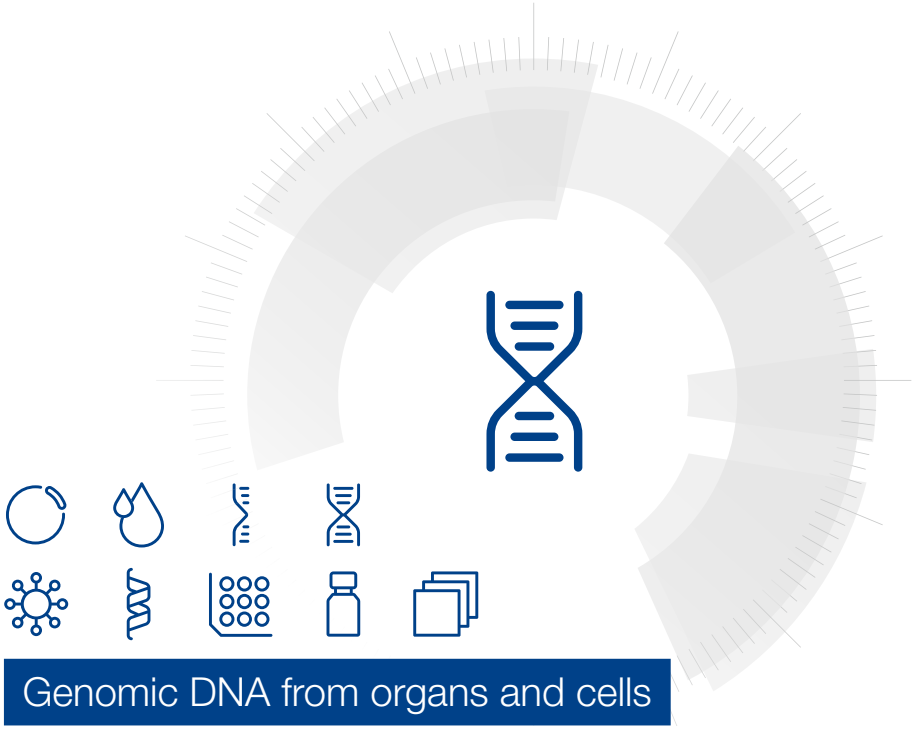
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA from organs and cells

■ NucleoSpin® DNA RapidLyse

May 2022 / Rev. 05

Genomic DNA from organs and cells

Protocol at a glance (Rev. 05)

		NucleoSpin® DNA RapidLyse	
1 Lyse sample		Up to 40 mg wet weight sample or 1 x 10^6 cells in a 2 mL tube 150 µL RLY 10 µL Liquid Proteinase K 56 °C, 1 h, thermomixer at maximum speed	
2 Adjust DNA binding conditions		440 µL RLB Vortex **5 s**	
3 Bind DNA	 	Load 640 µL lysate on a NucleoSpin® DNA RapidLyse Column 11,000 x *g*, **1 min**	
4 Wash silica membrane	 	**1st**	500 µL RLW — 11,000 x *g*, **1 min**
		2nd	500 µL RLW — 11,000 x *g*, **1 min**
5 Dry silica membrane	 	11,000 x *g*, **1 min**	
6 Elute DNA	 	100 µL RLE 11,000 x *g*, **1 min**	

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1 Components

1.1 Kit contents

	NucleoSpin® DNA RapidLyse		
REF	**10 preps 740100.10**	**50 preps 740100.50**	**250 preps 740100.250**
Lysis Buffer RLY	13 mL	13 mL	60 mL
Binding Buffer RLB	25 mL	25 mL	125 mL
Wash Buffer RLW (Concentrate)*	6 mL	12 mL	3 x 25 mL
Elution Buffer RLE**	13 mL	13 mL	30 mL
Liquid Proteinase K	120 µL	600 µL	2 x 1.5 mL
NucleoSpin® DNA RapidLyse Columns (light green rings)	10	50	250
Collection Tubes (2 mL)	20	100	500
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* For preparation of working solutions and storage, see section 3.

**Composition of Elution Buffer RLE: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol (for preparation of Wash Buffer RLW)

Consumables

- 2 mL microcentrifuge tubes for sample lysis
- 1.5 mL microcentrifuge tubes for DNA elution
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer (e.g., Vortex-Genie® 2 from Scientific Industries)
- Thermomixer (e.g., ThermoMixer® C from Eppendorf for 2 mL tubes)
- Personal protection equipment (lab coat, gloves, goggles)
- For challenging samples (protocol 5.2): MN Bead Tube Holder and Bead Tubes Type F

1.3 About this user manual

It is strongly recommeded for first time users to read the detailed protocol sections of the **NucleoSpin® 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.

2 Product description

2.1 The basic principle

The **NucleoSpin® DNA RapidLyse** kit is designed for fast and efficient isolation of 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® 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. An incubation over night or for several hours is not necessary.

2.2 Kit specifications

Kit specifications at a glance

Parameter	NucleoSpin® DNA RapidLyse
Technology	Silica membrane technology
Format	Mini spin column
Sample material	Fresh, frozen, dried, and ethanol preserved tissue samples (e.g., organs), eukaryotic cells
Sample amount	Up to 40 mg fresh weight (sample dependent)
Typical yield	1 – 30 µg (depending on sample source)
A_{260}/A_{280}	1.7 – 1.9
Elution volume	60 – 100 µL
Preparation time	25 min (6 preps, excluding lysis)
Lysis time	Maximal 1 h
Binding capacity	60 µg

2.3 Handling, preparation, and storage of starting materials

Fresh, frozen, and ethanol preserved samples can be used. Make sure not to use more than 40 mg sample.

2.4 Lysis of sample material

In order to obtain optimal DNA yields and a smooth processing, sample material should be thoroughly lysed.

Lysis time depends upon sample material and may vary from a couple of minutes to one hour.

Sample material	Lysis time (optimal)	DNA yield (typical)	Specification
Cells	15 min	5 µg	10^6 Hela cells
Bacteria (Gram-negative)	15 min	9 – 10 µg	30 mg *Pseudomonas fluorescens* (wet weight)
Bacteria (Gram-positive)	60 min	5 µg	30 – 40 mg *Corynebacterium glutamicum* (wet weight)
Blood	30 min	1 µg	200 µl EDTA whole blood
Organs (kidney)	60 min	30 µg	10 mg mouse kidney

Table 1 Optimal lysis time and typical yield for different samples types.
Genomic DNA was isolated with the NucleoSpin ® DNA RapidLyse kit from the following: 10^6 Hela cells; 30 mg Gram-negative bacteria *Pseudomonas fluorescens*; 30 – 40 mg Gram-positive bacteria *Corynebacterium glutamicum*, and 200 µl whole blood treated with EDTA. DNA was measured via OD after extraction according to the protocol for fresh, frozen and ethanol-preserved samples. *Note: For 200 µl blood samples 2 x binding buffer RLB was used.*

Most samples can be processed according to procedure 5.1. However, some sample materials (e.g., spleen or lung) need to be processed according to procedure 5.2 which requires additional material (see section 5.2 and 6.2).

2.5 Elution procedures

In addition to the standard method, several modifications are possible to increase yield, concentration, and convenience.

- **Convenient elution (standard elution):** Elution can be performed by a single addition of 100 µL Elution Buffer onto the column.
- **High yield:** Elution can be performed in two serial elutions of 100 µL each, resulting in a total volume of 200 µL.
- **High concentration:** Elution can be performed by application of 100 µL Elution Buffer, which is then re-used in a second elution step, resulting in 100 µL eluate with a high DNA concentration. Alternatively, the elution volume can be reduced down to 60 µL.

3 Storage conditions and preparation of working solutions

Attention:

Binding Buffer RLB contains chaotropic salts! Wear gloves and goggles!

CAUTION: Buffer RLB contains chaotropic salt 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.

- All kit components can be stored at 15 – 25 °C and are stable until: see package label.

Prior to the **NucleoSpin® 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 15 – 25 °C for at least one year.
- **Liquid Proteinase K** is ready to use. After first time use, store **Liquid Proteinase K** at 4 °C or -20 °C.

	NucleoSpin® DNA RapidLyse		
REF	**10 preps 740100.10**	**50 preps 740100.50**	**250 preps 740100.250**
Wash Buffer RLW (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	3 x 25 mL Add 100 mL ethanol to each bottle

4 Safety instructions

When working with the NucleoSpin® 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 ***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® 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 Protocol for fresh, frozen, and ethanol-preserved samples

Before starting the preparations:

- Check if Buffer RLW was prepared according to section 3.

1 Lyse sample

+ 150 µL RLY

+ 10 µL Liquid Proteinase K

Place the sample into a 2 mL tube.

Note: Do not use 1.5 mL conical tubes. The shape of the tube will impair thorough mixing. Use common 2 mL tubes which will facilitate proper sample and lysis buffer agitation.

Add 150 µL Buffer RLY.

Note: While mechanical homogenization of the sample is unnecessary in most cases, for some materials (e.g. fibrous tissue) a homogenization step in Buffer RLY prior to lysis may be beneficial for obtaining an optimal yield and quality.

Add 10 µL Liquid Proteinase K.

Incubate at 56 °C on a heated shaking device (e.g. thermomixer) at maximum speed for a maximum time of 1 hour, or until the sample appears visually lysed (e.g. mostly cleared of particulates).

Note: Incubation times longer than 1 h can increase degree of lysis, but might impair DNA quality (sample dependent).

Note: If the sample is incubated in a heated water bath or heating block without agitation, vortex the sample frequently to ensure optimal lysis conditions.

Make sure that the tissue sample is submerged in the lysis buffer during incubation!

Centrifuge the tube at 11,000 x *g* for approx. 5 s (short spin), in order to clean the lid.

Note: If unlysed sample material remains after lysis, an additional centrifugation step is recommended to recover a cleared lysate. In this case, centrifuge 30 s at 14,000 x g.

2 Adjust DNA binding conditions

Add **440 µL Buffer RLB** and **mix** (e.g., vortex 3 s).

+ 440 µL RLB

Mix

3 Bind DNA

Apply the mixture (ca. 640 µL) onto the **NucleoSpin® DNA RapidLyse Column** placed into a 2 mL Collection Tube (provided).

Centrifuge for **1 min** at **11,000 x *g***.

Discard Collection Tube with flow through. Put column into a fresh 2 mL Collection Tube (provided).

Load samples

11,000 x *g*, 1 min

4 Wash silica membrane

1st wash

Add **500 µL Buffer RLW**.

Centrifuge for **1 min** at **11,000 x *g***.

Discard flowthrough and place column back into the Collection Tube.

+ 500 µL RLW

11,000 x *g*, 1 min

2nd wash

Add **500 µL Buffer RLW**.

Centrifuge for **1 min** at **11,000 x *g***.

Discard flowthrough and place column back into the Collection Tube.

+ 500 µL RLW

11,000 x *g*, 1 min

5 Dry silica membrane

Centrifuge for **1 min** at **11,000 x *g***.

<u>Note:</u> Residual wash buffer is removed in this step.

11,000 x *g*, 1 min

6 Elute highly pure DNA

+ 100 µL RLE

Place the NucleoSpin® DNA RapidLyse Column into a 1.5 mL nuclease-free tube (not provided) and add **100 µL Buffer RLE** onto the column.

11,000 x *g*, 1 min

Centrifuge for **1 min** at **11,000 x *g***.

Note: DNA yield can be increased by an incubation for 4 min at room temperature before centrifugation.

For alternative elution procedures see section 2.5.

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

Before starting the preparations:

- The following items are additionally required for this protocol: MN Bead Tube Holder, Bead Tubes Type F (see ordering information).
- Check if Buffer RLW was prepared according to section 3.

1 Lyse sample

Place the sample into a **Bead Tube Type F**.

Add **100 µL Buffer RLE**.

Add **40 µL Buffer RLB**.

Add **10 µL 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.

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 beads) high impact disruption devices will cause steel abrasion and possible demolition of the bead tubes!

+ 100 µL RLE

+ 40 µL RLB

+ 10 µL Liquid Proteinase K

Shake 20 min, full speed

2 Adjust DNA binding conditions

Add **420 µL Buffer RLB** and **mix** (e.g., vortex 3 s).

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.

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 beads.

+ 420 µL RLB

Mix

11,000 x *g*, 5 s

3 Bind DNA

Apply cleared supernatant (approximately 500 µL) onto the **NucleoSpin® DNA RapidLyse Column** placed into a 2 mL Collection Tube (provided).

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

Centrifuge for **1 min** at **11,000 x *g***.

Discard Collection Tube with flow through. Put column into a fresh 2 mL Collection Tube (provided).

Load samples

11,000 x *g*, 1 min

4 Wash silica membrane

1st wash

Add **500 µL Buffer RLW**.

Centrifuge for **1 min** at **11,000 x *g***.

Discard flowthrough and place column back into the Collection Tube.

+ 500 µL RLW

11,000 x *g*, 1 min

2nd wash

Add **500 µL Buffer RLW**.

Centrifuge for **1 min** at **11,000 x g**.

Discard flowthrough and place column back into the Collection Tube.

+ 500 µL RLW

11,000 x *g*, 1 min

5 Dry silica membrane

Centrifuge for **1 min** at **11,000 x *g***.

Note: Residual wash buffer is removed in this step.

11,000 x *g*, 1 min

6 Elute highly pure DNA

Place the NucleoSpin® DNA RapidLyse Column into a 1.5 mL nuclease-free tube (not provided) and add **100 µL Buffer RLE** onto the column.

Centrifuge for **1 min** at **11,000 x *g***.

For alternative elution procedures see section 2.5.

+ 100 µL RLE

11,000 x *g*, 1 min

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
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.
Clogged column	*Too much sample material used* • Reduce the sample amount or follow procedure 5.2 for the next preparation. • Increase centrifugation time.
No or poor DNA yield	*Reagents not applied properly* • Prepare Buffer RLW according to the instructions (section 3). *Suboptimal elution of DNA from the column* • For certain sample types, 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 at a pH < 7.0. Use slightly alkaline elution buffers like Buffer RLE (pH 8.5). • Especially when expecting high yields from large amounts of material, we recommend elution with 200 µL RLE and incubation of the closed columns in an incubator at 70 °C for 5 min before centrifugation.

Problem	Possible cause and suggestions
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 column. *Reagents not applied properly* • Prepare Buffer RLW according to instructions (see section 3). *Carry-over of impurities* • Residual liquid can be removed from the lid at any step of the protocol by an additional brief centrifugation step (approx. 1 s at 2,000 x *g*).
Suboptimal performance of gDNA in enzymatic reactions	*Carry-over of ethanol or salt* • Make sure to centrifuge ≥ 1 min at 11,000 x *g* in order to remove all of ethanolic Buffer RLW before eluting the DNA. • If, for any reason, the level of Buffer RLW has reached the column outlet after drying, repeat the centrifugation. *Contamination of DNA with inhibitory substances* • Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Re-purify DNA and elute in Buffer BE.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® DNA RapidLyse	740100.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® DNA Insect	740470.10 / .50	10 / 50 preps
NucleoSpin® Soil	740780.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® DNA Stool	740472.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® DNA Lipid Tissue	740471.10 / .50	10 / 50 preps

Product	REF	Pack of
NucleoSpin® Microbial DNA	740235.10 / .50	10 / 50 preps
MN Bead Tube Holder	740469	1 piece
NucleoSpin® Bead Tubes Type A (0.6 – 0.8 mm ceramic beads, recommended for soil and sediments)	740786.50	50 pieces
NucleoSpin® Bead Tubes Type B (40 – 400 µm glass beads, recommended for bacteria)	740812.50	50 pieces
NucleoSpin® Bead Tubes Type C (1 – 3 mm corundum, recommended for yeast)	740813.50	50 pieces
NucleoSpin® Bead Tubes Type D (3 mm steel beads, recommended for insects)	740814.50	50 pieces
NucleoSpin® Bead Tubes Type E (40 – 400 µm glass beads and 3 mm steel beads, recommended for hard-to-lyse bacteria within insect samples)	740815.50	50 pieces
NucleoSpin® Bead Tubes Type F (1 – 3 mm corundum and 3 mm steel beads, recommended for challenging samples in conjunction with NucleoSpin® DNA RapidLyse – use only with MN Bead Tube Holder)	740816.50	50 pieces
NucleoSpin® Bead Tubes Type G (5 mm steel beads, recommended for plant material)	740817.50	50 pieces
Liquid Proteinase K	740396	5 mL
RNase A	740505 740505.50	50 mg 100 mg
Collection Tubes (2 mL)	740600	1000

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

6.3 Product use restriction / warranty

NucleoSpin® DNA RapidLyse 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.

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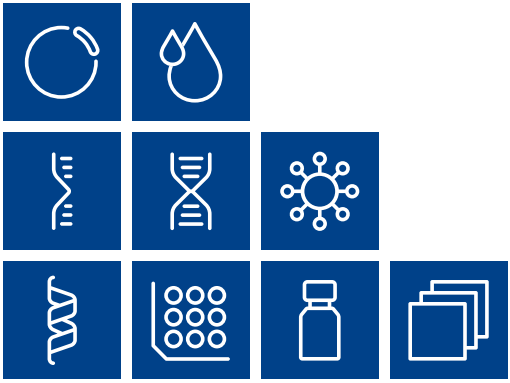

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