Bioanalysis

MACHEREY-NAGEL

User manual

Genomic DNA from organs and cells

■ NucleoSpin® 96 DNA RapidLyse

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1 Components

1.1 Kit contents

	NucleoSpin® 96 DNA RapidLyse	
REF	**1 × 96 preps 740110.1**	**4 × 96 preps 740110.4**
Lysis Buffer RLY	30 mL	70 mL
Binding Buffer RLB	2 × 25 mL	2 × 100 mL
Wash Buffer RLW (Concentrate)[1]	50 mL	200 mL
Elution Buffer RLE[2]	13 mL	60 mL
Liquid Proteinase K	1250 µL	4,5 mL
NucleoSpin® 96 DNA RapidLyse Binding Plates (green rings)	1	4
Square-well Blocks with self-adhering PE Foil	2	8
MN Wash Plates[3]	1	4
User manual	1	1

1.2 Reagents to be supplied by user

- 96 – 100 % ethanol (for preparation of working solutions; see section 3)

For more detailed information regarding special hardware required for centrifuge or vacuum processing, please see section 2.3.

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoSpin® 96 DNA RapidLyse** kit before using this product. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available online at ***www.mn-net.com***.

Please contact Technical Service regarding information about any changes to the current user manual compared with previous revisions.

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

[2] Elution Buffer RLE: 5 mM Tris/HCl, pH 8.5

[3] For use with vacuum only.

2 Product description

2.1 The basic principle

The **NucleoSpin® 96 DNA RapidLyse** kit is designed for the efficient isolation of high molecular weight genomic DNA from cells and organs like liver, kidney, heart, muscle, spleen, and lung. Processing of mouse tail and ear clippings is also possible. Fresh, frozen, and ethanol-preserved samples can be used.

The **NucleoSpin® 96 DNA RapidLyse** kit lyses samples in maximal one hour agitated incubation at 56 °C. This is enabled by a thoroughly designed lysing setup with well balanced parameters that comprise a special lysis buffer in combination with Liquid Proteinase K. Incubation over night or for several hours is not necessary.

2.2 Kit specifications

Kit specifications at a glance	
Parameter	**NucleoSpin® 96 DNA RapidLyse**
Technology	Silica-membrane technology
Format	96-well plates
Processing	Manual and automated, vacuum, positive pressure or centrifugation
Sample material	Fresh, frozen, dried, and ethanol preserved tissue samples (e.g., organs), eukaryotic cells
Sample amount	Up to 30 mg fresh weight (sample dependent)
Typical yield	Up to 4 µg DNA per mg tissue (sample dependent)
A_{260}/A_{280}	1.7 – 1.9
Elution volume	100 µL
Preparation time	60 min (96 preps, excluding lysis)
Lysis time	Maximal 1 h
Binding capacity	40 µg
Use	For research use only

- **NucleoSpin® 96 DNA RapidLyse** can be processed by vacuum, positive pressure or in a centrifuge and is designed for manual or automated use. The kit allows easy automation on common liquid handling instruments.

- The **NucleoSpin® 96 DNA RapidLyse** kit allows the purification of multiples of 96 samples. The kits are supplied with accessory plates for highest convenience. Further Accessory components (e.g., lysis plates, elution plates) can be individually selected from a variety of suitable accessories to provide highest flexibility (see section 2.3 for further information).

2.3 Required hardware

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

Centrifugation

For centrifugation, a microtiterplate centrifuge is required. This centrifuge must be able to accommodate the NucleoSpin® 96 DNA RapidLyse Binding Plate stacked on a Square-well Block and need to reach accelerations of 4,000 x *g* (bucket height: min. 75 mm).

Consumables for waste collection need to be ordered separately (e.g., MN Square-well Blocks). When processing two 96-well plates at once, six MN Square-well blocks are sufficient to collect the entire liquid waste without the need of emptying and reusing the MN Square-well Blocks (see ordering information, section 6.2). Alternatively, it is possible to use a single MN Square-well Block, which needs to be emptied after every centrifugation step.

Vacuum processing

The **NucleoSpin® 96 DNA RapidLyse** kit can be used with the NucleoVac 96 Vacuum Manifold. When using **NucleoSpin® 96 DNA RapidLyse** with less than 96 samples, Self-adhering PE Foil should be used in order to close and protect non-used wells of the NucleoSpin® 96 DNA RapidLyse Binding Plate and thus guarantee proper vacuum (see ordering information, section 6.2).

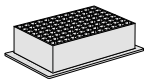
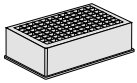
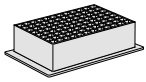
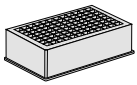
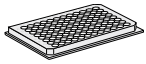
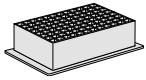
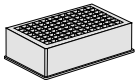
The manifold may be used with a vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of -0.2 to -0.6 bar (reduction of atmospheric pressure). The use of the NucleoVac Vacuum Regulator (see ordering information, section 6.2) is recommended. Alternatively, adjust the vacuum in a way that 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.

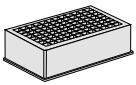
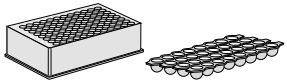
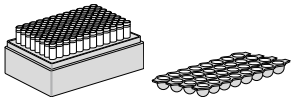
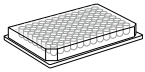
In addition, a suitable centrifuge for sample preparation steps may be required.

For general consumables and equipment needed, please see section 1.2.

Additional accessories for use of the NucleoSpin® 96 DNA RapidLyse Kit

Additional accessories for use of the **NucleoSpin® 96 DNA RapidLyse Kit** are available from MACHEREY-NAGEL (see ordering information, section 6.2).

Protocol step	Additional consumables	Remarks
Lyse samples	MN-Square-well Block 	For sample lysis
	Square-well Block 	
Adjust binding conditions	MN Square-well Block 	Recommended for automated processing only
	Square-well Block 	Self-adhering PE-Foil is required
Bind DNA	MN Wash Plate 	MN Wash Plate minimizes the risk of cross contamination (vacuum processing only)
	MN Square-well Block 	For waste collection during centrifugation (reusable)
	Square-well Block 	

Protocol step	Additional consumables	Remarks
Elute DNA	Square-well Block 	
	Round-well Block with Cap Strips 	
	Rack of Tube Strips with Cap Strips 	
	Elution plates 	

2.4 Automated processing on robotic platforms

NucleoSpin® 96 DNA RapidLyse can be used fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting the kit on a certain workstation, please contact MN. Full processing under vacuum or positive pressure enables complete automation without the need for centrifugation steps for drying of the binding membrane or for elution. However, a final elution step by centrifugation is recommended in order to achieve a DNA solution with higher concentration.

The risk of cross-contamination is reduced by optimized vacuum or positive pressure settings during the elution step and by the improved shape of the outlets of the NucleoSpin® 96 DNA RapidLyse Binding Plate.

Drying of the NucleoSpin® 96 DNA RapidLyse Binding Plates under vacuum or positive pressure is sufficient because the bottom of the plate is protected by the MN Wash Plate during the washing steps. Therefore it is recommended to integrate the MN Wash Plate into the automation procedure. The MN Frame (see ordering information, section 6.2) can be used to position 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.

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 materials

Fresh, frozen, and ethanol preserved samples can be used. Make sure not to use more than 30 mg sample.

2.6 Lysis of sample material

In order to obtain optimal DNA yields and a smooth processing, sample material should be thoroughly lysed. Most samples can be processed according to procedure 5.1. However, some sample materials (e.g., spleen or lung) need to be lysed according to an alternative protocol, which requires additional material (see section 5.2 and 6.2).

2.7 Elution procedures

It is possible to adjust the elution method and the volume of the elution buffer to the subsequent application of interest. In addition to the standard method described in the protocols (recovery rate about 70 – 90 %), there are several modifications possible. Use elution buffer preheated at 70 °C for one of the following procedures:

- **High yield**: Perform two elution steps with the volume indicated in the individual protocol. About 90 – 100 % of bound nucleic acids can be eluted.
- **High concentration**: Perform one elution step with only 60 % of the volume indicated in the individual protocol. Concentration of DNA will be about 30 % higher than with the standard elution procedure. Maximum yield of bound nucleic acids is about 80 %.
- **High yield and high concentration**: Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85 – 100 % of bound nucleic acids are eluted in the standard elution volume at a high concentration.
- **Convenient elution**: For convenience, elution buffer of ambient temperature may be used. This will result in a slightly lower yield (approximately 20 %) compared to elution with heated elution buffer.

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability during long term or multi-use storage at 4 °C (or ambient temperature) by inhibiting omnipresent DNases. However, high EDTA concentrations may interfere with certain downstream applications.

For optimal performance of isolated DNA in downstream applications, we recommend eluting with the supplied Elution Buffer RLE. For long term storage, the Elution Buffer RLE should be kept at - 20 °C. Several freeze-thaw cycles will not interfere with most downstream applications.

Performance of long-range PCR (e.g., > 10 kb) or the detection limit of trace amount of DNA species, may be reduced after multiple freeze-thaw cycles or prolonged storage of eluted DNA at 4 °C or room temperature. This is due to shearing of DNA or adsorption to surfaces.

Due to the dead volume of the silica membrane, please note that the difference between the dispensed elution buffer volume and the recovered elution buffer volume containing genomic DNA is approximately 40 µL (recovered elution volume = dispensed elution volume - 40 µL).

3 Storage conditions and preparation of working solutions

Attention*: Buffer RLB contains chaotropic salts! Wear gloves and goggles!*

CAUTION: Buffer RLB contains chaotropic salts 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:

- All kit components can be stored at room temperature (18 – 25 °C) and are stable for at least one year.
- Storage at lower temperatures may cause precipitation of salts. If a salt precipitation is observed, incubate the bottle at 30 – 40 °C for some minutes and mix well until all of the precipitate is redissolved. The performance of the kits is not affected by the salt precipitates.

Prior to the **NucleoSpin® 96 DNA RapidLyse** procedure, prepare the following:

- **Wash Buffer RLW**: Add the indicated volume of ethanol (96 – 100 %) to Wash **Buffer RLW Concentrate**. Mark the label of the bottle to indicate that ethanol was added. **Wash Buffer RLW can be stored** at room temperature (18 – 25 °C) for up to one year.
- **Liquid Proteinase K** is ready to use. After first time use, store **Liquid Proteinase K** at 4 °C or -20 °C.

	NucleoSpin® 96 DNA RapidLyse	
REF	**1 × 96 preps** **740110.1**	**4 × 96 preps** **740110.4**
Wash Buffer RLW (Concentrate)	50 mL Add 200 mL ethanol	200 mL Add 800 mL ethanol

4 Safety instructions

When working with the NucleoSpin® 96 DNA RapidLyse 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 RLB 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 DNA RapidLyse** 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 and Proteinase K 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 DNA RapidLyse – centrifuge processing

- For hardware requirements, refer to section 2.3.
- For storage conditions, refer to section 3.
- For use of recommended accessories, refer to section 2.4.

Before starting the preparation:

- Check if Buffer RLW was prepared according to section 3.
- Set incubator or oven to 56 °C.
- Set incubator or oven to 70 °C after lysis step.

1 Lyse sample

Place the samples into the wells of the **Square-well Block** or an appropriate lysis container (e.g. U-bottom plate or 2 mL tube).

Add **150 µL Buffer RLY** to each sample. — **+ 150 µL RLY**

Add **10 µL Liquid Proteinase K to each sample.** — **+ 10 µL Liquid Proteinase K**

Seal the plate with a Self-adhering Foil and **mix** by vigorous shaking for 10 – 15 s. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the wells. — **Mix**

Incubate at **56 °C** on a thermomixer at maximum speed until the sample appears visually lysed. — **56 °C, max. 1 h**

Note: Lysis time depends on sample material and may vary from a couple of minutes up to one hour (for mammalian cells reduce incubation to 30 min).

Do not use conical plates or 1.5 mL tubes, as their shape will impair thorough mixing. Use the Square-well Block or 2 mL tubes which will facilitate proper sample and lysis buffer agitation. Make sure that the tissue sample is submerged in the lysis buffer!

Take care not to moisten the rims of the individual wells while dispensing the buffer.

Spin briefly the Square-well Block or lysis container (15 s; 1,500 x *g*) to collect any condensate at the bottom of the wells.

Residual hair and / or bones in the lysate can be removed by centrifugation (2 min; 5,600 – 6,000 x g) and transfer of the supernatant to a new Square-well Block (not supplied with the kit).

2 Adjust DNA binding conditions

Add **440 µL Buffer RLB to each sample** and **mix** (e.g., pipetting up and down, pulse vortexing or shaking). Seal the plate with a Self-adhering Foil if necessary.

+ 440 µL RLB

Mix

Take care not to moisten the rims of the individual wells while dispensing the buffer. Spin briefly the Square-well Block or lysis container (15 s; 1,500 x *g*) to collect any condensate at the bottom of the wells.

Never centrifuge at higher g-forces or for longer periods as DNA will precipitate.

Place a NucleoSpin® 96 DNA RapidLyse Binding Plate on a Square-well Block.

If using more than one plate, label the plates for later identification. The use of a second plate placed on a MN Square-well Block avoids the need to balance the centrifuge.

3 Transfer lysates

Transfer the **lysates** resulting from step 3 carefully from the Square-well Block into the wells of the **NucleoSpin® 96 DNA RapidLyse Binding Plate**.

Transfer lysate

Take care to dispense the lysate centric onto the silica membrane.

Do not moisten the rims of the individual wells while dispensing the samples – moistened rims may cause cross contamination during centrifugation.

We recommend the use of an electronic eight-channel pipetting device with extra long tips capable of holding approx. 1000 µL, for transfer of the lysate from the Square-well Block to the NucleoSpin® 96 DNA RapidLyse Binding Plate.

4	**Bind DNA**	
	After transfer, seal the openings of the plate with Self-adhering PE-Foil and incubate the lysates for **1 min** on the NucleoSpin® 96 DNA RapidLyse Binding Plate at **room-temperature**.	**1 min, RT**
	Place the Square-well Blocks with NucleoSpin® 96 DNA RapidLyse Binding Plates onto the centrifuge carriers and insert them into the rotor buckets. Centrifuge for **2 min** at **4,000 x *g***.	**4,000 x *g*, 2 min**
	Typically, the lysates will have passed through the silica membrane within a few minutes. The centrifugation process can be extended to 20 min, if the lysates have not passed completely.	
	Discard Square-well block with flow through. Put NucleoSpin® 96 DNA RapidLyse Binding Plate onto a new Square-well Block (not provided).	
	When using NucleoSpin® 96 DNA RapidLyse with less than 96 samples, Self-adhering PE Foil should be used in order to close and protect non-used wells of the NucleoSpin® 96 DNA RapidLyse Binding Plate and thus guarantee proper vacuum.	

5 Wash silica membrane

1st wash

Remove the Self-adhering PE Foil and add **750 µL Buffer RLW** to each well of the NucleoSpin® 96 DNA RapidLyse Binding Plate. Seal the plate with a new Self-adhering PE Foil and centrifuge again for **2 min** at **4,000 x *g*.**	**+ 750 µL RLW** **4,000 x *g*, 2 min**
Discard flow through and place NucleoSpin® 96 DNA RapidLyse Binding Plate back onto the Square-well Block.	

2nd wash

Remove the Self-adhering PE Foil and add **750 µL Buffer RLW** to each well of the NucleoSpin® 96 DNA RapidLyse Binding Plate. Seal the plate with a new Self-adhering PE Foil and centrifuge again for **2 min** at **4,000 x *g*.**	**+ 750 µL RLW** **4,000 x *g*, 2 min**
Discard flow through and place NucleoSpin® 96 DNA RapidLyse Binding Plate back onto the Square-well Block.	

3rd wash

Remove the Self-adhering PE Foil and add **750 µL Buffer RLW** to each well of the NucleoSpin® 96 DNA RapidLyse Binding Plate. Seal the plate with a new Self-adhering PE Foil and centrifuge again for **2 min** at **4,000 x *g*.**	**+ 750 µL RLW** **4,000 x *g*, 2 min**
Discard flow through and place NucleoSpin® 96 DNA RapidLyse Binding Plate back onto the Square-well Block (not provided).	

6 Dry silica membrane

Remove the Self-adhering PE Foil and place the NucleoSpin® 96 DNA RapidLyse Binding Plate onto a Square-well Block. Place it in an incubator for **10 min** at **70 °C** to evaporate residual ethanol.	**70 °C, 10 min**
Removal of ethanol by evaporation at 70 °C is more effective than prolonged centrifugation.	**or**
Optional:	
Centrifuge for **5 min** at **4,000 x *g***. *Note: Residual wash buffer is removed in this step.*	**4,000 x *g*, 5 min**

7	**Elute highly pure DNA**	
	Place the NucleoSpin® 96 DNA RapidLyse Binding Plate onto a fresh Square-well Block and dispense **100 µL Buffer RLE** to each well of the NucleoSpin® 96 DNA RapidLyse Binding Plate. Dispense the buffer directly onto the membrane. Incubate at room temperature for **1 min**.	**+ 100 µL RLE**
	Centrifuge for **5 min** at **4000 x *g***.	**1 min, RT**
	If elution in small volume tubes is desired, place a 96 PCR plate (not supplied) on top of a Round-well Block or a Rack of Tube Strips and elute into the PCR plate.	**4,000 x *g*, 5 min**
	Note: DNA yield can be increased by incubation for 4 min at room temperature before centrifugation.	
	For alternative elution procedures see section 2.5.	

Setup of vacuum manifold:

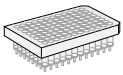
5.2 NucleoSpin® 96 DNA RapidLyse – vacuum processing

- For hardware requirements, refer to section 2.3.
- For storage conditions, refer to section 3.
- For use of recommended accessories, refer to section 2.4.

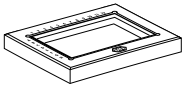
Before starting the preparation:

- Check if Buffer RLW was prepared according to section 3.
- Set incubator or oven to 56 °C.

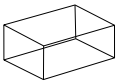
Binding setup



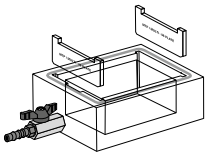
NucleoSpin 96 DNA RapidLyse Binding Plate (green rings)



MN Wash Plate

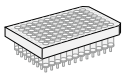


Waste Container

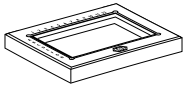


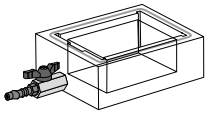
Manifold base with spacers 'MTP/MULTI-96 PLATE' inserted

Drying setup



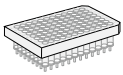
NucleoSpin 96 DNA RapidLyse Binding Plate (green rings)



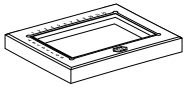


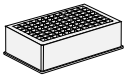
Manifold base without spacers

Elution setup

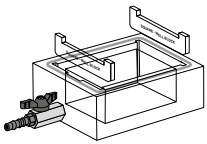


NucleoSpin 96 DNA RapidLyse Binding Plate (green rings)





Square-well Block



Manifold base with spacers 'SQUARE-WELL BLOCK' inserted

1 Lyse sample

Place the samples into the wells of the **Square-well Block** or an appropriate lysis container (e.g. U-bottom plate or 2 mL tube).

Add **150 µL Buffer RLY** to each sample. **+ 150 µL RLY**

Add **10 µL Liquid Proteinase K to each sample.** **+ 10 µL Liquid Proteinase K**

Seal the plate with a Self-adhering Foil and **mix** by vigorous shaking for 10 – 15 s. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the wells. **Mix**

Incubate at **56 °C** on a thermomixer at maximum speed until the sample appears visually lysed.

Note: Lysis time depends on sample material (for mammalian cells reduce incubation to 30 min) and may vary from a couple of minutes up to one hour. **56 °C, max. 1 h**

Do not use conical plates or 1.5 mL tubes as their shape will impair thorough mixing. Use the Square-well Block or 2 mL tubes which will facilitate proper sample and lysis buffer agitation. Make sure that the tissue sample is submerged in the lysis buffer!

Take care not to moisten the rims of the individual wells while dispensing the buffer.

Spin briefly the Square-well Block or lysis container (15 s; 1,500 x *g*) to collect any condensate at the bottom of the wells.

Residual hair and / or bones in the lysate can be removed by centrifugation (2 min; 5,600 – 6,000 x g) and transfer of the supernatant to a new Square-well block (not supplied with the kit).

2 Adjust DNA binding conditions

Add **440 µL Buffer RLB to each sample** and **mix** (e.g., pipetting up and down, pulse vortexing or shaking). Seal the plate with a Self-adhering Foil if necessary. **+ 440 µL RLB** **Mix**

Take care not to moisten the rims of the individual wells while dispensing the buffer. Spin briefly the Square-well Block or lysis container (15 s; 1,500 x *g*) to collect any condensate at the bottom of the wells.

3 Transfer lysates

Transfer the **lysates** resulting from step 3 carefully from the Square-well block into the wells of the **NucleoSpin® 96 DNA RapidLyse Binding Plate**.

Transfer lysates

Take care to dispense the lysate centric onto the silica membrane.

Do not moisten the rims of the individual wells while dispensing the samples – moistened rims may cause cross contamination during centrifugation.

We recommend the use of an electronic eight-channel pipetting device with extra long tips capable of holding approx. 1000 µL, for transfer of the lysate from the Square-well block to the NucleoSpin® 96 DNA RapidLyse Binding Plate.

Seal non-used wells of the NucleoSpin® 96 DNA RapidLyse Binding Plate with a Self-adhering PE-foil

Prepare the NucleoVac 96 Vacuum Manifold:

Place waste tray into vacuum manifold base. Insert spacers labeled “MTP/Multi-96 plate” notched side up and place the MN Wash Plate on them. Close the manifold with the manifold lid and place a NucleoSpin® 96 DNA RapidLyse Binding Plate on top of the manifold.

4 Bind DNA

Incubate the lysates for 1 min on the **NucleoSpin® 96 DNA RapidLyse Binding Plate**.

1 min, RT

Apply vacuum until all lysates have passed through the wells of the NucleoSpin® DNA RapidLyse Binding Plate **(-0.4 bar*; 2 min)**. Release the vacuum.

-0.4 bar*, 2 min

* reduction of atmospheric pressure

5	**Wash silica membrane**	
	1st wash	
	Add **750 µL Buffer RLW** to each well of the NucleoSpin® 96 DNA RapidLyse Binding Plate. Apply vacuum until all buffer has passed through the wells **(-0.4 bar*; 2 min)**. Release the vacuum.	**+ 750 µL RLW** **-0.4 bar*, 2 min**
	2nd wash	
	Add **750 µL Buffer RLW** to each well of the NucleoSpin® 96 DNA RapidLyse Binding Plate. Apply vacuum until all buffer has passed through the wells **(-0.4 bar*; 2 min)**. Release the vacuum.	**+ 750 µL RLW** **-0.4 bar*, 2 min**
	3rd wash	
	Add **750 µL Buffer RLW** to each well of the NucleoSpin® 96 DNA RapidLyse Binding Plate. Apply vacuum until all buffer has passed through the wells **(-0.4 bar*; 2 min)**. Release the vacuum.	**+ 750 µL RLW** **-0.4 bar*, 2 min**
	Remove MN Wash Plate After the final washing step, close the valve, release the vacuum and remove the NucleoSpin® 96 DNA RapidLyse Binding Plate. 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.	
6	**Dry silica membrane**	
	Insert the NucleoSpin® 96 DNA RapidLyse Binding Plate onto the lid, and close the manifold (without waste container). Apply maximum vacuum **(at least -0.6 bar*)** for **10 min** to dry the membrane completely. *Note: Residual wash buffer is removed in this step.* Finally, release the vacuum.	**-0.6 bar*, 10 min**

* reduction of atmospheric pressure

7	**Elute highly pure DNA**	
	Insert spacers “Square-well Block” into the NucleoVac Vacuum Manifold’s short sides. Place a Square-well Block onto the spacer. Close the vacuum manifold and place the NucleoSpin® 96 DNA RapidLyse Binding Plate on top. Dispense **100 µL Buffer RLE** to each well of the NucleoSpin® 96 DNA RapidLyse Binding Plate. Dispense the buffer directly onto the membrane. Incubate at room temperature for **1 min**.	**+ 100 µL RLE** **1 min, RT**
	Apply vacuum for elution **(-0.6 bar*; 5 min)**. Release the vacuum and seal the Square-well block with Self-adhering Foil. Spin the Square-well block briefly to collect all samples at the bottom of the plate.	**-0.6 bar*, 5 min**
	For alternative elution procedures see section 2.5.	

* reduction of atmospheric pressure

5.3 Protocol for challenging samples (e.g., spleen and lung)

- For hardware requirements, refer to section 2.3.
- For storage conditions, refer to section 3.
- For use of recommended accessories, refer to section 2.4.

Before starting the preparation:

- Check if Buffer RLW was prepared according to section 3.
- Set incubator or oven to 56 °C.

The following items are additionally required for this protocol:

- MN Bead Tube Holder, NucleoSpin® Bead Tubes Type F (see ordering information, section 6.2).

1	**Lyse sample**	
	Place the sample into a **NucleoSpin® Bead Tube Type F**.	
	Add **120 µL Buffer RLE**.	**+ 120 µL RLE**
	Add **20 µL Buffer RLB**.	**+ 20 µL RLB**
	Add **10 µL Proteinase K**.	**+ 10 µL Liquid Proteinase K**
	Insert the Bead Tube into the **MN Bead Tube Holder** and **shake 20 min** at **full speed** on a Vortex-Genie® 2. Up to 30 mg of wet weight sample can processed.	**Shake 20 min, full speed**
	Note: The use of other disruption devices is not recommended in conjunction with Bead Tube Type F. Due to the lysing matrix (corundum and steel balls) high impact disruption devices will cause steel abrasion and possible demolition of the bead tubes!	
2	**Adjust DNA binding conditions**	
	Add **420 µL Buffer RLB** and **mix** (e.g., vortex 3 s).	**+ 420 µL RLB**
	Centrifuge the tube at **11,000 x *g*** for approx. **5 s** (short spin), in order to clean the lid and sediment the lysing matrix.	**Mix**
	DO NOT centrifuge for longer times and/or higher g-force, as this might damage the Bead Tubes due to the high density of the steel balls.	**11,000 x *g*, 5 s**

3 Bind DNA

Load supernatant

Load cleared **supernatant** (approximately 500 µL) on the wells of the **NucleoSpin® 96 DNA RapidLyse Binding Plate**.

Note: Do not disturb the lysing matrix. Make sure not to transfer corundum matter from the lysing tube onto the column!

Take care to dispense the lysate centric onto the silica membrane.

Do not moisten the rims of the individual wells while dispensing the samples – moistened rims may cause cross contamination during centrifugation.

We recommend the use of an electronic eight-channel pipetting device with extra long tips capable of holding approx. 1000 µL, for transfer of the lysate from the Square-well block to the NucleoSpin® 96 DNA RapidLyse Binding Plate.

Follow step 4 (see section 5.1) of the vacuum processing protocol, starting with the preparation of the NucleoVac 96 Vacuum Manifold or step 4 (see section 5.2) of the centrifuge processing protocol starting.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield	*Incomplete lysis* • Sample has not completely been submerged during heat incubation. Cut samples into small pieces. Mix well. Be sure that the samples are fully submerged in Buffer RLY / Proteinase K mixture. *Reagents not applied properly* • Prepare Wash Buffer RLW according to instructions (section 3). *Suboptimal elution of DNA from the column* • Preheat Buffer RLE to 70 °C before elution. Apply Buffer RLE directly onto the center of the silica membrane. • Elution efficiencies decrease dramatically if elution is done with buffers with pH < 7. Use slightly alkaline elution buffer like Buffer RLE (pH 8.5). • Perform a second elution step using 100 µL RLE and incubate for 5 min at RT before applying vacuum. *For alternative elution procedures see section 2.5.*
Poor DNA quality	*High A_{260}/A_{280} ratio* • Ratios > 1.9 can be caused by RNA contamination. Usually, such RNA contamination does not interfere with downstream applications. Depending on sample type, amount, and disruption procedure, preparations might contain small amounts of RNA. If it is necessary to reduce RNA contamination to the lowest possible level, incubate the lysate after disruption for 5 min at 70 °C in order to inactivate the Proteinase K. After cooling to room temperature, add 20 µL RNase A (20 mg/mL) and incubate 5 min. Continue with the application of the lysate onto the silica membrane. *Reagents not applied properly* • Prepare Buffer RLW according to instructions (see section 3). *Low A_{260}/A_{230} ratio* • *Increase the volume of Wash Buffer RLW to 900 µL per washing steps to remove residual impurities.*

Problem	Possible cause and suggestions
RNA contamination	*RNA in sample* • If DNA free of RNA is desired, cool down to room temperature after lysis incubation and add 20 µL of an RNase A solution (20 mg/mL; see ordering information 6.2). Incubate for 15 min with moderate shaking.
Poor performance of genomic DNA in enzymatic reactions	*Carry-over of ethanol* • After washing with Buffer RLW, centrifuge ≥ 4 min at 4000 x *g* in order to remove ethanolic Buffer RLW completely and evaporate residual ethanol by incubating the NucleoSpin® 96 DNA RapidLyse Binding Plate at 70 °C for 10 min. • Increase vacuum drying time to 15 min. *Contamination of DNA with inhibitory substances* • Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer RLE.
Clogged wells	*Too much sample material used* • Reduce the sample amount or follow procedure 5.2 for the next preparation. *Hair or bones left in the lysate after step 2* • Centrifuge the Square-well Block for 3 min at *5,600 – 6,000 x g*. Transfer lysates to a new Square-well Block without disturbing the debris pellet. *Incomplete passage of lysate in step 4* • If no more than 300 – 500 µL of lysate is remaining in the columns, continue with step 5. Through the addition of Buffer RLW the sample is diluted and thus the sample will pass the column more easily. *Poor vacuum pressure* • Check the vacuum manifold for a leaking air-flow. Press the NucleoSpin® DNA Binding Plate slightly onto the vacuum manifold lid before applying vacuum.
Grayish lysate or membrane	*Lysis with Bead Tube Type F for 20 min on the MN Bead Tube Holder might cause a slight grayish color of the lysate, which is tolerable. Prolonged shaking or use of other disruption devices can cause steel abrasion.* • Do not perform prolonged incubation, do not use other disruption devices with Bead Tube Type F.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 96 DNA RapidLyse	740110.1 740110.4	1 × 96 preps 4 × 96 preps
Liquid Proteinase K	740396	5 mL
RNase A	740505 740505.50	50 mg 100 mg
Square-well Block	740481 740481.24	4 24
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
Round-well Block with Cap Strips (1 set consists of 1 Round-well Block and 12 Cap Strips)	740475 740475.24	4 sets 24 sets
MN Wash Plate	740479 740479.24	4 24
Cap Strips	740478 740478.24	48 288
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Self-adhering PE Foil	740676	50
Rubber Pad	740640	2
MN Bead Tube Holder	740469	1 piece
NucleoSpin® Bead Tubes Type F (1 – 3 mm corundum and 3 mm steel balls, recommended for challenging samples in combination with NucleoSpin® DNA RapidLyse – use only with MN Bead Tube Holder)	740816.50	50 pieces

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 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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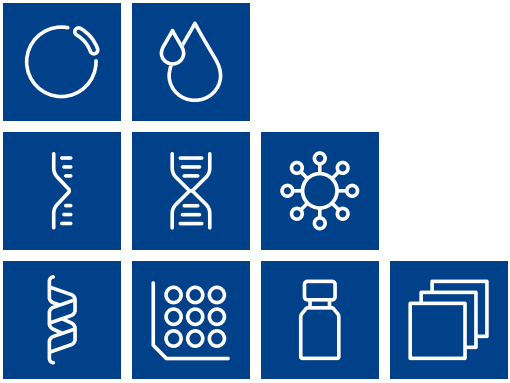

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