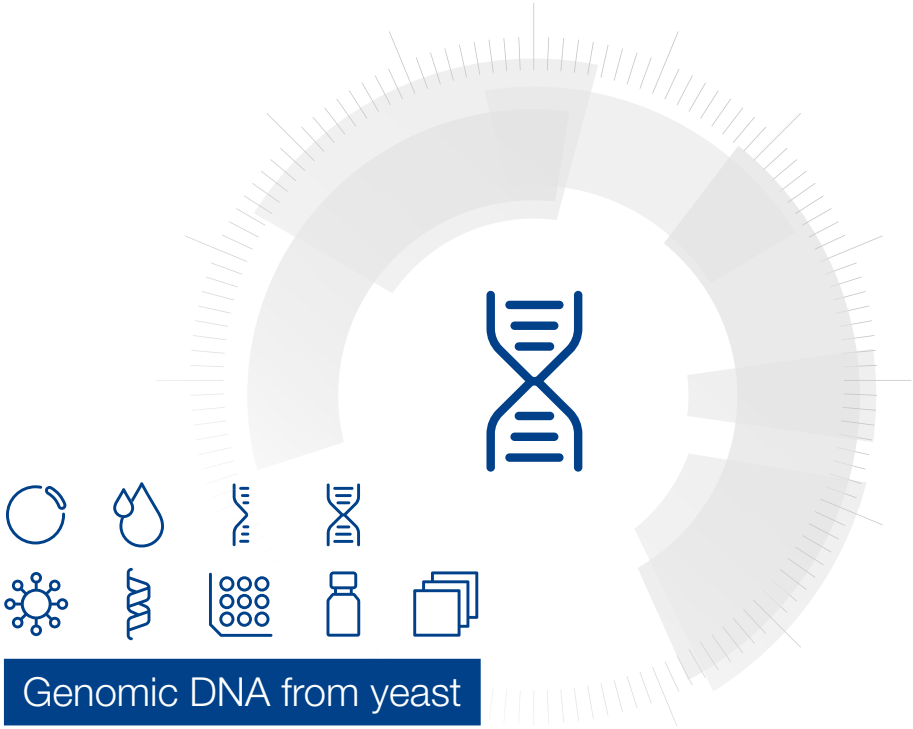
Bioanalysis

MACHEREY-NAGEL

User manual



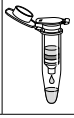
Genomic DNA from yeast

■ NucleoSpin® DNA Yeast

January 2023 / Rev. 03

Genomic DNA from yeast

Protocol at a glance (Rev. 03)

		NucleoSpin® DNA Yeast
1 Prepare sample		< 100 mg yeast pellet (wet weight) 100 µL BE
2 Lyse sample	 	Transfer sample in NucleoSpin® Bead Tube Type C 40 µL Buffer MG 10 µL Liquid Proteinase K Agitate on MN Bead Tube Holder 15–20 min or on a swing mill for a shorter period 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 650 µL supernatand onto the NucleoSpin DNA Yeast 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 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*

Table of contents

1 Components

1.1 Kit contents

	NucleoSpin® DNA Yeast	
REF	**10 preps 740236.10**	**50 preps 740236.50**
Lysis Buffer MG	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 Tube Type C	10	50
NucleoSpin® DNA Yeast Columns (light green rings)	10	50
Collection Tubes (2 mL)	20	100
User Manual	1	1

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

Reagents

- 96 – 100 % ethanol (to adjust binding conditions and to prepare Wash Buffer B5)

Consumables

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

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Sample disruption device: MN Bead Tube Holder (REF 740469) or a 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 (QIAGEN))
- 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® DNA Yeast** 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 Yeast kit is designed for efficient isolation of genomic DNA from yeast samples. DNA can be isolated from a wide variety of yeasts such as *Saccharomyces cerevisiae, Schizosaccharomyces pombe* and *Pichia pastoris (Komagataella phaffii)*. Preparation of the collected samples containing the mircobial sample 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.

2.2 Kit specifications

Kit specifications at a glance	
Parameter	NucleoSpin® DNA Yeast
Technology	Silica membrane technology
Format	Mini spin column
Sample material	Yeast cell culture pellets, fungal mycelium
Sample amount	Up to approximately 100 mg wet weight
Typical yields	Varies by sample quality, species, and disruption device. Approximately one up to 20 µg DNA from 10 – 100 mg wet weight yeast pellet can be obtained.
$A_{260/280}$	1.6 – 2.0*
Elution volume	100 – 200 µL
Preparation time	35 min / 6 preps
Theoretical binding capacity	60 µg
Use	For research use only

2.3 Handling, preparation, and storage of starting materials

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

* Quality ratio determination strongly depends on a sufficient amount of DNA measuredpresent in the eluate. Make sure to use sufficient amount of DNA that has been validated to enable a meaningful $A_{260/280}$ ratio determination with the photometer used.

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 the following suggestions outlined in the subsequent sections.

2.4.1 Disruption device

The following devices are compatible with NucleoSpin® Bead Tubes. Please check whether NucleoSpin® 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 2.1) 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

For DNA isolation from yeast, MN Bead Tube Type C – as included in this kit – is recommended.

If other types of sample material will be used, bead type, disruption time, and frequency / speed must be optimized for a given sample to obtain maximal DNA yield and quality.

- MN Bead Tubes Type A (0.6 – 0.8 mm ceramic beads)
  Recommended for soil, sediment, and stool (included in NucleoSpin® Soil).
- MN Bead Tube Type B (40 – 400 µm glass beads)
  Recommended for gram positive and negative bacteria (included in NucleoSpin® Microbial DNA).
- MN Bead Tube Type C (1 – 3 mm corundum)
  Recommended for yeast, as included in this kit here.
- MN Bead Tube Type D (3 mm steel beads, included in NucleoSpin® DNA Insect kit)
  Recommended for insects, crustaceans, and lipid-rich tissue.
- MN Bead Tube Type E (combination of 3 mm steel beads and 40 – 400 µm glass beads)
  Recommended for had to lyse bacteria within insect or tissue samples.
- MN Bead Tube type Type F (combination of 1 – 3 mm corundum and 3 mm steel beads)
  Recommended for challenging tissues, e.g., spleen, or lung tissue in combination with NucleoSpin® DNA RapidLyse.
- MN Bead Tube Type G (5 mm steel beads)
  Recommended for plant material.

See ordering information for above mentioned kits and bead tubes.

For more information, please visit the following websites.

www.mn-net.com/culturedmicroorganism
www.mn-net.com/beadtubeoverview
www.mn-net.com/BeadTubeOverview-BeadTubeC

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 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 yeast samples for 15 – 20 min using MN Bead Tube Holder on a Vortex Genie® 2.

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

As a general starting point disrupt yeast samples for approximately 5 min using a Retsch® Mixer Mill MM300 operating at 30 Hz.

Note: Performance and stability testing has been conducted for the MN Bead Tube Type C on a Retsch Mixer Mill MM300 at highest frequency (30 Hz) for up to 15 minutes for optimal sample disruption, avoidance of DNA fragmentation, and tube durability. Other disruption devices 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 a Retsch Mixer Mill is important for optimal performance – please consult instruction manual of the machine.

WARNING: The use of other disruption devices like FastPrep® System (MP-Biomedicals), Precellys® (Bertin Technologies), MagNA Lyser (Roche), TissueLyser (QIAGEN), Bullet Blender® (Next Advance), MiniBeadbeater (Biospec Products), Speed Mill (Analytik Jena), or similar devices might cause bead tube damage. Such disruption devices can cause high mechanical stress on the bead tubes. Depending on bead tube type and content (liquid volume, sample type), high frequency of shaking and / or long shaking duration can cause damage 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 MN Bead Tubes during the individual experimental setup (e.g., intensity of agitation). This is especially important for MN Bead Tubes that contain steel beads. 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 within the bead tube during cell disruption will severely increase the mechanical impact of the grinding matrix and can result in damage of DNA and tube (especially if MN Bead Tubes Type D, E, F, or G 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 a 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 (15 – 25 °C) and are stable until: see package label.

Before starting any **NucleoSpin® DNA Yeast** 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 Yeast	
REF	**10 preps 740236.10**	**50 preps 740236.50**
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 Yeast** 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 *www.mn-net.com/msds*).

Caution: Guanidine hydrochloride in buffer BW, guanidinium thiocyanate in buffer MG 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 Yeast** 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 DNA isolation from yeast

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) in order to provide a **yeast cell pellet**. Discard supernatant.

Note: Up to approximately 100 mg of wet weight yeast cell culture pellet can be used as sample material.

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

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

Note: The liquid is completely absorbed by the corundum particles. Do not increase the volume of Buffer BE or water; increased liquid volumes during agitation cause reduced efficiency of cell lysis!

2 Lyse sample

+40 µL MG

+10 µL Liquid Proteinase K

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

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

Agitate the MN Bead Tube in the MN Bead Tube Holder on a Vortex-Genie 2, a swing mill, or similar device.

Recommended time and frequency of disruption:

- MN Bead Tube Holder on a Vortex Genie® 2: Agitate 15 – 20 min at full speed.
- Retsch® Mixer Mill MM300: Agitate for approximately 5 min at 30 Hz. The position of the tube in the mill can considerably influence the result – please consult the instruction manual of the device used.
- Other disruption devices: see section 2.4.3.

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

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 approx. 3 s).

Note: Corundum should be resuspended in order to dispense DNA evenly in Buffer MG; 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 an sediment corundum and cell debris.

+600 µL MG

Mix

11,000 x *g*, 30 s

4 Bind DNA

Withdraw **650 µL of supernatant and transfer** it onto a **NucleoSpin® DNA Yeast Column**, placed in a 2 mL Collection Tube (provided).

Note: Recover the supernatant from above the corundum; do not recover liquid from within the corundum to avoid transfer of cell debris and corundum.

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

Load samples

11,000 x *g*, 30 s

5 Wash silica membrane

1 st wash

Add **500 µL Buffer BW** and centrifuge for **30 s** at **11,000 x *g***. Discard flow through 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** onto the column and 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

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 Yeast Column into a 1.5 mL nuclease-free tube (not provided) and add **100 µL Buffer BE** onto the column.

+100 µL BE

Incubate at **room temperature** for **1 min**.

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 DNA isolation from mycelium

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.
- Check if a 500 mM solution of TCEP is available (see ordering information; TCEP has to be ordered separately)

1 Prepare sample

Harvest mycelium and transfer it without excess of water or medium into Bead Tube Type C (provided).

2 Lyse sample

Add 100 µL Elution Buffer BE and resuspend mycelium.

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

Add **40 µL Buffer MG** into the tube.

Add **20 µL of a 500 mM TCEP solution** and mix.

Incubate for approx. **10 min at room temperature.**

Add **10 µL Liquid Proteinase K** and close the tube.

Agitate the MN Bead Tube in the MN Bead Tube Holder on a Vortex-Genie 2, a swing mill, or similar device.

Recommended time and frequency of disruption:

- MN Bead Tube Holder on a Vortex Genie® 2: Agitate 15 – 20 min at full speed.
- Retsch® Mixer Mill MM300: Agitate for approximately 5 – 10 min at 30 Hz. The position of the tube in the mill can considerably influence the result – please consult the instruction manual of the device used.
- Other disruption devices: see section 2.4.3.

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

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

Continue with the addition of 600 µL Buffer MG in order to adjust the binding conditions according to protocol 5.1 step 3 and continue with this protocol.

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. • Low $A_{260/280}$ or low $A_{260/230}$ ratio Make sure that a sufficient amount / concentration of DNA is used for quantification so that the A_{280} or A_{230} value is significantly higher than the background level. Measurement of low amount / concentration of DNA will cause unstable ratio values. *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 transfer 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 Yeast	740236.10 / .50	10 / 50 preps
NucleoSpin® DNA Bacteria	740235.10 / .50 / .250	10 / 50 / 250 preps
MN Bead Tube Holder	740469	1 piece
MN Bead Tube Type A	740786.50	50 pieces
MN Bead Tube Type B	740812.50	50 pieces
MN Bead Tube Type C	740813.50	50 pieces
MN Bead Tube Type D	740814.50	50 pieces
MN Bead Tube Type E	740815.50	50 pieces
MN Bead Tube Type F	740816.50	50 pieces
MN Bead Tube Type G	740817.50	50 pieces
TCEP	740395.107	107 mg
RNase A	740505.50, 740505	50 mg, 100 mg

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.

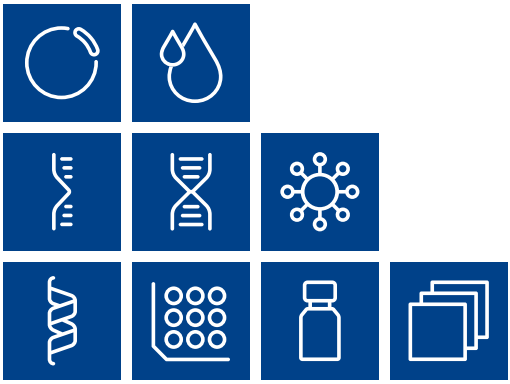
This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No 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.

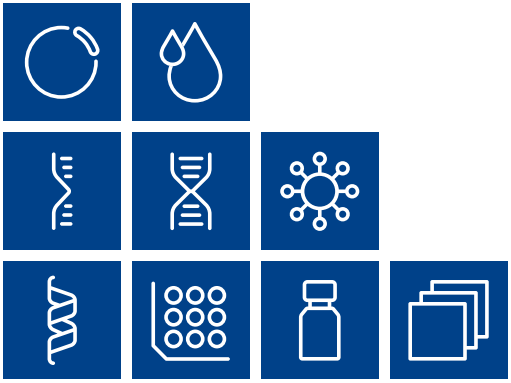
Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

Last updated: 08/2022, Rev. 04

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



Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



www.mn-net.com

MACHEREY-NAGEL

Management System EN ISO 13485:2016 ISO 9001:2015
www.tuv.com ID 9000003338

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

A066926/0130.3