96 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® 96 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® 96 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.

Kit specifications at a glance	
Parameter	**NucleoSpin® 96 RNA Blood**
Format	96-well plate
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	~ 100 min/plate
Binding capacity	100 µg

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® 96 RNA Blood** kit can be used with either the NucleoVac 96 Vacuum Manifold (see ordering information). When using **NucleoSpin® 96 RNA Blood** 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 Blood Binding Plate and thus guarantee a 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.

Centrifugation

For centrifugation a microtiterplate centrifuge is required, This centrifuge must be able to accommodate the NucleoSpin® RNA Blood 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.

2.4 Automated processing on robotic platforms

NucleoSpin® 96 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® 96 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).
- 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® 96 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 **10 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® 96 RNA Blood	
	12 x 8 preps	**60 x 8 preps**
REF	**740220**	**740220.5**
rDNase, RNase-free (lyophilized)	4 vials (size D) Add 540 µL RNase-free H_2O to each vial	8 vials (size D) Add 540 µL RNase-free H_2O to each vial
Wash Buffer RB3 Concentrate	100 mL Add 400 mL ethanol	2 x 100 mL Add 400 mL ethanol to each bottle
Wash Buffer RB4 Concentrate	65 mL Add 150 mL ethanol	2 x 65 mL Add 150 mL ethanol to each bottle

4 Safety instructions

The following components of the **NucleoSpin® 96 RNA Blood** kits contain hazardous contents. *Wear gloves and goggles and follow the safety instructions given in this section.*

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
rDNase, RNase-free	rDNase, lyophilized *rDNase, lyophilisiert*	 Warning *Achtung*	317, 334	261, 304+340, 342+311, 301+312, 280, 302+352, 333+313
DL	Guanidinium thiocyanate 30–60 % *Guanidiniumthiocyanat 30–60 %*	 Warning *Achtung*	302, 412, EUH031	260, 273, 301+312, 330
RB2	Guanidinium thiocyanate 24–36 % + ethanol 20–35 % *Guanidiniumthiocyanat 24–36 % + Ethanol 20–35 %*	 Warning *Achtung*	226, 302,	210, 233, 301+312, 330, 403+235
Liquid Proteinase K	Proteinase K, liquid (1–10 %) *Proteinase K, flüssig (1–10 %)*	 Danger *Gefahr*	317, 334	261, 272, 280, 302+352, 304+340, 333+313, 342+311, 363

Hazard phrases

H 226	Flammable liquid and vapour. *Flüssigkeit und Dampf entzündbar.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 317	May cause an allergic skin reaction. *Kann allergische Hautreaktionen verursachen.*
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled. *Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.*
H 412	Harmful to aquatic life with long lasting effects. *Schädlich für Wasserorganismen, mit langfristiger Wirkung.*
EUH 031	Contact with acids liberates toxic gas. *Entwickelt bei Berührung mit Säure giftige Gase.*

Precaution phrases

P 261	Avoid breathing dust. *Einatmen von Staub vermeiden.*
P 272	Contaminated work clothing should not be allowed out of the workplace. *Kontaminierte Arbeitskleidung nicht außerhalb des Arbeitsplatzes tragen.*
P 280	Wear protective gloves / eye protection. *Schutzhandschuhe / Augenschutz tragen.*
P 301+312	IF SWALLOWED: Call a POISON CENTER/ doctor/…/if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /… anrufen.*
P 302+352	IF ON SKIN: Wash with plenty of water/… *BEI KONTAKT MIT DER HAUT: Mit viel Wasser/… waschen.*
P 304+340	IF INHALED: If breathing is difficult, remove to fresh air and keep at rest in a position comfortable for breathing. *BEI EINATMEN: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.*
P 333+313	If skin irritation occurs: Get medical advice / attention. *Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER/ doctor/… *Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM /Arzt/… anrufen.*
P 363	Wash contaminated clothing before reuse. *Kontaminierte Kleidung vor erneutem Tragen waschen.*

For further information please see Material Safety Data Sheets ***(www.mn-net.com).***
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ***(www.mn-net.com)****.*

⚠ The symbol shown on labels refers to the precaution phrases of this section.
Das auf Etiketten dargestellte Symbol weist auf die P-Sätzen dieses Kapitels hin.

5 Protocols

5.1 NucleoSpin® 96 RNA Blood – vacuum processing

- For hardware requirements refer to section 2.3.
- For detailed information regarding the vacuum manifold set-up see page 15.
- For detailed information on each step see page 16.

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 Plate	
4	**Bind** RNA to silica membrane of the NucleoSpin® RNA Blood Binding Plate	**-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**

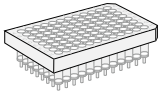
* Reduction of atmospheric pressure

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 H_2O** **RT, 2 min** **-0.5 bar*, 1 min**

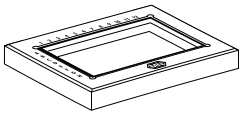
* Reduction of atmospheric pressure

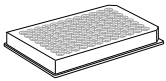
Setup of vacuum manifold:

Binding / Washing / Elution steps

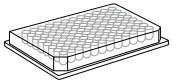


NucleoSpin® Binding Plate

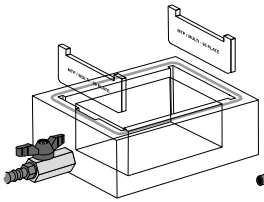




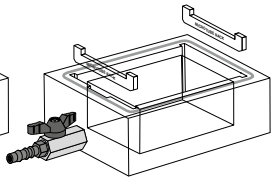
MN Wash Plate



Elution Plate U-bottom



Manifold base with spacers
'MTP/Multi-96 Plate' inserted



Manifold base with spacers for
'Microtube Rack' inserted

Binding / Washing step

Elution step

Detailed protocol

For vacuum processing of the **NucleoSpin® 96 RNA Blood** kit all necessary consumables are included. 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 prevents this contamination effected by droplets at the outlets of the individual wells of the NucleoSpin® RNA Blood Binding Plate. This consistent and effective tool is highly recommended for vacuum processing.

When using the **NucleoSpin® 96 RNA Blood** kit under vacuum, the NucleoVac 96 Vacuum Manifold is required (see ordering information). Place NucleoSpin® 96 RNA Blood Binding Plate on NucleoVac 96 Vacuum Manifold. If processing less than 96 samples, seal unused wells with a Self-adhering PE Foil in order to ensure proper vacuum during the filtration steps.

This standard protocol is recommended for purification of RNA from fresh or frozen whole blood stabilized with (e.g., EDTA, Na-citrate, or Li-heparin).

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.

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 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. Place the MN Wash Plate on the spacers in the manifold base.

3 Transfer lysates to NucleoSpin® RNA Blood Binding Plate

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

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 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 comes into contact with the silica membrane and that the membrane is wet completely.

7 Wash silica membrane

1st wash

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

* Reduction of atmospheric pressure

3rd wash

Add **500 µL Buffer RB4** to each well of the NucleoSpin® RNA Blood Binding Plate. 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 NucleoSpin® RNA Blood Binding Plate 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 Plate. If necessary, tap the outlets of the NucleoSpin® RNA Blood Binding Plate onto a clean Paper Sheet (supplied with the MN Wash Plate) or soft tissue until there are no more drops observed.

Insert the NucleoSpin® RNA Blood 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 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 standard PCR plates is possible. Elution into PCR plates can be performed by placing a PCR plate onto a Square-well Block resting on the spacers *'Square-well Block'* in the manifold.

* Reduction of atmospheric pressure

5.2 NucleoSpin® 96 RNA Blood – centrifuge processing

- For hardware requirements refer to section 2.3.
- For detailed information on each step see page 21.

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 Plate	
4	**Bind** RNA to silica membrane of the NucleoSpin® RNA Blood Binding Plate	**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 H_2O** **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 (e.g., EDTA, Na-citrate, or Li-heparin). Place the NucleoSpin® RNA Blood Binding Plate on an MN Square-well Block (not supplied). The use of a second plate placed on a MN Square-well Block avoids the need to balance the centrifuge.

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 emptying and reuse of MN-Square-well Blocks we recommend to use 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 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 Plate

Place the NucleoSpin® RNA Blood Binding Plate on an MN Square-well Block (not supplied) and transfer lysates into the wells of the NucleoSpin® RNA Blood Binding Plate.

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 Plate onto 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 Plate 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 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.

7 Wash silica membrane

1st wash

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

Place NucleoSpin® RNA Blood Binding Plate 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 Plate and centrifuge for **2 min** at **5,600–6,000 x *g***. Empty MN Square-well Block. Place NucleoSpin® RNA Blood Binding Plate back onto the MN Square-well Block.

3rd wash

Add **500 µL Buffer RB4** to each well of the NucleoSpin® RNA Blood Binding Plate 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 Plate 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 the NucleoSpin® RNA Blood Binding Plate onto a Round-well block (included in the kit) 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 for **3 min** at **5,600–6,000 x *g***.

Alternatively, elution in a MN Square-well Block (see ordering information) or standard PCR plates is possible. For elution, place the NucleoSpin® RNA Blood Binding Plate on top of a MN Square-well Block and centrifuge. For direct elution onto PCR plates, place a PCR plate between the NucleoSpin® RNA Blood Binding Plate and the MN Square-well Block before centrifugation.

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 x 50 µL).

Problem	Possible cause and suggestions
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 5 min *(at 5,600–6,000 x g).*
Colored membrane after last wash step with RB3	*Insufficient washing* • Repeat RB3 wash step (800 µL).
Contamination of RNA 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 Plates 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 Self-adhering PE Foil (supplied).

Problem	Possible cause and suggestions
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 Plate* • 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 Blood	740225 .2 740225 .4	2 x 96 preps 4 x 96 preps
NucleoSpin® 8 RNA Blood	740220 740220 .5	12 x 8 preps 60 x 8 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	740637	5 racks
Cap Strips	740478 740478 .24	48 288
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
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
MN Frame	740680	1

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

6.3 Product use restriction/warranty

NucleoSpin® 96 RNA Blood kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

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ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN VITRO*-diagnostic use. Please pay attention to the package of the product. *IN VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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Last updated: 07/2010, Rev. 03

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