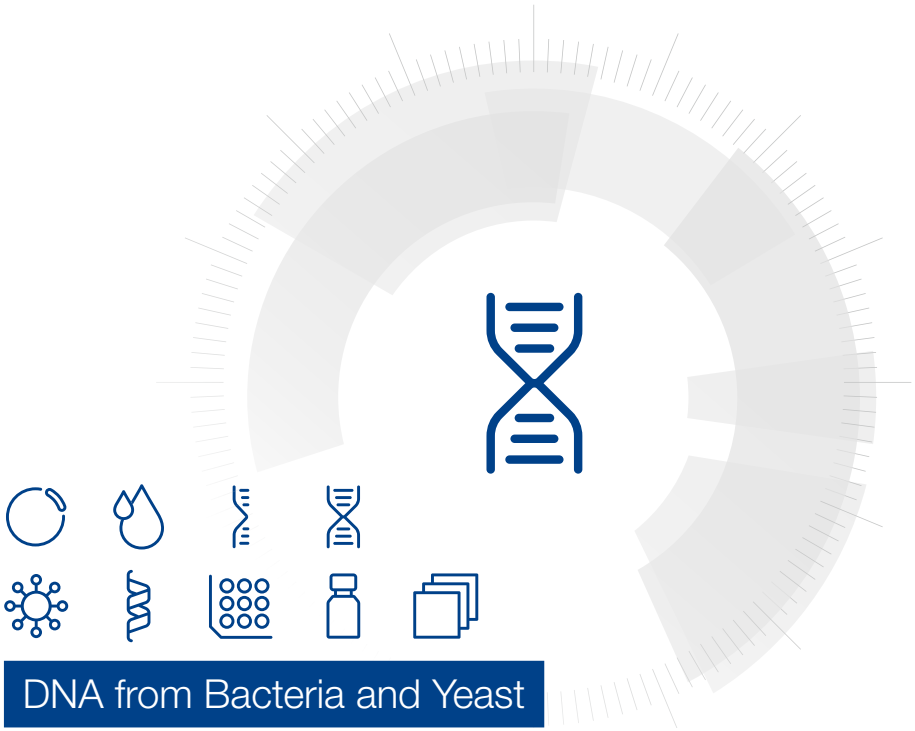
Bioanalysis

MACHEREY-NAGEL

User manual



DNA from Bacteria and Yeast

■ NucleoMag® DNA Bacteria

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1 Components

1.1 Kit contents

	NucleoMag® DNA Bacteria	
REF	**1 × 96 preps 744310.1**	**4 × 96 preps 744310.4**
NucleoMag® B-Beads	2 × 1.5 mL	12 mL
Buffer IML	60 mL	250 mL
Binding Buffer IMB*	40 mL	2 × 75 mL
Wash Buffer IMW (Concentrate)**	50 mL	200 mL
Buffer IME***	60	250 mL
Liquid Proteinase K	1.25 mL	4.5 mL
Liquid RNase A	0.3 mL	1.25 mL
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*Store Binding Buffer IMB upon arrival at 4 °C.

**For preparation of working solutions and storage, see section 3.

***Composition of Buffer IME: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables and equipment to be supplied by user

Reagents

- 80 % ethanol
- 96 – 100 % ethanol

Consumables

- MN Bead Tubes Type A for mechanical disruption of yeast pellets in single tube format
- MN Bead Tubes Type B for mechanical disruption of bacterial pellets in single tube format
- MN 96 Bead Plate Type B for mechanical disruption of bacterial pellets in a convenient 96-well format
- Suitable reaction container for magnetic bead based purification (e.g., 1.5 mL reaction tubes or 96-well plate (see table on the next page)

Equipment

- Centrifuge for microcentrifuge tubes of plates depending on disruption consumable
- Vortex mixer
- Personal protection equipment (e.g., lab coat, gloves, goggles)

Sample disruption device:

- MN Bead Tube Holder (see table on the next page) is recommended to be used in combination with the Vortex-Genie® 2 for cost efficient and convenient sample disruption in combination with MN Bead Tubes.
- Alternatively, a swing mill or similar device can be used considering precautions of section 2.4.3 and compability with disruption consumables (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 or bead plate destruction. Such disruption devices can cause high mechanical stress on the bead tubes or bead plates. Depending on bead tube / bead plate type and content (beads like steel beads, liquid volume, sample type), especially high frequency of shaking and / or long shaking duration can cause destruction of the bead tubes or bead plates. If using such a disruption device, it is the responsibility of the user to perform initial stability tests to ensure stability of bead tubes or bead plates during the individual experimental setup (e.g., intensity of agitation). See also section 2.4.3!

Product	REF	Pack of
Magnetic separation system		
e.g., NucleoMag® SEP (suitable for 96-(deep)-well plates)	744900	1
e.g., NucleoMag® SEP Mini (suitable for 1.5 – 2 mL tubes)	744901	1
e.g., NucleoMag® SEP Maxi (suitable for 50 mL tubes)	744902	1
e.g., NucleoMag® SEP 24 (suitable for 24-deep-well plates)	744903	1
Separation plate for magnetic beads separation		
e.g., Square-well Block (96-well block with 2.1 mL square-wells)	740481 740481.24	4 sets 24 sets
Sample homogenization system		
e.g., **MN Bead Tubes Type A** Bead tubes containing 0.6 – 0.8 mm ceramic beads; suitable in conjunction with MN Bead Tube Holder or mixer mill	740786.50	50
e.g., **MN Bead Tubes Type B** Bead tubes containing 40 – 400 µm glass beads; suitable in conjunction with MN Bead Tube Holder or mixer mill	740812.50	50
e.g., **MN 96 Bead Plate Type B** Rack of prefilled tube strips (8 × 12) containing 40 – 400 µm glass beads; suitable in conjunction with mixer mill	740851.4 740851.24	4 24
e.g., **MN Bead Tube Holder** Tube holder for Vortex-Genie® instrument and a 3-inch platform in order to house up to 12 bead tubes	740469	1
Additional enzymes (if necessary)		
Liquid Proteinase K	740396	5 mL
Liquid RNase A	740397	2 × 1.25 mL
High throughput consumables		
e.g., **Rack of Tube Strips** 1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 mL wells) each, and 12 Cap Strips	740477 740477.24	4 sets 24 sets
e.g., **Elution Plate U-bottom** Elution plate for collecting purified nucleic acids (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)	740486.24	24
For use of kit on KingFisher® instruments		
e.g., KingFisher® 96 / Flex platform: **KingFisher® 96 Accessory Kit A** (Square-well Blocks, Deep-well tip Combs, plates for 4 × 96 NucleoMag® DNA Bacteria preps)	744950	1 set
e.g., KingFisher® DUO / DUO Prime platform: **KingFisher® DUO Accessory Kit** (Square-well Blocks, Deep-well tip Combs, plates for 8 × 12 NucleoMag® DNA Bacteria preps)	744952	1 set

1.3 About this user manual

It is strongly recommended that first time users of the **NucleoMag® DNA Bacteria** kit read the detailed protocol sections of this user manual. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used as a tool for quick referencing while performing the purification procedure.

All technical literature is available at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

1.4 Automation support

MN extraction kits are designed for streamlined automation, offering compatibility with a range of leading open robotic systems. Whether you're using magnetic rod systems or liquid handlers like Hamilton, Tecan, Eppendorf, or other platforms, our kits ensure efficient and reliable extraction processes. Reach out to us for comprehensive support and tailored automation solutions, making your extraction experience seamless and effortless.

Questions about MACHEREY-NAGEL's scripting support or automation service?

Please contact us for personal assistance:
Phone: +49 2421 969 333
Email: support@mn-net.com

2 Product description

2.1 The basic principle

The **NucleoMag® DNA Bacteria** kit is designed for the isolation of DNA from microorganisms, such as Gram-positive or Gram-negative bacteria and yeast. For optimal usage of the **NucleoMag® DNA Bacteria** kit for yeast or fungal mycelia samples specific bead tubes might be required (see section 2.4.2).

The **NucleoMag® DNA Bacteria** kit utilizes a unique buffer chemistry (patent pending), which does not require harmful and corrosive chaotropic salts or high concentrations of alcohols during the binding step.

Microbial samples such as Gram-positive bacteria, yeast and spores can be difficult to lyse due to their strong complex cell wall structures. The **NucleoMag® DNA Bacteria** kits combines enzymatic lysis and mechanical disruption of sample material with MN Bead Tubes or MN 96 Bead Plates.

The procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. After sample lysis (enzymatic treatment combined with mechanical disruption of the sample material using MN Bead Tubes or MN 96 Bead Plates), lysis mixtures can be cleared by centrifugation and binding conditions are adjusted by adding the Buffer IML. For binding of nucleic acids to the paramagnetic beads, Binding Buffer IMB and the NucleoMag® B-Beads are added to the transferred lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants using Wash Buffers IMW and 80 % ethanol. Residual ethanol from previous wash steps is removed by air drying. Next, highly purified DNA is eluted with the Buffer IME and can be used directly for downstream applications. The **NucleoMag® DNA Bacteria** kit can be used either manually, or automated on standard liquid handling instruments and automated magnetic separators.

We can provide personalized support, protocol information, or verified scripts for numerous platforms. For more information, please contact our technical support or visit ***www.mn-net.com/automation***.

2.2 Kit specifications

- **NucleoMag® DNA Bacteria** is recommended for the rapid manual and automated isolation of total DNA from microorganisms. DNA from bacteria or yeast is isolated simultaneously using magnetic bead based technology and can be directly subjected for subsequent downstream applications such as real-time PCR, Next Generation Sequencing, etc.
- **NucleoMag® DNA Bacteria** kit allows easy automation on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 120 minutes using the NucleoMag® SEP on a common liquid handling platform or less than 30 minutes using a magnetic rod based system (excluding sample lysis). For more information about the automation process and the availability of ready to run scripts for certain platforms, please contact your local distributor or MN directly.

Kit specifications at a glance	
Parameter	**NucleoMag® DNA Bacteria**
Technology	Magnetic bead technology
Format	Highly reactive superparamagnetic beads
Sample material	Microbial cell culture pellets of Gram-positive and Gram-negative bacteria and yeast
Sample amount	Up to approx. 40 mg wet weight
Typical yield	Varies by sample and disruption device.
Elution volume	50 – 200 µL
Use	For research use only

2.3 Handling, preparation, and storage of starting material

Microbial cells should be harvested from fresh microbial cultures by sedimentation via centrifugation. Supernatant should be removed carefully by aspiration. Microbial cell pellets can be used fresh or frozen (-20 °C to -80 °C) before starting the preparation.

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 the suggestions outlined in the subsequent sections.

2.4.1 Disruption device

The following devices are compatible with MN Bead Tubes or MN 96 Bead Plates. Please check whether MN Bead Tubes or MN 96 Bead Plates can be accommodated by the available tube adapters prior to starting the procedure.

MN Bead Tube Holder (for MN Bead Tubes) in combination with the Vortex-Genie® 2 (recommended).

Mixer Mill MM200, MM300, MM400 (Retsch®) (suitable for MN Bead Tubes or MN 96 Bead Plates; Please check the compability of the available adaptors prior to starting the procedure).

If other disruption devices (section 1.2) are intended to be used, consider section 2.4.3 and read carefully the WARNING note.

2.4.2 Type of bead tubes or bead plates

- 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	Recommended for	REF
MN Bead Tubes Type A (0.6 – 0.8 mm ceramic beads)	Soil and sediment, yeast cells	740786.50
MN Bead Tubes Type B (40 – 400 µm glass beads)	Gram-positive and -negative bacteria	740812.50
MN Bead Tubes Type D (3 mm steel beads)	Insect samples	740814.50
MN 96 Bead Plate Type B (40 – 400 µm glass beads)	Gram-positive and -negative bacteria	740851.4 740851.24
MN 96 Bead Plate Type D (3 mm steel beads)	Insect samples	740853.4 740853.24
Other types of bead tubes available for various applications		
MN Bead Tubes Type E (combination of 3 mm steel beads and 40 – 400 µm glass beads)	Difficult to lyse tissue containing Gram-positive bacteria	740815.50
MN Bead Tube Type G (5 mm steel beads)	Plant material	740817.50

2.4.3 Time and frequency of disruption

MN Bead Tubes

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**.

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

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

Sample material	MN Bead Tube	Disruption time / speed
Gram-negative bacteria E.g., *Escherichia coli, Vibrio fischeri*	MN Bead Tubes Type B (Alternative: Type A)	Approx. 12 min, full speed
Gram-positive bacteria E.g., *Bacillus subtilis, Corynebacterium glutamicum*	MN Bead Tubes Type B (Alternative: Type A)	Approx. 14 min, full speed
Yeast E.g., *Saccharomyces cerevisiae*	MN Bead Tubes Type A	Approx. 15 min, full speed
Filamentous fungi E.g., *Aspergillus nidulans. melon mold; Citrus mold; Potato mold*	MN Bead Tubes Type D	Approx. 15 min, full speed
Insect sample material E.g. fresh, frozen, dried or ethanol preserved	MN Bead Tubes Type D (Alternative: Type G)	Approx. 12 – 20 min, full speed
Lipid rich tissues E.g. brain, adipose or fatty fish tissue	MN Bead Tubes Type D	Approx. 20 min, full speed
Bacteria from insect sample material	MN Bead Tubes Type E	Approx. 20 min, full speed

Time and frequency of disruption using a Retsch Mixer Mill MM300

Sample material	MN Bead Tube	Disruption time / frequency
Gram-negative bacteria E.g., *Escherichia coli, Vibrio fischeri*	MN Bead Tubes Type B (Alternative: Type A,)	Approx. 6 min 30Hz
Gram-positive bacteria E.g., *Bacillus subtilis, Corynebacterium glutamicum*	MN Bead Tubes Type B (Alternative: Type A)	Approx. 6 min 30Hz
Yeast E.g., *Saccharomyces cerevisiae*	MN Bead Tubes Type A	Approx. 7 min 30 Hz
Filamentous fungi E.g., *Aspergillus nidulans*. melon mold; Citrus mold; Potato mold	MN Bead Tubes Type D	Approx. 1 – 2 min 25 Hz

Sample material	MN Bead Tube	Disruption time / frequency
Insect sample material E.g. fresh, frozen, dried or ethanol preserved	MN Bead Tubes Type D (Alternative: Type G)	Approx. 40 s, 20 Hz
Lipid rich tissues E.g. brain, adipose or fatty fish tissue	MN Bead Tubes Type D	Approx.1 min 10 Hz followed by Approx. 10 s, 20 Hz.

Note: Performance and stability testing has been conducted on the MN Bead Tubes Type A, B, C and D on a Retsch® Mixer Mill MM300 at highest frequency (30 Hertz) for up to 15 minutes (Type A, B and C) or up to 30 min (Type D). For optimal sample disruption, avoidance of DNA fragmentation, and highest DNA yield, see recommendation table above for adequate disruption conditions. Other disruption devices (see e.g. 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 respective device.

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. Integrity and tightness of the tube need to be controlled after every run.

***WARNING:** In section 5 – 8 certain liquid volume during the 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).*

MN 96 Bead Plate

The following recommendations have been established for the 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 a Retsch Mixer Mill MM300

Sample material	MN 96 Bead Plate	Disruption time / frequency
Gram-negative bacteria E.g., *Escherichia coli, Vibrio fischeri*	MN 96 Bead Plate Type B	Approx. 2 × 4 min*, 30 Hz
Gram-positive bacteria E.g., *Bacillus subtilis, Corynebacterium glutamicum*	MN 96 Bead Plate Type B	Approx. 2 × 4 min*, 30 Hz
Yeast	MN 96 Bead Plate Type B	Approx. 2 × 4 min*, 30 Hz
Insect sample material E.g. fresh, frozen, dried or ethanol preserved	MN 96 Bead Plate Type D	Approx. 2 × 20 s*, 20 Hz
Filamentous fungi E.g., *Aspergillus nidulans*, melon mold; Citrus mold; Potato mold	MN 96 Bead Plate Type D	Approx. 2 × 1 min*, 20 Hz

Note: Performance and stability testing has been conducted on the MN 96 Bead Plate Type B and D on a Retsch® Mixer Mill MM300 at highest frequency (30 Hertz) for up to 15 minutes (Type B and D). For optimal sample disruption, avoidance of DNA fragmentation, and highest DNA yield, see recommendation table above for adequate disruption conditions. 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 respective device.

WARNING:** Many modern disruption devices can cause very high energy input on the MN 96 Bead Plate. Depending on bead plate type and content (beads, liquid volume, sample type), especially high frequency of shaking and / or long shaking duration can cause breaking up of individual tubes or plates!* ***Never use the MN 96 Bead Plate with less than 12 tube strips. It is the responsibility of the user to perform initial stability test for the used MN 96 Bead Plates 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. Integrity and tightness of the tube need to be controlled after every run.

* Re-orient MN 96 Bead Plates vertically for 180° after the first disruption time. Ensure that all wells are sealed properly before and after each disruption. Samples which have been the closest to the machine body should be now the furthest apart.

WARNING: *In section 5 – 8 certain liquid volume during the 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 Magnetic separation systems

For use of **NucleoMag® DNA Bacteria**, the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information). The kit can also be used with other common separators.

Magnetic separator	Separation plate or tube
NucleoMag® SEP (MN REF 744900)	Square-well Block (MN REF 740481)
NucleoMag® SEP Mini (MN REF 744901)	1.5 mL or 2 mL reaction tubes (Sarstedt)
NucleoMag® SEP Maxi (MN REF 744902)	50 mL tubes (Falcon)
NucleoMag® SEP 24 (MN REF 744903)	24-Square-well Block U-bottom (MN REF 740448.4/.24)
Tecan Te-MagS™	1.5 mL tubes without lid (Sarstedt)

Static magnetic pins

Separators with static magnetic pins, for example, NucleoMag® SEP or other common magnetic separators are suitable for manual use and for use on liquid handling workstations: This type of separator is recommended in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully automated use on liquid handling workstations, a gripper tool is required; the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

Movable magnetic systems

Separators with moving magnetic pins: Magnetic pins/rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

Automated separators

Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

2.6 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and shaker to prevent cross contamination from well to well. Proceed as follows:

Adjusting shaker speed for binding and wash steps:

- Load 1000 µL dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check the plate surface for droplets again.
- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again, and use this setting for the washing step.

Adjusting shaker speed for the elution step:

- Load 100 – 200 µL dyed water to the wells of the collection plate and proceed as described above.

2.7 Handling of magnetic beads

Distribution of magnetic beads

A homogeneous distribution of the magnetic NucleoMag® B-Beads to the individual wells of the separation plate is essential for a high well to well consistency. Therefore, before distributing the magnetic beads, make sure that they are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. During automation, a premix step before aspirating the magnetic beads is recommended to keep them resuspended.

Magnetic separation time

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the magnetic beads to the magnetic pins should be checked and adjusted on each system. It is recommended to use the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the magnetic beads

Washing the magnetic beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down, however, is more efficient than mixing by shaking or magnetic mixing.

Method	Resuspension efficiency	Speed	Small elution volume possible	Number of tips needed
Magnetic mix	+	++	+	Low
Shaker	++	++	+++	Low
Pipetting	+++	+*	++	High

+: acceptable, ++: good, +++: excellent, * 8-channel pipetting device

2.8 Elution procedures

Purified DNA can be eluted directly with the supplied Buffer IME. Elution can be carried out in a volume of ≥ 50 µL. It is essential to cover the NucleoMag® B-Beads completely with Buffer IME during the elution step. The volume of dispensed Buffer IME depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution, the magnetic bead pellet should be resuspended completely in Buffer IME. For some separators, higher elution volumes might be necessary to cover the whole pellet.

3 Storage conditions and preparation of working solutions

- Store Buffer IMB upon arrival at 4 °C.
- Liquid Proteinase K is ready to use. After first time use, store Liquid Proteinase K at 4 °C or -20 °C
- Liquid RNase is ready to use. After first time use, store Liquid RNase at 4 °C or -20 °C.
- Storage of Buffer IML below 20 °C may cause precipitation of components. If precipitation is observed, incubate buffer at 30 – 40 °C for several minutes and mix well until all precipitate is dissolved completely. Cool down to room temperature before use.
- All other kit components should be stored at room temperature (15 – 25 °C) and are stable until: see package label. Storage at lower temperatures may cause precipitation of salts.

Before starting any **NucleoMag® DNA Bacteria protocol, prepare the following:**

- Wash Buffer IMW: Add the indicated volume of ethanol (96 – 100 %) to Wash Buffer IMW Concentrate. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer IMW can be stored at room temperature for at least one year.

	NucleoMag® DNA Bacteria	
REF	**1 × 96 preps** **744310.1**	**4 × 96 preps** **744310.4**
Wash Buffer IMW (Concentrate)	50 mL Add 25 mL ethanol	200 mL Add 95 mL ethanol

4 Safety instructions

It is always recommended to follow the rules of good laboratory practice.

Wear gloves and goggles and follow the laboratory safety instructions.

WARNING: *The use of other disruption devices like FastPrep® System (MPBiomedicals), 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 or bead plate destruction. Such disruption devices can cause high mechanical stress on the bead tubes or bead plates. Depending on bead tube/ bead plate 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 or bead plates.* ***If using such a disruption device, it is the responsibility of the user to perform initial stability tests to ensure stability of bead tubes or bead plates during the individual experimental setup (e.g., intensity of agitation).*** *See also section 2.4.3!*

When working with the **NucleoMag® DNA Bacteria** 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 ***http://www.mn-net.com/msds***).

The waste generated with the **NucleoMag® DNA Bacteria** 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.

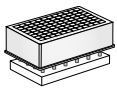
5 Protocol for isolation of DNA from bacterial and yeast samples

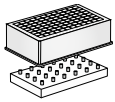
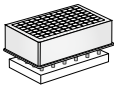
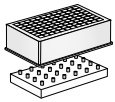
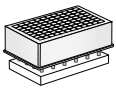
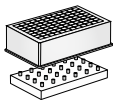
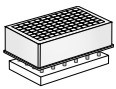
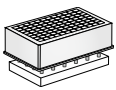
5.1 Protocol at a glance using MN Bead Tube Type B

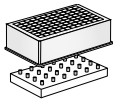
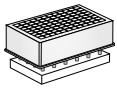
For additional equipment and hardware requirements, refer to section 1.2, 2.4.1, and 2.4.2 respectively.

MN Bead Tube Type A is recommended for optimal disruption of yeast cell pellets. MN Bead Tube Type D is recommended for optimal disruption of filamentous fungi. See section 2.4.3for additional information on sample disruption.

For detailed information on each step, see section 5.2.

1	**Prepare sample**	< 40 mg microbial pellet (wet weight) 140 µL IME	
2	**Lyse sample**	Transfer sample to a MN Bead Tube Type B 10 µL Liquid Proteinase K 2.5 µL Liquid RNase A Agitate on a swing mill or similar device 0.5 – 15 min 11,000 x *g*, 30 s	
3	**Adjust binding conditions**	460 µL IML Vortex 3 s, 11.000 x *g*, 30 s	
4	**Bind** DNA to NucleoMag® B-Beads	Up to 500 µL lysate 24 µL NucleoMag® B-Beads 320 µL IMB	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
			
		Remove supernatant after 5 min separation	

5	**Wash** with IMW	Remove Square-well Block from NucleoMag® SEP 600 µL IMW	
		Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
			
		Remove supernatant after 2 min separation	
6	**Wash** with 80 % ethanol	Remove Square-well Block from NucleoMag® SEP 600 µL 80 % ethanol	
		Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
			
		Remove supernatant after 2 min separation	
7	**Wash** with 80 % ethanol	Remove Square-well Block on NucleoMag® SEP 600 µL 80 % ethanol	
		Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
			
		Remove supernatant after 2 min separation	
8	**Dry** the beads	10 min at RT	

9	**Elute** DNA	Remove Square-well Block from NucleoMag® SEP **50-200 µL IME** *(Optional: Elute at 56 °C)*	
		Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Separate 2 min and transfer DNA into elution plate	

5.2 Detailed protocol using MN Bead Tube Type B

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.5). It is recommended to use a Square-well Block for separation (see section 2.5). Alternatively, isolation of DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

Before starting the preparation:

- Check if Buffer IMW was prepared according to section 3.
- MN Bead Tube Type A is recommended for optimal disruption of yeast cell pellets. MN Bead Tube Type D is recommended for optimal disruption of filamentous fungi.
- Check section 2.4.3 for additional information regarding time and frequency of disruption for sample material lysis

1 Prepare sample

Harvest microbial cells from a culture by centrifugation. Discard the supernatant.

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

Add **140 µL Buffer IME** and resuspend the pellet.

2 Lyse sample

Transfer the cell suspension into a MN Bead Tube Type B or another bead beating tube or plate suitable for the disruption of microorganisms.

Add **10 µL of Liquid Proteinase K, 2.5 µL Liquid RNase A** and close the tube.

Agitate the MN Bead Tube Type B on the MN Bead Tube Holder in conjunction with Vortex-Genie®, swing mill or similar device for **0.5 – 15 min** (e.g., 4 min for the MN Bead Tube Holder).

Note: Please refer to section 5.3 for sample disruption in a 96-well format.

Note: Optimal agitation duration, speed / frequency depends on the device used. For the MN Bead Tube Holder it is approximately 12 – 14 min; in a mixer mill MM200, MM300, MM400 (Retsch®), e.g., 6 min at maximal frequency (30 Hertz) is suitable (see section 2.4.3). 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!

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

Attention: Do not centrifuge at higher g-force, nor for longer than 30 s as this might damage the MN Bead Tube.

3 Adjust binding conditions

Add **460 µL Buffer IML** 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.

4 Bind DNA to NucleoMag® B-Beads

Transfer **up to 500 µL lysate to a Square-well Block**. Add **24 µL of NucleoMag® B-Beads** and **320 µL of Binding Buffer IMB**. Mix by pipetting up and down 6 times and **shake** for **5 min** at **room temperature**. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for **5 min** at room temperature.

Pipette gently in order to avoid foaming. If using electronic pipettes, use only 40 % of the total volume as mixing volume.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 5 min until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet might not be visible in this step. Remove supernatant from the opposite side of the well.

5 Wash with IMW

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer IMW** to each well and resuspend the beads by shaking until the beads are resuspended completely **(1 – 3 min)**. Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 – 5 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

6 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely **(1 – 3 min)**. Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

7 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely **(1 – 3 min)**. Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

8 Air dry magnetic beads

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Air dry the magnetic beads for **10 – 15 min** at **room temperature.**

9 Elute DNA

Add desired volume of **Buffer IME (50 – 200 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5 min** at room temperature. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5 – 10 min** at **room temperature** or **56 °C**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified DNA to suitable elution plate.

5.3 Detailed protocol using MN 96 Bead Plate Type B

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.5). It is recommended using a Square-well Block for separation (see section 2.5). Alternatively, isolation of DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

- For hardware requirements, refer to section 2.4.1 and 2.4.2.

1 Prepare sample

Place individual insect samples into each well of the MN 96 Bead Plate Type B.

Spin the MN 96 Bead Plate B briefly down 1 sec in order concentrate the beads at the bottom.

Up to approximately 40 mg of wet weight can be processed. Remove excess liquid (e.g., growth medium) from the sample.

Add **140 µL Buffer IME** to each sample.

2 Lyse sample

Add **10 µL of Liquid Proteinase K, 2.5 µL Liquid RNase A** and seal the tubes strips tightly with the cap strips.

Agitate the MN 96 Bead Plate Type B on a swing mill or similar device for **2 × 0.5 – 5 min** at **30 Hertz**.

Note: Re-orient MN 96 Bead Plates vertically for 180° after the first disruption time. Samples which have been the closest to the machine body should be now the furthest apart. Ensure that each well is sealed properly before and after each disprution.

Note: Optimal agitation duration, speed / frequency depends on the device used. For the mixer mill MM300 (Retsch®), e.g., 2 × 4 min at maximal frequency (30 Hertz) is suitable (see section 2.4.3). 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!

Centrifuge the MN 96 Bead Plate B for **5 min** at **2,000 x *g*** to clean the lid

Attention: Do not centrifuge at higher g-force, nor for longer than 30 s as this might damage the MN 96 Bead Plate.

3 Adjust binding conditions

Add **460 µL Buffer IML,** seal the tubes strips with cap strips and **mix** (e.g., vortex for 10 s or invert).

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

Centrifuge for **5 min** at **2,000 x** *g*.

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

Attention: Do neither centrifuge at higher g-force, nor for longer than 30 s because this might damage the MN 96 Bead Plate.

4 Bind DNA to NucleoMag® B-Beads

Transfer **up to 500 µL lysate to a Square-well block**. Avoid aspiration of cellular debris.

Note: Individual tubes strips can be removed from the Rack of Tube Strips allowing an easier access for supernatant aspiration

Add **24 µL of NucleoMag® B-Beads** and **320 µL of Binding Buffer IMB**. Mix by pipetting up and down 6 times and **shake** for **5 min** at **room temperature**. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

Pipette gently in order to avoid foaming. If using electronic pipettes, use only 40 % of the total volume as mixing volume.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 5 min until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet might not be visible in this step. Remove supernatant from the opposite side of the well.

5 Proceed with step 5 of detailed protocol for isolation of DNA from microbes (see section 5.2).

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	*Insufficient mechanical treatment of sample material* • For most sample sources, we recommend mechanical disruption with the specialized MN Bead Tubes on the MN Bead Tube Holder or with commercial bead mills, mixers or homogenizers. See section 2.4.1 and 2.4.2 for further recommendations. *Insufficient extraction of nucleic acids during lysis* • To obtain higher yields of nucleic acids, an additional heat incubation for 10 min at 56 °C can be performed prior mechanical treatment. *Sample contains too much RNA* • Add 2.5 µL Liquid RNase A following mechanical lysis. If this is not successful, add the enzyme to the cleared lysate and incubate for 10 min at room temperature. *Insufficient Elution buffer volume* • Beads pellet must be covered completely with Buffer IME *Insufficient performance of elution buffer during elution step* • Remove residual buffers during the separation steps completely. Remaining buffers decrease efficiency of following wash steps and elution step. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic pellet is not visible in the lysate. *Aspiration and loss of beads* • Time for magnetic separation was too short or aspiration speed was too high.

Problem	Possible cause and suggestions
Low purity / Low sensitivity	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down. • Repeat washing step with wash Buffer IMW
Suboptimal performance of DNA in downstream applications	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down. • Repeat washing step with wash buffer IMW. *Carry-over of ethanol from wash buffers* • Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications. *Ethanol evaporation from wash buffers* • Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs.
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

Problem	Possible cause and suggestions
Cross contamination	*Contamination of the rims* • Do not moisten the rims of the Square-well Block when transferring the lysate. If the rim of the wells is contaminated, seal the Square-well Block with Self-adhering PE Foil (see ordering information) before starting the shaker.

6.2 Ordering information

Product	REF	Pack of
NucleoMag® DNA Bacteria	744310.1 744310.4	1 × 96 preps 4 × 96 preps
MN Bead Tube Type A (0.6 – 0.8 mm ceramic beads, recommended for yeast, soil and sediments)	740786.50	50
MN Bead Tube Type B (40 – 400 µm glass beads, recommended for bacteria)	740812.50	50
MN Bead Tube Type D (3 mm steel beads, recommended for insects)	740814.50	50
MN Bead Tube Type E (40 – 400 µm glass beads and 3 mm steel beads, recommended for hard to lyse bacteria within insect samples)	740815.50	50
MN Bead Tube Type G (5 mm steel beads, recommended for plant material)	740817.50	50
MN Bead Tube Holder (Tube Holder for Vortex-Genie® instrument and a 3-inch platform in order to house up to 12 MN Bead Tubes)	740469	1
MN 96 Bead Plate Type A (Rack of prefilled tube strips (8 × 12) containing 0.6-0.8 mm ceramic beads; suitable in conjunction with mixer mill)	740853.4 740853.24	4 24
MN 96 Bead Plate Type B (Rack of prefilled tube strips (8 × 12) containing 40 – 400 µm glass beads; suitable in conjunction with mixer mill)	740851.4 740851.24	4 24

Product	REF	Pack of
MN 96 Bead Plate Type D (Rack of prefilled tube strips (8 × 12) containing 3 mm steel beads; suitable in conjunction with mixer mill)	740853.4 740853.24	4 24
Liquid Proteinase K	740396	5 mL
NucleoMag® SEP	744900	1
NucleoMag® SEP Mini	744901	1
NucleoMag® SEP Maxi	744902	1
NucleoMag® SEP 24	744903	1
Square-well Blocks	740481 740481.24	4 24
Self adhering PE Foil	740676	50 sheets
Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)	740486.24	24
KingFisher® 96/Flex Accessory Kit A (set consists of 16 Square-well Blocks, 4 Deep-well tip combs, 4 Elution Plates; for 4 × 96 NucleoMag® DNA Bacteria preps using KingFisher® 96/Flex platform)	744950	1 set
KingFisher® DUO Accessory Kit (8 Square-well Blocks, 8 Tip Combs, 8 Elution Strips; for 8 × 12 NucleoMag® DNA Bacteria preps using KingFisher® DUO/DUO Prime platform.	744952	1 set
96 Deep-well plates for magnetic rod systems	744955	25
8-well Tip Combs for magnetic rod system	744960	50
8-well Accessory Kit magnetic rod systems	744961	1 set

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-NAGEL's 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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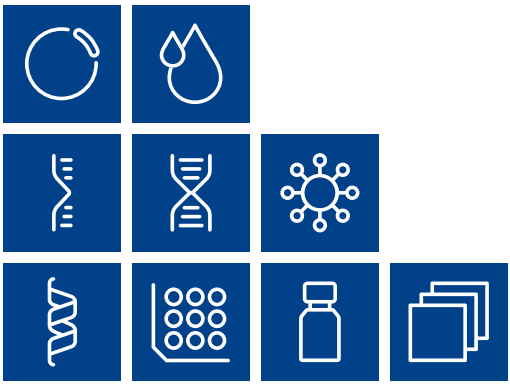
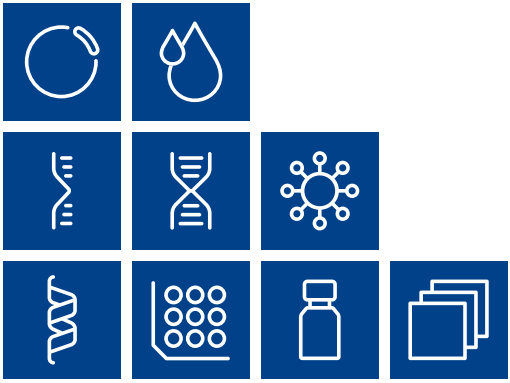


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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