

Genomic DNA from organs and cells

User manual

NucleoSpin® DNA RapidLyse

This product distributed by Takara Bio USA, Inc.

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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
User manual	1	1	1

 $^{^{\}ast}$ For preparation of working solutions and storage, see section 3.

^{**}Composition of Elution Buffer RLE: 5 mM Tris/HCI, 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 μ g 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	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Inhalt	Gefahrstoff	GHS-Symbol	H-Sätze	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		317	261, 272, 280, 302+352, 333+313, 363
	CAS 39450-01-6	WARNING ACHTUNG		333+313, 36

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.

Protocols 5

Protocol for fresh, frozen, and ethanol-preserved 5.1 samples

Before starting the preparations:

Check if Buffer RLW was prepared according to section 3.

1 Lyse sample

3

Bind DNA

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.

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!

2 Adjust DNA binding conditions

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



+ 150 µL RLY

+ 10 µL Liquid

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



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 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.

<u>Note:</u> 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).

Centrifuge the tube at $11,000 \times g$ 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.



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

<u>Note:</u> 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).



+ 120 µL RLE

+ 20 µL RLB

+ 10 µL Liquid

Proteinase K

Shake 20 min,

full speed





4	Wash silica membrane		
	1 st wash		+ 500 μL RLW
	Add 500 µL Buffer RLW.		
	Centrifuge for 1 min at 11,000 x g.	Ò	11,000 x <i>g</i> ,
	Discard flow-through and place column back into the Collection Tube.		1 min
	2 nd wash		+ 500 µL RLW
	Add 500 µL Buffer RLW.		
	Centrifuge for 1 min at 11,000 x g.	Ò	11,000 x <i>g</i> ,
	Discard flow-through and place column back into the Collection Tube.		1 min
5	Dry silica membrane		
	Centrifuge for 1 min at 11,000 x <i>g</i> .	Ĩ	
	Note: Residual wash buffer is removed in this step.	Ē	
		Ö	11,000 x <i>g</i> , 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.		+ 100 μL RLE
	Centrifuge for 1 min at 11,000 x g.	Ċ	11,000 x <i>g</i> ,
	For alternative elution procedures see section 2.5.		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.			
	Too much sample material used			
Clogged column	• Reduce the sample amount or follow procedure 5.2 for the next preparation.			
	Increase centrifugation time.			
	Reagents not applied properly			
	• Prepare Buffer RLW according to the instructions (section 3).			
	Suboptimal elution of DNA from the column			
No or poor DNA	 For certain sample types, preheat Buffer RLE to 70 °C before elution. Apply Buffer RLE directly onto the center of the silica membrane. 			
yield	 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			
	High A ₂₆₀ /A ₂₈₀ ratio			
Poor DNA quality	 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). 			
Ca	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). 			
	Carry-over of ethanol or salt			
Suboptimal	 Make sure to centrifuge ≥ 1 min at 11,000 x g in order to remove all of ethanolic Buffer RLW before eluting the DNA. 			
performance of gDNA in enzymatic	 If, for any reason, the level of Buffer RLW has reached the column outlet after drying, repeat the centrifugation. 			
reactions	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.

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