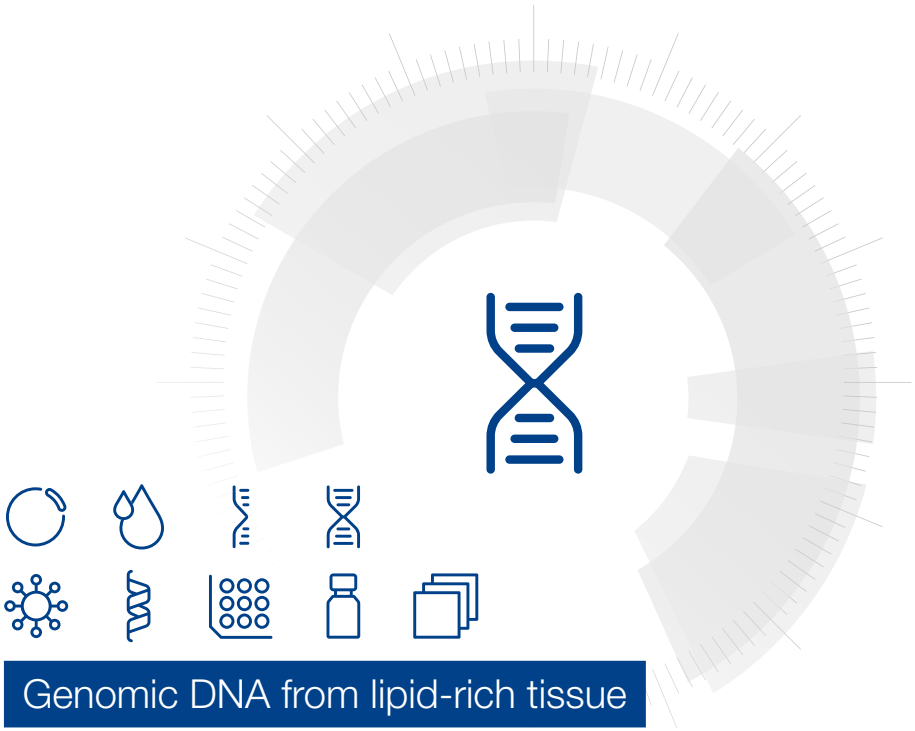
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA from lipid-rich tissue

■ NucleoSpin® DNA Lipid Tissue

Januar 2023 / Rev. 02

Genomic DNA from lipid-rich tissue

Protocol at a glance (Rev. 02)

	NucleoSpin® DNA Lipid Tissue	
1 Prepare sample		< 40 mg lipid tissue (wet weight) in MN Bead Tube Type D 100 µL Buffer BE
2 Lyse sample	 	40 µL Buffer LT 10 µL Liquid Proteinase K Agitate on MN Bead Tube Holder for approx. 20 min 11,000 x *g*, 30 s
3 Adjust binding conditions	 	600 µL LT Mix 11,000 x *g*, 30 s
4 Bind DNA	 	Load samples 11,000 x *g*, 30 s
5 Wash silica membrane	 	**1st** 500 µL BW 11,000 x *g*, 30 s **2nd** 500 µL B5 11,000 x *g*, 30 s
6 Dry silica membrane	 	11,000 x *g*, 30 s
7 Elute DNA	 	100 µL BE RT, 1 min 11,000 x *g*, 30 s

MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany
Tel.: +49 24 21 969-333 · support@mn-net.com · *www.mn-net.com*

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1 Components

1.1 Kit contents

	NucleoSpin® DNA Lipid Tissue	
REF	**10 preps 740471.10**	**50 preps 740471.50**
Lysis Buffer LT	10 mL	38 mL
Wash Buffer BW	6 mL	30 mL
Wash Buffer B5 (Concentrate)*	6 mL	6 mL
Elution Buffer BE**	13 mL	30 mL
Liquid Proteinase K	120 µL	600 µL
MN Bead Tubes Type D	10	50
NucleoSpin® DNA Lipid Tissue Columns (light green rings)	10	50
Collection Tubes (2 mL)	20	100
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* For preparation of working solutions and storage, see section 3

** Composition of Elution Buffer BE: 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 B5)

Consumables

- Disposable tips
- 1.5 mL or 2 mL microcentrifuge tubes for elution

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Personal protection equipment (lab coat, gloves, goggles)
- Sample disruption device:
  The MN Bead Tube Holder (REF 740469, see ordering information, section 6.2) is recommended to be used in combination with the Vortex-Genie® 2 for cost efficient and convenient disruption of lipid tissue samples. The Vortex Adapter (MoBio) for Vortex-Genie® 2 X is also suitable.

Alternatively, a swing mill can be used considering precautions of section 2.4.3 (e.g., mixer mill MM200, MM300, MM400 (Retsch®).

WARNING: *The use of other disruption devices like FastPrep® System (MP-Biomedicals), Precellys® (Bertin Technologies), MagNA™ Lyser (Roche), TissueLyser (QIAGEN), Bullet Blender® (Next Advance), Mini-Beadbeater™ (Biospec Products), Speed Mill (Analytik Jena), or similar devices might cause bead tube destruction. Such disruption devices can cause high mechanical stress on the bead tubes. Depending on bead tube type and content (beads like steel balls, liquid volume, sample type), especially high frequency of shaking and / or long shaking duration can cause destruction of the bead tubes.* ***If using such a disruption device, it is the responsibility of the user to perform initial stability tests to ensure stability of bead tubes during the individual experimental setup (e.g., intensity of agitation).*** *See also section 2.4.3!*

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoSpin® DNA Lipid Tissue** 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 com-pared with previous revisions.

2 Product description

2.1 The basic principle

The **NucleoSpin® DNA Lipid Tissue** kit is designed for efficient isolation of genomic DNA from lipid-rich samples of human / animal origin. DNA can be isolated from a wide variety of fresh or frozen samples, such as brain, adipose tissue, and other tissue types that are rich in lipids, like fatty fish tissue.

Lipid-rich tissue can cause difficulties in DNA isolation due to lipids interfering with tissue disruption or by influencing the chemistry of the DNA isolation buffers.

The **NucleoSpin® DNA Lipid Tissue** kit combines enzymatic lysis and mechanical disruption of lipid-rich tissues with the MN Bead Tubes. The MN Bead Tubes can be used in combination with the MN Bead Tube Holder (REF 740469) and the Vortex-Genie® 2. They are also compatible with other disruption devices (see section 1.2 and section 2.4.1). High DNA yields can be obtained with the MN Bead Tubes from a large variety of sample types – enabling the procedure to be convenient, fast, and easy. Alternative bead types can be ordered separately for selected sample types (see section 2.4.3 for recommendations).

2.2 Kit specifications

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® DNA Lipid Tissue
Technology	Silica-membrane technology
Format	Mini spin column
Sample material	Fresh or frozen, lipid-rich tissues (e.g., brain, adipose tissue, fatty fish tissue)
Sample amount	Up to approx. 40 mg (wet weight)
Typical yield	Varies by sample and disruption device. Up to 25 µg DNA can be obtained.
A_{260} / A_{280}	1.7 – 1.9
Elution volume	25 – 200 µL
Preparation time	35 min/6 preps
Binding capacity	60 µg

2.3 Handling, preparation, and storage of starting materials

Fresh or frozen tissue samples. Make sure not to use more than 40 mg starting material.

2.4 Lysis and disruption of sample material

In order to obtain optimal DNA yields, a complete disruption of the sample material is essential. The efficiency of sample disruption depends on the parameters listed below and suggestions for optimization are outlined in the subsequent sections.

2.4.1 Disruption device

The following devices are compatible with MN Bead Tubes. Please check whether MN Bead Tubes can be accommodated by the available tube adapters prior to starting the procedure.

- MN Bead Tube Holder in combination with the Vortex-Genie® 2 (recommended).
- Mixer mill MM200, MM300, MM400 (Retsch®) (suitable).

If other disruption devices (section 1.2) are intended to be used, consider section 2.4.2 and WARNING note in section 2.4.3!

2.4.2 Type of bead tube

Bead type, disruption time, and frequency / speed must be optimized for a given sample to obtain maximal DNA yield and quality.

Type of bead tube

- MN Bead Tubes Type D (3 mm steel balls)
  Recommended for lipid–rich tissue samples (included in NucleoSpin® DNA Lipid Tissue kit).

Other types of bead tubes are available for other applications:

- MN Bead Tubes Type A (0.6 – 0.8 mm ceramic beads)
  Recommended for soil and sediment (included in NucleoSpin® Soil, see ordering information, section 6.2).
- MN Bead Tubes Type B (40 – 400 µm glass beads)
  Recommended for gram-positive and -negative bacteria (included in NucleoSpin® Microbial DNA, see ordering information, section 6.2).
- MN Bead Tubes Type C (1 – 3 mm corundum)
  Recommended for yeast (see ordering information, section 6.2).
- MN Bead Tubes Type E (combination of 3 mm steel balls and 40 – 400 µm glass beads)
  Recommended for difficult-to-lyse tissue containing gram-positive bacteria (see ordering information, section 6.2).

2.4.3 Time and frequency of disruption

The following recommendations have been established for the MN Bead Tube Holder in combination with a Vortex-Genie® 2 or a Retsch® Swingmill MM300 operating at highest frequency (30 Hertz). For using other disruption devices, and other sample materials, time and frequency have to be optimized.

Sample material	Disruption device	Disruption time	Speed / intensity / frequency
Fresh or frozen, lipid-rich tissues (e.g., brain, adipose tissue, fatty fish tissue)	MN Bead Tube Holder in conjunction with a Vortex-Genie® 2	approximately 20 min	full speed
Fresh or frozen, lipid-rich tissues (e.g., brain, adipose tissue, fatty fish tissue)	Mixer mill (Retsch®)	approximately 0.5 – 5 min	30 Hz
Fresh or frozen, lipid-rich tissues (e.g., brain, adipose tissue, fatty fish tissue)	other device	to be optimized by user	see recommendations below

Note: Stability testing has been performed on the MN Bead Tubes Type D with the MN Bead Tube Holder on a Vortex-Genie® 2 and with a mixer mill MM300 (Retsch®) at highest frequency (30 Hertz). MN Bead Tubes Type D withstand shaking for several hours in the MN Bead Tube Holder on a Vortex-Genie® 2 and for up to 30 minutes on a mixer mill MM300 (Retsch®) at highest frequency (30 Hertz). For optimal sample processing, avoidance of DNA fragmentation, and highest DNA yield, see table above for recommendations of adequate disruption times. Other disruption devices (see section 2.4.1) will require different settings regarding frequency and duration for optimal performance with the selected sample material. Please note that the position of the tube within the machine (mixer mill, Retsch®) is important for optimal performance! Please refer to the user manual of the disruption device.

WARNING: *Many disruption devices (see section 1.2) can cause high mechanical stress on the bead tubes. Depending on bead tube type and content (beads, liquid volume, sample type), especially high frequency of shaking and / or long shaking duration can cause breaking-up of the bead tubes.* ***It is the responsibility of the user to perform initial stability tests to ensure stability of bead tubes during the individual experimental setup!***

These tests should be performed with water instead of lysis buffer in order to avoid spillage of chaotropic lysis buffer in case of tube breakage. Integrity and tightness of the tube need to be controlled after every run.

WARNING: *In section 5 a certain liquid volume during disruption is recommended. The reduction of liquid content will severely increase the mechanical impact by the steel balls, and can result in damage of DNA and tube.*

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 elution steps 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 high DNA concentration. Alternatively, the elution volume can be reduced down to 25 µL.

3 Storage conditions and preparation of working solutions

Attention:

Lysis Buffer LT and Wash Buffer BW contain chaotropic salt! Wear gloves and goggles!

CAUTION: Buffers LT and BW contain 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 waster!

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

Before starting any **NucleoSpin® DNA Lipid Tissue** protocol, prepare the following:

- **Wash Buffer B5:** Add the indicated volume of ethanol (96 – 100 %) to **Wash Buffer B5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer B5 can be stored at room temperature 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 Lipid Tissue	
REF	**10 preps** **740471.10**	**50 preps** **740471.50**
Wash Buffer B5 (Concentrate)	6 mL Add 24 mL ethanol	6 mL Add 24 mL ethanol

4 Safety instructions

When working with the **NucleoSpin® DNA Lipid Tissue** 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: Guanidine hydrochloride in buffer BW, and guanidinium thiocyanate in buffer LT 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 Lipid Tissue** 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 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 or frozen lipid-rich tissue samples

Before starting the preparation:

- Check if Buffer B5 was prepared according to section 3.
- Check section 2.4 for lysis and disruption of sample material.

1 Prepare sample

Place the lipid-rich tissue sample in a **MN Bead Tube Type D** (provided).

Up to approximately 40 mg of wet weight tissue sample can be processed.

Add **100 µL Elution Buffer BE** to the sample.

Alternatively, molecular biology-grade water (not provided) can be used.

+100 µL BE

2 Lyse sample

Add **40 µL Buffer LT**. Then, add **10 µL Liquid Proteinase K** and close the tube.

+40 µL LT

+10 µL Liquid Proteinase K

Agitate the MN Bead Tube in the MN Bead Tube Holder on a Vortex-Genie® 2. Alternatively a swing mill (Retsch®) can be used (see section 2.4.3).

Note: Optimal agitation duration, speed / frequency depends on the device used. For the MN Bead Tube Holder it is approximately 20 min; in a mixer mill MM200, MM300, MM400 (Retsch®), e.g., 0.5 – 5 min at maximal frequency (30 Hertz) is suitable (see section 2.4). On a swing mill, position of the tube in the mill can considerably influence the result. Please refer to the instruction manual of the device used. Respect warnings in section 1.2 and 2.4.3 if other devices are intended to be used!

Agitate

Centrifuge the MN Bead Tube **30 s** at **11,000 x *g*** to clean the lid.

11,000 x *g*, 30 s

Note: In this step foam is removed from the screw cap to allow clean opening of the tube.

Note: Depending on the fat amount of the sample, a fatty layer might form on top of the lysate. This layer does not have to be removed.

***Attention:** Do neither centrifuge at higher g-force, nor longer because this might damage the MN Bead Tubes.*

3 Adjust DNA binding conditions

Add **600 µL Buffer LT** and **mix** (e.g., vortex for 3 s).

+600 µL LT

Mix

Note: Steel balls should be agitated in the tube; some residual pellet (cell debris) may remain on the bottom of the tube.

Centrifuge for **30 s** at **11,000 x *g***.

11,000 x *g*, 30 s

Note: This centrifugation step is performed in order to clean the lid and sediment beads and cell debris. Depending on the fat amount of sample, fatty layer might form on top of the liquid. This fat layer is typically observed with adipose tissue but not with brain or fatty fish tissue.

***Attention:** Do neither centrifuge at higher g-force, nor longer because this might damage the MN Bead Tube.*

Alternatively, the lysate can be transfered without steel balls to a fresh and transparent centrifugation tube (not provided) before centrifugation in order to simplify supernatant transfer in step 5.

4 Bind DNA

Transfer the cleared liquid supernatant (~500 – 600 µL) onto the **NucleoSpin® DNA Lipid Tissue Column**, placed in a 2 mL Collection Tube (provided).

Load samples

Note: Do not to transfer the semi-liquid to soft-solid fatty layer typically observed with adipose tissue samples. Pierce the fatty layer with the pipet tip in order to aspirate the cleared liquid. The fatty matter can stay within the tube and / or will partially stick to the outside of the pipet tip.

11,000 x *g*, 30 s

Centrifuge for **30 s** at **11,000 x *g***. Discard Collection Tube with flow through. Put column into a fresh Collection Tube (2 mL, provided).

5 Wash silica membrane

+500 µL BW

11,000 x *g*, 30 s

1 st wash

Add **500 µL Buffer BW**. Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the Collection Tube.

+500 µL B5

11,000 x *g*, 30 s

2nd wash

Add **500 µL Buffer B5** to the column and centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the Collection Tube.

6 Dry silica membrane

Centrifuge the column for **30 s** at **11,000 x *g***.

Note: Residual wash buffer is removed in this step.

11,000 x *g*, 30 s

7 Elute highly pure DNA

Place the NucleoSpin® DNA Lipid Tissue Column into a 1.5 mL nuclease-free tube (not provided) and add **100 µL Buffer BE** onto the column. Incubate at room temperature for 1 min.

100 µL BE

RT, 1 min

Centrifuge **30 s** at **11,000 x *g***.

11,000 x *g*, 30 s

For alternative elution procedures see section 2.5.

5.2 Protocol for purification of DNA from hard-to-lyse bacteria in (lipid-rich) tissue samples

The purification of DNA from hard-to-lyse bacteria (e.g., gram-positive bacteria) in tissue samples can be challenging as disruption of different organisms requires individual mechanical forces.

Therefore, MACHEREY-NAGEL has developed the MN Bead Tubes Type E, which contain 40 – 400 µm glass beads as well as 3 mm steel balls. MN Bead Tubes Type E can be used according to the protocol described for MN Bead Tubes Type D in section 5.1.

However, the use of MN Bead Tubes Type E is a very harsh method in terms of sample disruption. Please note that processing time on a selected disruption device (e.g., MN Bead Tube Holder or mixer mill (Retsch®)) has to be optimized by the user with regard to sample type, amount, and downstream application. Long disruption duration on high impact machines (e.g., mixer mill (Retsch®)) can cause total DNA loss due to massive DNA fragmentation. Please contact the technical service for further information. **Respect warnings in section 1.2 and 2.4.3 when using MN Bead Tubes Type E.**

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Damaged bead tubes	*Unsuitable disruption device or intensity* • High force disruption devices can damage MN Bead Tubes Type D and E. Respect warnings in section 1.2 and 2.4.3. Use the recommended MN Bead Tube Holder.
No or poor DNA yield	*Incomplete lysis* • Adjust lysis conditions (bead tube type, agitation device, duration, or frequency). *Reagents not applied properly* • Prepare Buffer B5 according to instructions (section 3). *Suboptimal elution of DNA from the column* • For certain sample types, preheat Buffer BE to 70 °C before elution. Apply Buffer BE directly onto the center of the silica membrane. • Elution efficiencies decrease dramatically, if elution is done with buffers with a pH < 7.0. Use slightly alkaline elution buffers like Buffer BE (pH 8.5). • Especially when expecting high yields from large amounts of material, we recommend elution with 200 µL Buffer BE and incubation of the closed columns in an incubator at 70 °C for 5 min before centrifugation.
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 the disruption step 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 B5 according to instructions (see section 3).

Problem	Possible cause and suggestions
Clogged columns	*Too much sample material used* • Make sure to centrifuge the lysate after cell disruption in order to sediment beads and cell debris. Only apply cleared supernatant onto the column.
Suboptimal performance of genomic DNA 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 B5 before eluting the DNA. If, for any reason, the level of Buffer B5 has reached the column outlet after drying, repeat the centrifugation. • Do not chill Buffer B5 before use. Cold buffer will not remove salt effectively. Equilibrate Buffer B5 to room temperature before use. *Contamination of DNA with inhibitory substances* • Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer BE.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® DNA Lipid Tissue	740471.10 / .50	10 / 50 preps
NucleoSpin® DNA Insect	740470.10 / .50	10 / 50 preps
MN Bead Tube Holder	740469	1 piece
NucleoSpin® Soil	740780.10 / .50 / .250	10 / 50 / 250 preps
MN Bead Tubes Type A (0.6 – 0.8 mm ceramic beads recommended for soil and sediments)	740786.50	50 pieces
MN Bead Tubes Type B (40 – 400 µm glass beads recommended for bacteria)	740812.50	50 pieces
MN Bead Tubes Type C (1 – 3 mm corundum recommended for yeasts)	740813.50	50 pieces
MN Bead Tubes Type D (3 mm steel balls recommended for insects)	740814.50	50 pieces
MN Bead Tubes Type E (40 – 400 µm glass and 3 mm steel balls recommended for hard-to-lyse bacteria within insect or tissue samples)	740815.50	50 pieces
Buffer BE	740306.100	125 mL
Buffer B5 Concentrate (for 125 mL Buffer B5)	740921	25 mL
Buffer BW	740922	100 mL
Liquid Proteinase K	740396	5 mL
RNase A	740505 740505.50	100 mg 50 mg
Collection Tubes (2 mL)	740600	1000

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-NAGELS 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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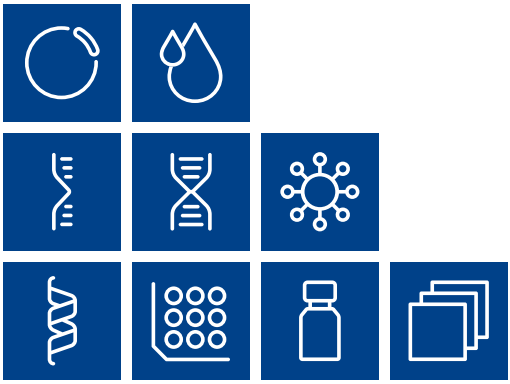
Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 (0) 24 21 969 333 support@mn-net.com



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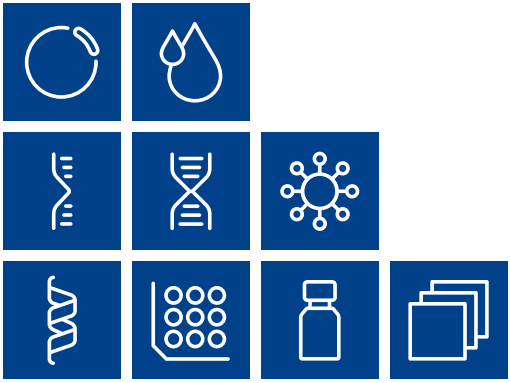

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MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11
52355 Düren · Germany

DE Tel.: +49 24 21 969-0 info@mn-net.com
CH Tel.: +41 62 388 55 00 sales-ch@mn-net.com
FR Tel.: +33 388 68 22 68 sales-fr@mn-net.com
US Tel.: +1 888 321 62 24 sales-us@mn-net.com

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