Genomic DNA from organs and cells

User manual

NucleoSpin® DNA RapidLyse

February 2017 / Rev. 01

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Table of contents

1 Components 4
- 1.1 Kit contents 4
- 1.2 Reagents, consumables, and equipment to be supplied by user 5
- 1.3 About this user manual 5

2 Product description 6
- 2.1 The basic principle 6
- 2.2 Kit specifications 6
- 2.3 Handling, preparation, and storage of starting materials 6
- 2.4 Lysis of sample material 6
- 2.5 Elution procedures 7

3 Storage conditions and preparation of working solutions 8

4 Safety instructions 9

5 Protocols 11
- 5.1 Protocol for fresh, frozen, and ethanol-preserved samples 11
- 5.2 Protocol for challenging samples (e.g., spleen and lung) 13

6 Appendix 15
- 6.1 Troubleshooting 15
- 6.2 Ordering information 16
- 6.3 Product use restriction / warranty 18

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
User manual	1	1	1

* 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	Up to 4 µg DNA per mg tissue (sample dependent)
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. 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 room temperature (18–25 °C) and are stable for at least one year.

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 room temperature (18–25 °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

The following components of the **NucleoSpin® DNA RapidLyse** 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*
RLB	Guanidinium thiocyanate 30–60 % and ethanol 5–20 % *Guanidinhydrochlorid 30–60 % und Ethanol 5–20 %* CAS 593-84-0, 64-17-5	WARNING *ACHTUNG*	H226, H302, H412, EUH031	P210, P233, P260, P273, P301+312, P330, P370+378, P403+235
Proteinase K	Proteinase K, liquid 1–3 % *Proteinase K flüssig 1–3 %* CAS 39450-01-6	WARNING *ACHTUNG*	317	261, 272, 280, 302+352, 333+313, 363

Hazard phrases

H226 Flammable liquid and vapour.
Flüssigkeit und Dampf entzündbar.

H302 Harmful if swallowed.
Gesundheitsschädlich bei Verschlucken.

H317 May cause an allergic skin reaction.
Kann allergische Hautreaktionen verursachen.

H412 Harmful to aquatic life with long lasting effects.
Schädlich für Wasserorganismen, mit langfristiger Wirkung.

EUH031 Contact with acids liberates toxic gas.
Entwickelt bei Berührung mit Säure giftige Gase.

Precaution phrases

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
Von Hitze, heissen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.

P233 Keep container tightly closed.
Behälter dicht verschlossen halten.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.
Staub/Rauch/Gas/Nebel/Dampf/Aerosol nicht einatmen.

P261	Avoid breathing dust/fume/gas/mist/vapours/spray. *Einatmen von Staub/Rauch/Gas/Nebel/Dampf/Aerosol vermeiden.*
P272	Contaminated work clothing should not be allowed out of the workplace. *Kontaminierte Arbeitskleidung nicht außerhalb des Arbeitsplatzes tragen.*
P273	Avoid release to the environment. *Freisetzung in die Umwelt vermeiden.*
P280	Wear protective gloves/protective clothing/eye protection/face protection. *Schutzhandschuhe/Schutzkleidung/Augenschutz/Gesichtsschutz tragen.*
P301+312	IF SWALLOWED: Call a POISON CENTER/ doctor/…/ if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt/… anrufen.*
P302+352	IF ON SKIN: Wash with plenty of water/… *BEI BERÜHRUNG MIT DER HAUT: Mit viel Wasser/… waschen.*
P330	Rinse mouth. *Mund ausspülen.*
P333+313	If skin irritation or rash occurs: Get medical advice/attention. *Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.*
P363	Wash contaminated clothing before reuse. *Kontaminierte Kleidung vor erneutem Tragen waschen.*
P370+378	In case of fire: Use … to extinguish. *Bei Brand: … zum Löschen verwenden.*
P403+235	Store in a well-ventilated place. Keep cool. *An einem gut belüfteten Ort aufbewahren. Kühl halten.*

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 further safety information in this section.
Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

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.

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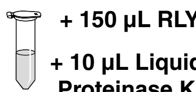
1 Lyse sample

Place the sample into a 2 mL tube.

Add **150 µL Buffer RLY**.

Add **10 µL Liquid Proteinase K**.



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.

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

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2 Adjust DNA binding conditions

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

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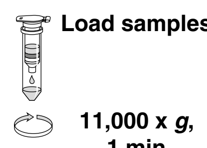
3 Bind DNA

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).

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4 Wash silica membrane

1st wash

Add **500 µL Buffer RLW**.

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

11,000 x *g*, 1 min

Discard flow-through and place column back into the Collection Tube.

2nd wash

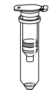
Add **500 µL Buffer RLW**.

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

11,000 x *g*, 1 min

Discard flow-through and place column back into the Collection Tube.

5 Dry silica membrane

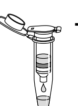


Centrifuge for **1 min** at **11,000 x *g***.

Note: Residual wash buffer is removed in this step.

11,000 x *g*, 1 min

6 Elute highly pure DNA



+ 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.

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

11,000 x *g*, 1 min

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.

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1 Lyse sample

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

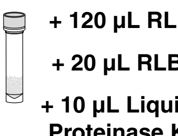
Add **120 µL Buffer RLE**.

Add **20 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 balls) high impact disruption devices will cause steel abrasion and possible demolition of the bead tubes!



Shake 20 min, full speed

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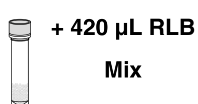
2 Adjust DNA binding conditions

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 balls.



11,000 x *g*, 5 s

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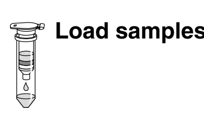
3 Bind DNA

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).



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 flow-through and place column back into the Collection Tube.

11,000 x *g*, 1 min

2nd wash

Add **500 µL Buffer RLW**.

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

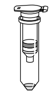
Discard flow-through and place column back into the Collection Tube.

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.



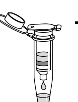
11,000 x *g*, 1 min

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 balls, recommended for insects)	740814.50	50 pieces
NucleoSpin® Bead Tubes Type E (40–400 µm glass beads and 3 mm steel balls, 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 balls, recommended for challenging samples in conjunction with NucleoSpin® DNA RapidLyse – use only with MN Bead Tube Holder)	740816.50	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.

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.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for IN VITRO-USES ONLY!

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.

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Last updated: 07/2010, Rev. 03

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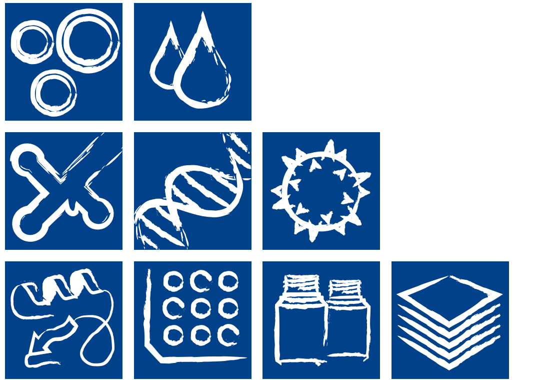


Plasmid DNA

Clean-up

RNA

Genomic DNA

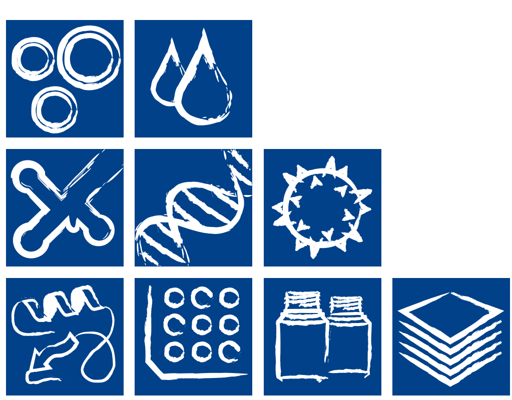
Viral RNA and DNA

Protein

High throughput

Accessories

Auxiliary tools



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