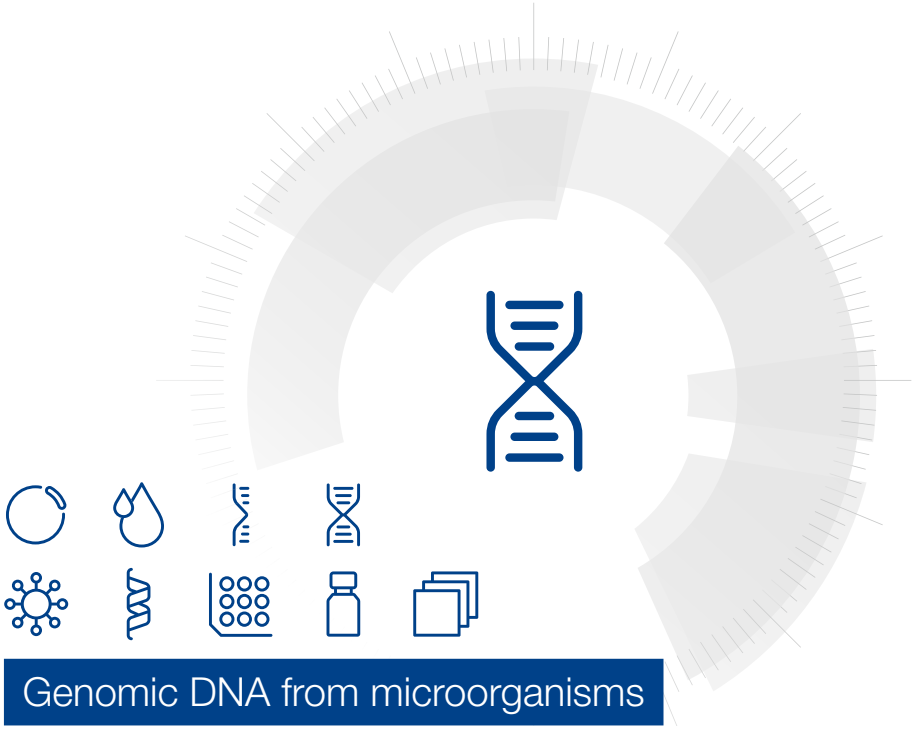
Bioanalysis

MACHEREY-NAGEL

User manual



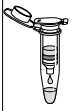
Genomic DNA from microorganisms

■ NucleoSpin® Microbial DNA

Ferbruary 2021 / Rev. 04

Genomic DNA from microorganisms

Protocol at a glance (Rev. 04)

		NucleoSpin® Microbial DNA
1 Prepare sample		< 40 mg microbial pellet (wet weight) 100 µL Elution Buffer BE
2 Lyse sample	 	Transfer sample in MN Tube Type B 40 µL Buffer MG 10 µL Liquid Proteinase K Agitate on a swing mill or similar device 4–12 min 11,000 x *g*, 30 s
3 Adjust binding conditions	 	600 µL Buffer MG Vortex 3 s 11,000 x *g*, 30 s
4 Bind DNA	 	Load 500–600 µL sample on NucleoSpin® Microbial DNA Column 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 Elution Buffer BE RT, 1 min 11,000 x *g*, 30 s

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1 Components

1.1 Kit contents

	NucleoSpin® Microbial DNA		
REF	**10 preps 740235.10**	**50 preps 740235.50**	**250 preps 740235.250**
Lysis Buffer MG	10 mL	38 mL	5 x 38 mL
Wash Buffer BW	6 mL	30 mL	150 mL
Wash Buffer B5 (Concentrate)*	6 mL	6 mL	50 mL
Elution Buffer BE**	13 mL	30 mL	125 mL
Liquid Proteinase K	120 µL	600 µL	2 x 1.5 mL
MN Bead Tubes Type B	10	50	250
NucleoSpin® Microbial DNA 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 BE: 5 mM Tris/HCl, pH 8.5

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

Reagents

- 96–100 % ethanol

Consumables

- 1.5 mL or 2 mL microcentrifuge tubes for microbial sample sedimentation
- Disposable tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Sample disruption device: swing mill or similar device (e.g., Mixer Mill MM200, MM300, MM400 (Retsch®); FastPrep® System (MP-Biomedicals); Precellys® (Bertin Technologies); MagNA Lyser (Roche); TissueLyser (QIAGEN); Bullet Blender® (Next Advance); Mini-Beadbeater (Biospec Products); Speed Mill (Analytik Jena); Vortex Adapter for Vortex-Genie® 2 X (MoBio))
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoSpin® Microbial DNA** 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® Microbial DNA** kit is designed for efficient isolation of genomic DNA from microbial samples. DNA can be isolated from a wide variety of microorganisms such as gram negative, and gram positive bacteria as well as yeast, e.g., *Escherichia coli, Bacillus subtilis, Corynebacterium glutamicum, Saccharomyces cerevisiae.* Preparation of the collected samples containing the microbes of interest should be in pellet format.

Preliminary data also indicate the usability of the kit for DNA isolation from fungal mycelia, e.g., *Aspergillus nidulans*, from bacterial spore suspensions, e.g., *Geobacillus stearothermophilus*, and from plant pollen, e.g., honey bee pollen baskets. For optimal DNA yield, bead tubes different from the ones included in the kit might be required for such applications (see section 2.4).

Microbial samples such as gram positive bacteria, yeast, and spores can be difficult to lyse due to their strong complex cell wall structures. The NucleoSpin® Microbial DNA kit replaces enzymatic lysis by utilizing mechanical disruption of cell wall structures with the MN Bead Tubes. The MN Bead Tubes can be used in combination with many compatible disruptive devices (see 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 select sample types (see section 2.4.2 for recommendations).

2.2 Kit specifications

Kit specifications at a glance	
Parameter	**NucleoSpin® Microbial DNA**
Technology	Silica membrane technology
Format	Mini spin column
Sample material	Microbial cell culture pellets of gram positive and gram negative bacteria, yeast
Sample amount	Up to approx. 40 mg wet weight
Typical yield	Varies by sample and disruption device. 5–25 µg DNA from approx. 30 mg wet weight microbial pellet can be obtained
A_{260} / A_{280}	1.6–2.0
Elution volume	100–200 µL
Preparation time	35 min/6 preps
Binding capacity	60 µg

2.3 Handling, preparation, and storage of starting materials

Cells should be harvested from fresh microbial cultures by sedimentation via centrifugation. Supernatant should be removed by aspiration. Microbial cell pellets can be used fresh or stored at -20 °C to -80 °C before starting DNA isolation.

2.4 Lysis and disruption of sample material

In order to obtain optimal yields of DNA from sample material, a complete disruption of the sample material is necessary. Sample disruption efficiency depends on the following parameters and can be achieved by following suggestions 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 A (0.6–0.8 mm ceramic beads)
  Recommended for soil, sediment, and stool (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 D (3 mm steel beads; included in NucleoSpin® DNA Insect kits)
  Recommended for insects, crustaceans, and lipid-rich tissue.
- MN Bead Tubes Type E (combination of 3 mm steel beads and 40–400 µm glass beads)
  Recommended for hard to lyse bacteria within insect or tissue samples (see ordering information, section 6.2).
- MN Bead Tubes Type F (combination of 1-3 mm corundum and 3 mm steel beads; included in NucleoSpin® RapidLyse kits)
  Recommended for challenging tissues, e.g., spleen, or lung tissue.
- MN Bead Tubes Type G (5 mm steel beads)
  Recommended for plant material (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® Mixer Mill MM300 operating at highest frequency (30 Hertz). **For using other disruption devices, and other sample materials, time and frequency have to be optimized.**

Time and frequency of disruption using MN Bead Tube Holder on a Vortex Genie® 2

As a general starting point disrupt microbial samples for 20 min using MN Bead Tube Holder on a Vortex Genie® 2.

Time an frequency of disruption using a Retsch® Mixer Mill MM300

Sample material	MN Bead Tube	Disruption time
Gram negative bacteria E.g., *Escherichia coli*, *Vibrio fischeri*	MN Bead Tubes Type B (Alternative: Type A, Type C)	4 min
Gram positive bacteria E.g., *Bacillus subtilis*, *Corynebacterium glutamicum*	MN Bead Tubes Type B (Alternative: Type A)	12 min
Yeast E.g., *Saccharomyces cerevisiae*	MN Bead Tubes Type C	12 min
Filamentous fungi E.g., *Aspergillus spec.*, *Rhizopus spec.*	MN Bead Tubes Type C	12 min

Note: Performance and stability testing has been conducted on the MN Bead Tubes A, B, and C on a Retsch® Mixer Mill MM300 at highest frequency (30 Hertz) for up to 15 minutes for optimal sample disruption, avoidance of DNA fragmentation, and tube durability. 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 (Retsch® Mixer Mill) is important for optimal performance! Please consult instruction manual of the machine.

WARNING:** Many modern disruption devices can cause very high energy input in 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 test for the used bead tubes under the conditions used! *Perform initial test with water instead of lysis buffer and moderate machine setting (low frequency, short time) in order to avoid spillage of chaotropic lysis buffer in case of tube breakage.*

***WARNING:** In section 5 a certain liquid volume during disruption is recommended. The reduction of liquid will severely increase the mechanical impact of the grinding matrix and can result in damage of DNA and tube (especially if MN Bead Tubes D and E are used).*

2.5 Elution procedures

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

- **Convenient elution (standard elution)**: For convenience, elution can be performed by one time addition of 100 µL elution buffer onto the column.
- **High yield**: Two serial elutions of 100 µL each for total elution volume of 200 µL.
- **High concentration**: Use initial 100 µL eluate for second elution – 100 µL total elution volume, 2 elutions.

3 Storage conditions and preparation of working solutions

Attention:

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

CAUTION: Buffers MG and BW contain chaotropic salts which can form highly reactive compounds when combines 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 (18–25 °C) and are stable for at least one year.

Before starting any **NucleoSpin® Microbial DNA** 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 (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® Microbial DNA			
REF	**10 preps** **740235.10**	**50 preps** **740235.50**	**250 preps** **740235.250**
Wash Buffer B5 (Concentrate)	6 mL Add 24 mL ethanol	6 mL Add 24 mL ethanol	50 mL Add 200 mL ethanol

4 Safety instructions

The following components of the **NucleoSpin® Microbial DNA** kits contain hazardous contents.
Wear gloves and goggles and follow the safety instructions given in this section.

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*
BW	Guanidine hydrochloride 36–50% + 2-propanol 20–35% *Guanidinhydrochlorid 36–50% + 2-Propanol 20–35%* CAS 50-01-1	 WARNING *ACHTUNG*	226, 302, 319, 336	210, 260D, 264W, 280sh, 301+312, 330
MG	Guanidinium thiocyanate 30–45 % *Guanidinthiocyanat 30–45 %* CAS 593-84-0	 WARNING *ACHTUNG*	302, 412	260W, 273, 301+312, 330

Hazard phrases

H226 Flammable liquid and vapour.
Flüssigkeit und Dampf entzündbar.

H302 Harmful if swallowed.
Gesundheitsschädlich bei Verschlucken.

H319 Causes serious eye irritation.
Verursacht schwere Augenreizung.

H336 May cause drowsiness or dizziness.
Kann Schläfrigkeit und Benommenheit verursachen.

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

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.

P260D Do not breathe vapors.
Dampf nicht einatmen.

P264W Wash with water thoroughly after handling.
Nach Gebrauch mit Wasser gründlich waschen.

P273	Avoid release to the environment. *Freisetzung in die Umwelt vermeiden.*
P280sh	Wear protective gloves/eye protection. *Schutzhandschuhe/Augenschutz tragen.*
P301+312	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt anrufen.*
P330	Rinse mouth. *Mund ausspülen.*

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 gram positive and gram negative bacteria

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

+ 100 µL BE

Harvest cells from a culture by centrifugation in a microcentrifuge tube (not provided). Discard supernatant.

Up to approximately 40 mg of wet weight microbial cell culture pellet can be used as sample material.

Add **100 µL Elution Buffer BE** and resuspend cells.

Alternatively, high quality grade water (not provided) can be used.

2 Lyse sample

+ 40 µL MG

+ 10 µL Liquid Proteinase K

Transfer the cell suspension into the **MN Bead Tube Type B** (provided).

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

Note: It is not necessary to vortex here.

Agitate the MN Bead Tube on a swing mill or similar device.

Agitate

Note: Optimal agitation duration, speed / frequency depends on the machine used. On a Retsch® Mixer Mill MM200, MM300, MM400, e.g., 4 min at maximal frequency (30 Hertz) is adequate for E. coli, 12 min for B. subtilis (see section 2.4). On the swing mill, position of the tube in the mill can considerably influence the result. Please consult the instruction manual of the device used.

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 displaced from the screw cap, so that the cap can be removed in a clean way.

3 Adjust DNA binding conditions

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

Note: Glass beads should be resuspended; some residual pellet (cell debris) may remain on the bottom of the tube.

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

Note: This centrifugation step is performed in order to clean the lid and sediment glass beads and cell debris.

+ 600 µL MG

Mix

11,000 x *g*, 30 s

4 Bind DNA

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

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

Load samples

11,000 x *g*, 30 s

5 Wash silica membrane

1st wash

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

+ 500 µL BW

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 flowthrough and place the column back into the Collection Tube.

+ 500 µL B5

11,000 x *g*, 30 s

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® Microbial DNA Column into a 1.5 mL nuclease-free tube (not provided) and add **100 µL Elution Buffer BE** onto the column. Incubate **at room temperature** for **1 min**. Centrifuge **30 s** at **11,000 x *g***.

For alternative elution procedures see section 2.5.

5.2 Protocol for yeast (e.g., *Saccharomyces cerevisiae*)

Optimal DNA yields from yeast samples can be obtained by following the standard protocol using MN Bead Tube Type C (see ordering information on section 6.2) instead of MN Bead Tube Type B provided with the NucleoSpin® Microbial DNA kit.

The agitation is recommended at a Retsch® Mixer Mill MM300: 12 min at 30 Hz. For other disruption devices, please check section 2.4. Please note that the position of the tube within the machine is important for optimal performance, please consult instruction manual of the machine.

If bead carryover is observed in the eluate, transfer the eluate into a new 1.5 mL nuclease-free tube carefully avoid disturbing the pellet.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
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 do not interfere with downstream application. 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).
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 transfer cleared supernatant onto the column.

Problem	Possible cause and suggestions
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® Microbial DNA	740235.10 / .50 / .250	10 / 50 / 250 preps
MN Bead Tube Holder	740469	1 piece
NucleoSpin® Soil	740780.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® DNA Lipid Tissue	740471.10 / .50	10 / 50 preps
NucleoSpin® DNA Insect	740470.10 / .50	10 / 50 preps
NucleoSpin® DNA Stool	740472.10 / .50	10 / 50 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 yeast)	740813.50	50 pieces
MN Bead Tubes Type D (3 mm steel beads, recommended for insects)	740814.50	50 pieces
MN 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

Product	REF	Pack of
MN Bead Tubes Type F (1-3 mm corundum and 3 mm steel beads, recommended for challenging tissues, e.g., spleen, or lung tissue)	740816.50	50 pieces
MN Bead Tubes Type G (5 mm steel beads, recommended for plant material)	740817.50	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	740505.50 740505	50 mg 100 mg
Collection Tubes (2 mL)	740600	1000

6.3 Product use restriction / warranty

NucleoSpin® Microbial DNA 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.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or

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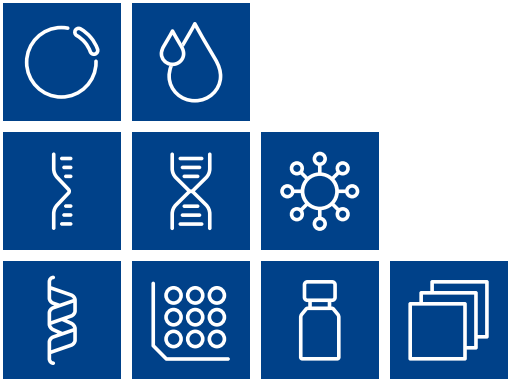

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