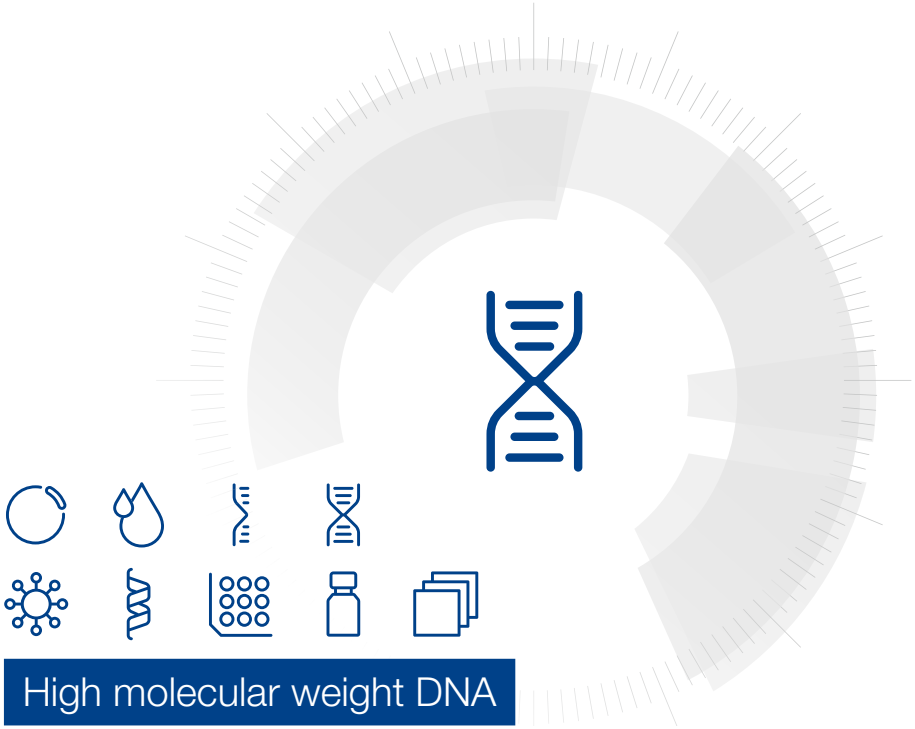
Bioanalysis

MACHEREY-NAGEL

User manual



High molecular weight DNA

- NucleoMag® HMW DNA

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1 Components

1.1 Kit contents

NucleoMag® HMW DNA	
REF	**1 × 96 preps 744160.1**
NucleoMag® B-Beads	2 × 1.25 mL
Lysis Buffer HM1	75 mL
Lysis Buffer HMb	75 mL
Precipitation Buffer HMc	25 mL
Binding Buffer HM2	40 mL
Wash Buffer HM3	100 mL
Wash Buffer HM4	125 mL
Rinsing Buffer HM5	60 mL
Elution Buffer HM6	13 mL
Liquid Proteinase K	2 × 1.25 mL
RNase A (lyophilized)	2 × 100 mg
Leaflet	1

1.2 Reagents, consumables and equipment to be supplied by user

Reagents:

- 70 % ethanol (v/v) (absolute or non-denatured ethanol)
- *Optional: Dedicated lytic enzymes and reaction buffers (e.g., lyticase, zymolyase, lysozyme, cellulase, chitinase, pectinase, driselase and others)*
- *Optional: Enzymatic DNA Repair Kit to reduce amount of nicked DNA and to increase average read length in third generation sequencers*

Consumables:

- Disposable wide bore pipette tips (aerosol barrier pipets tips are recommended to avoid cross-contaminations)
- 2 mL microtubes

Equipment:

- Heating block, incubator or water bath, alternatively: Suitable thermomixer such as Eppendorf or Starlab Thermomixer)
- Microcentrifuge for 2 mL reaction tubes, capable of reaching 11,000 x *g*
- Mortar and pestle plus liquid nitrogen

General:

- Personal protection equipment (e.g., lab coat, gloves, goggles)

Product	REF	Pack of
Magnet for magnetic beads separation e.g. NucleoMag® SEP Mini (suitable for 1.5 – 2 mL tubes)	744901	1

1.3 About this user manual

It is strongly recommended that first time users of the NucleoMag® HMW DNA 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.

2 Product description

2.1 The basic principle

The **NucleoMag® HMW DNA** kit is designed for the isolation of high molecular weight DNA from cells, tissue and plant material. Additionally, compatibility has been shown for whole blood (EDTA), saliva, buccal swabs as well as bacteria and yeast samples. The procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved enzymatically using Lysis Buffer HM1 or HMb and Proteinase K. For binding of nucleic acids to the paramagnetic beads, Binding Buffer HM2 and the NucleoMag® B-Beads are added to the transferred and cleared lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers HM3, HM4 and 70 % ethanol. Residual ethanol from previous wash steps is removed by using Rinsing Buffer HM5. Next, highly purified DNA is eluted with Elution Buffer HM6 and can be used directly for downstream applications. The **NucleoMag® HMW DNA** kit can be used either manually, or automated on standard liquid handling instruments and automated magnetic separators.

2.1.1 Kit specifications

Kit specifications at a glance	
Parameter	**NucleoMag® HMW DNA**
Technology	Magnetic bead technology
Format	Highly reactive superparamagnetic beads
Sample material	Cells, Tissue and plant material
Sample amount (wet weight)	Cells: Up to 1×10^6 Tissue: Up to 20 mg Plant: Up to 50 mg Bacteria: Up to 25 mg (gram-negative), up to 35 mg (gram-positive) Yeast: Up to 50 mg depending on species
Typical yield	Varies by sample type and quality
Average fragment length	≥ 50 kb depending on sample quality, sample lysis and processing
Elution volume	100 µL
Processing	Manual and automated
Processing time	Manual preparation time 1h for 24 samples, 1.5h for 48 samples Automated preparation time: Approx. 60 min on magnetic rod instrument
Use	For research use only

2.2 Working with high molecular weight DNA

Make sure to handle isolated DNA with wide bore pipette tips and minimize shearing forces such as vigorous vortexing or excessive pipetting steps wherever possible. Avoid repeated freeze-thawing cycles of sample material.

DNases will be removed by the preparation. Take care to use certified DNase-free centrifuge tubes and pipette tips.

The eluted DNA might be nicked, resulting in shorter average read lengths in single molecule sequencing reactions. Use of repair kits could reduce the amount of nicks and increase average reading lengths. Suitable kit recommendations might be given by the supplier of the sequencing machine.

Very high molecular weight DNA will increase the viscosity of the sample lysates, especially when using a gentle enzymatic lysis which returns the highest molecular weight. In combination with a higher sample input amount this high viscosity might lead to difficulties during the resuspension of the magnetic bead pellet. Add additional buffer HM1 if difficulties during the resuspension are observed. Alternatively, reduce sample input amount to 25 – 50 % of the maximum amount, especially when testing an unknown sample for the first time.

2.3 Sample Lysis

2.3.1 General recommendations

The most gentle and thus recommended lysis procedure is to lyse the sample enzymatically. An incubation of the sample in Lysis Buffer HM1, supplemented with Proteinase K, results in the release of DNA into the lysis mixture where DNA is stabilized. Independent of the chosen sample homogenization and lysis procedure, an incubation time of about 30 minutes at 50 °C is sufficient for most sample types. **If needed, the incubation time can be increased up to an overnight incubation** without negative effects on DNA integrity and molecular weight. This might be relevant for larger solid tissue biopsy blocks to ensure complete sample lysis. After complete lysis, residual RNA is digested by RNase A.

Due to the heterogeneity of sample materials, especially in respect to plant materials, two lysis buffer systems are included in this kit. The standard protocol utilizes lysis buffer HM1, based on the established CTAB procedure. Additionally, the SDS based Lysis Buffer HMb is provided, which requires a subsequent protein precipitation using buffer HMc.

Nevertheless, some sample types require a more dedicated lytic enzyme (e.g., lysozyme for bacteria, zymolyase for yeast, not supplied). The before mentioned enzymes will be active in Lysis Buffer HM1, but other enzymes might be inactivated. When using a specialized enzyme it is recommended to perform a small scale experimental test to check if this enzyme is active in Lysis Buffer HM1, HMb or requires a dedicated reaction buffer.

In case of the use of a dedicated lytic enzyme, use the reaction buffer supplied or recommended by the supplier of the enzyme and add Lysis Buffer HM1 and Proteinase K after the enzymatic digestion is complete.

Please keep in mind that excess substrate concentrations (i.e., too much sample) might decrease the enzymatic reaction speed. The optimal ratio of enzyme to substrate (sample) and necessary cofactors must be determined empirically or according to instructions provided by the supplier of the enzyme.

Make sure the contact surface area between reaction buffer and sample is as high as possible and agitate the reaction mixture continuously if possible.

Cells expressing thick cell walls like plant leaf cells cannot be completely lysed enzymatically. It is recommended to grind the sample material under liquid nitrogen with mortar and pestle in this case. Always use precooled material and do not let the sample thaw in between. Grinding of sample material with mortar and pestle under liquid nitrogen will result in a typical mean fragment length distribution starting at about 15 kb with a maximum at about 150 kb and above.

When grinding bacteria with mortar and pestle under liquid nitrogen make sure not to overload the magnetic beads later on. Intact cells and excessive sample material will complicate magnetic bead resuspension and might result in low purities and lower yields. It is preferred to lyse bacteria and yeast enzymatically.

Bead beating might also be an option for **bacteria** or **yeast cells**. Average fragment length might decrease by using a mechanical cell disruption down to around **50 kb or lower**, but total yield will increase.

Ultrasonic or high pressure devices for cell lysis will also result in decreased fragment lengths and are not recommended.

2.3.2 Lysis of solid tissue and cells

Solid tissues like biopsy samples or cells can be lysed directly in Lysis Buffer HM1 without mechanical disruption. Nevertheless, the solid sample should be cut into small pieces. The smaller the tissue pieces, the faster a complete cell lysis will occur and the less influence DNases / oxygen radicals will have.

2.3.3 Lysis of plant material

Plant cells (e.g., leaves or roots) express a strong cell wall which might be additionally coated by wax or lignin. If no specialized lytic enzymes are available, it is recommended to grind the cells with mortar and pestle under liquid nitrogen.

Plants are very heterogeneous and contain varying amounts of polyphenols, acidic components, or polysaccharides which can lead to suboptimal DNA extraction or performance in downstream applications. Therefore, two lysis buffer systems are included in this kit. The standard protocol utilizes lysis buffer HM1, based on the established CTAB procedure. Additionally, the SDS based lysis buffer HMb, which requires a subsequent protein precipitation using buffer HMc, is supplied. For some plant species Lysis Buffers HM1 and HMb can be used with similar results. However, for most plant material the lysis efficiency is different due to the negative charge of SDS and the positive charge of CTAB. Additionally, reducing agents, such as TCEP can be used as supplement within buffer HMb. For a large variety of plant species lysis buffer HM1 allows good results.

2.3.4 Lysis of liquid samples (e.g. blood and saliva samples)

Liquid samples, such as whole blood samples (EDTA) or saliva samples can be directly subjected to an enzymatic lysis in combination with the respective lysis buffers (e.g. HM1 for saliva or BQ1 for whole blood samples (EDTA). Therefore, 200 µL of whole blood EDTA sample are combined with 25 µL Liquid Proteinase K and 200 µL of Lysis buffer BQ1 (REF 740923). The mixture is then incubated for 10 min with moderate shaking at room temperature. Proceed then with step 4 (RNA digestion) of the detailed protocol in section 5.2.

2.3.5 Lysis of bacteria

Microbial samples such as gram-positive and gram-negative bacteria can be difficult to lyse due to their strong and complex cell wall structures. Therefore, the use of dedicated enzymes to specifically digest these cell walls is recommended. **Bacteria**, especially gram-positive bacteria, might be lysed by **lysozyme**, which is not included in the kit and have to be supplied by the user. **Please refer to the protocol supplied by the enzyme manufacturer / vendor** or contact MACHEREY-NAGEL (support@mn-net.com) if unsure how to use specialized lytic enzymes.

It is recommended to use lysozyme as a 100 mg/mL stock solution.

Harvest bacteria by centrifugation and resuspend the pellet (up to 25 mg for gram-negative bacteria, up to 35 mg for gram-positive bacteria) in 600 µL of lysis buffer HM1. Add 25 µL lysozyme (100 mg/mL) stock solution, mix and incubate for at least 30 – 60 min at 37 °C. Subsequently, add 25 µL of Liquid Proteinase K and proceed with the incubation at 56 °C.

2.3.6 Lysis of yeast

Yeast samples can be difficult to lyse due to their very complex and proteinaceous cell wall structures. Therefore, the use of dedicated enzymes to specifically digest these cell walls is recommended. **Yeast cells** might be lysed by **zymolyase (lyticase),** which is not included in the kit and has to be supplied by the user. **Please refer to the protocol supplied by the enzyme manufacturer / vendor** or contact MACHEREY-NAGEL (support@mn-net.com) if unsure how to use specialized lytic enzymes.

It is recommended to use zymolyase as a 100 mg/mL stock solution.

Harvest yeast by centrifugation and resuspend the pellet (up to 30 mg) in 600 µL of lysis buffer HM1. Add 10 µL zymolyase (50 U), mix and incubate for at least 60 – 120 min at 37 °C, preferably under constant agitation. Subsequently, add 25 µL of Liquid Proteinase K and proceed with the incubation at 56 °C.

2.3.7 Alternative lysis procedures

Due to wide variety of sample materials and species, which results varying amounts of polyphenols, acidic components, polysaccharides, fatty acid or varying composition of cell walls, two lysis buffer systems are included in this kit. The standard protocol utilizes lysis buffer HM1, based on the established CTAB procedure. Additionally, the SDS based lysis buffer HMb is provided. This lysis buffer can be combined with a subsequent protein precipitation using buffer HMc (recommended for plant material if using buffer HMb) or can be used as standalone lysis buffer without precipitation step (e.g. for bacteria).

Buffer HM1 will denature many proteins and reduce the reaction speed up to complete inhibition. Nevertheless, the reaction speed was found to be sufficiently high for Proteinase K, RNase A, zymolyase and lysozyme in Buffer HM1. When using a different enzyme, always use a reaction buffer recommended for this enzyme. Refer to manufacturers' recommendations or literature for buffer composition and necessary sample to enzyme ratios as well as incubation conditions. Do not combine other enzymes with Proteinase K. To prevent denaturation or degradation of those enzymes by the components of Buffer HM1 (detergents, Proteinase K) it is recommended to perform the lysis in the reaction buffer supplied or recommended by the manufacturer before the addition of Buffer HM1 and Proteinase K. Do not exceed a total lysis volume of 600 µL.

2.4 Handling of magnetic beads

Distribution of beads

A homogeneous distribution of the magnetic beads to the individual samples is essential for a high consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. During automation, a premix step before aspirating the beads is recommended to keep the beads resuspended.

Resuspension of beads

Resuspension of the magnetic beads during each step is essential for reliable extraction. Resuspension can be achieved by pipette-mixing using wide bore pipette tips or using a shaking device. The efficient resuspension on a shaking device is dependent on the speed and orbit. It is recommended to use a shaking device with an orbit of at least 2 – 3 mm (e.g. Eppendorf Thermoshaker). The top part of the NucleoMag® SEP Mini can be used to ease the resuspension of the magnetic beads. Therefore, the top parts of two NucleoMag® SEP Minis are combined resulting in a microtiter plate footprint, which fits on common plate shaking devices. Rotation of the 2 mL tubes within the top part of the NucleoMag® SEP Mini as well as the shaking facilitates a gentle bead resuspension.

When using a plate shaker in combination with a deep-well plate for the binding, washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and specific shaking devices to prevent cross-contamination from well to well.

2.5 Automation

Liquid handling systems

The NucleoMag® HMW DNA kit can be automated on various liquid handling platforms using wide bore pipette tips and the NucleoMag® SEP (MN REF: 744900) in combination with the Square-well Block (MN REF: 740481) and a suitable shaking device for optimal and gentle resuspension during binding, washing and elution steps. Additionally, a gripper tool is required for fully automated use on liquid handling workstations. The gripper needs to transfer the plate to the magnetic separator for the separation of the beads and then to the shaker module for resuspension of the beads. The complete resuspension of the magnetic beads during the extraction is essential for a reliable performance and should be checked during the validation on liquid handling systems. Alternatively, beads can be resuspended in the buffer by careful pipetting up and down using wide bore tips.

Magnetic rod systems

The NucleoMag® HMW DNA kit can be automated on magnetic rod systems such as the IsoPure Mini, MagnetaPure32+ or KingFisher® device using compatible consumables.

In order to reduce the impact of shearing forces, gentle movements of the magnetic rods are recommended.

Please contact our support for assistance regarding the automation of our kits.

2.6 Elution procedures

Purified DNA can be eluted directly with the supplied Elution Buffer HM6 (5 mM Tris/HCl, pH 8.5). Elution can be carried out in a volume of ≥ 100 µL. It is essential to cover the NucleoMag® B-Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer 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 the elution buffer. For some separators, higher elution volumes might be necessary to cover the whole pellet. A slow and gentle aspiration is recommended to avoid bead carry over into the final eluate. Furthermore, a second magnetic separation is recommended to reduce bead carry over.

3 Storage conditions and preparation of working solutions

Attention: *Buffer HM3 und HM4 contain chaotropic salts! Wear protective clothing, gloves and goggles!*

- Store Buffer HM2 upon arrival at 4 °C.
- All other components of the **NucleoMag® HMW DNA** kit should be stored at room temperature (15 – 25 °C) and are stable until: see package label.
- Once the enzymes have been opened it is recommended to store them at 4 °C.

All buffers are delivered ready to use.

Before starting any NucleoMag® HMW DNA protocol prepare the following:

Lysis Buffer HM1 / HMb: Check for precipitated detergent especially after storage at temperatures below 20 °C. If necessary, incubate the bottles for several minutes at 30 – 40 °C and mix well until the precipitate is re-dissolved completely.

RNase A: Before first use, add 1 mL of water to each vial of the lyophilized RNase A. Store RNase A at 4 °C.

4 Safety instructions

When working with the **NucleoMag® HMW DNA** 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***).

Caution: Guanidine hydrochloride in buffer HM3 and sodium perchlorate in buffer HM4 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 **NucleoMag® HMW DNA** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocol for isolation of high molecular weight DNA using Lysis Buffer HM1

5.1 Protocol at a glance

Carefully read the detailed protocol (section 5.2) as well as section 2 before starting the procedure. This protocol is designed for magnetic separators with static magnets (e.g., NucleoMag® SEP Mini). It is recommended to use 2 mL microcentrifuge tubes instead of 1.5 mL tubes. Alternatively, isolation of DNA can be performed in a Deep-well plate with suitable magnetic separators (e.g. Square-well Block and NucleoMag® SEP). This protocol is designed for manual use.

For hardware requirements and additional consumables, refer to sections 1.2.

Before starting the preparation:

- Check section 2.2 and 2.3 for additional information about the amount of starting material and sample material preparation.
- Check if wide bore pipette tips and 2 mL reaction tubes are used.
- Check Lysis Buffer HM1 for precipitates according to section 3.
- Check if 70 % ethanol was prepared according to section 1.2.
- Take precautions and wear the needed safety equipment when working with liquid nitrogen.

1	**Prepare and lyse sample**	Prepare sample material 600 µL HM1 25 µL Liquid Proteinase K Mix or invert 56 °C, 30 – 150 min, careful agitation (e.g. 900 rpm) **Note:** *Please check section 5.2 and 2.3. for alternative lysis recommendations*
2	**Precipitate debris**	11,000 x *g*, 1 min Transfer up to 500 µL to a fresh 2 mL tube
3	**Digest RNA**	20 µL RNase Mix or invert 15 min, RT Briefly centrifuge the 2 mL tube to remove drops from the lid
4	**Bind DNA to NucleoMag® B-Beads**	25 µL NucleoMag® B-Beads 400 µL HM2

		Mix by pipetting up and down *(Optional: Mix by shaking for 5 min, 1000 rpm at RT)* *Note: A phase formation can occur in this step. Depending on sample type, a full resuspension might not be possible.*
		Remove supernatant after 5 min separation
5	**Wash with HM3**	Remove reaction tubes from NucleoMag® SEP Mini 900 µL HM3
		Resuspend: Mix by pipetting up and down (10 x) *(Optional: Mix by shaking for 2 min, 1000 rpm at RT)*
		Remove supernatant after 2 min separation
6	**Wash with HM4**	Remove reaction tubes from NucleoMag® SEP Mini 900 µL HM4
		Resuspend: Mix by pipetting up and down (10 x) *(Optional: Mix by shaking for 2 min, 1000 rpm at RT)*
		Remove supernatant after 2 min separation
7	**1st Wash with 70 % EtOH**	Remove reaction tubes from NucleoMag® SEP Mini 900 µL 70 % EtOH
		Resuspend: Mix by pipetting up and down (10 x) *(Optional: Mix by shaking for 2 min, 1000 rpm at RT)*
		Remove supernatant after 2 min separation
8	**2nd Wash with 70 % EtOH**	Remove reaction tubes from NucleoMag® SEP Mini 900 µL 70 % EtOH
		Resuspend: Mix by pipetting up and down (10 x) *(Optional: Mix by shaking for 2 min, 1000 rpm at RT)*
		Remove supernatant after 2 min separation
	Rinse the beads	Leave reaction tubes on NucleoMag® SEP Mini 600 µL HM5
		Incubate for 30 – 60 sec *Note: Do not resuspend the beads in Buffer HM5*

		Remove supernatant
9	**Elute DNA**	Remove reaction tube from NucleoMag® SEP Mini 100 µL HM6
		Resuspend: *Mix by pipetting up and down (10 x)* *(Optional: Mix by shaking for 5 min, 1000 rpm at RT)*
		Separate 2 min and transfer DNA into elution plate Optional: Perform a second separation to remove residual magnetic beads from the eluate

5.2 Detailed protocol

This protocol is designed for magnetic separators with static magnets (e.g., NucleoMag® SEP Mini). It is recommended to use 2 mL microcentrifuge tubes instead of 1.5 mL tubes. Alternatively, isolation of DNA can be performed in a Deep-well plate with suitable magnetic separators (e.g. Square-well Block and NucleoMag® SEP). This protocol is designed for manual use.

For hardware requirements and additional consumables, refer to sections 1.2.

Before starting the preparation:

- Check section 2.2 and 2.3 for additional information about the amount of starting material and sample material preparation.
- Check if wide bore pipette tips and 2 mL reaction tubes are used.
- Check lysis buffer HM1 for precipitates according to section 3.
- Check if 70 % ethanol was prepared according to section 1.2.
- Take precautions and wear the needed safety equipment when working with liquid nitrogen.

1	**Prepare samples** • Cells: Pellet up to 1 × 10^6 cultured cells in 2 mL tube (not supplied) • Solid tissue: Cut solid tissue into small pieces and transfer up to 20 mg into a 2 mL tube (not supplied)**.** • Plant material: Homogenize about 20 – 50 mg plant material and transfer into a 2 mL tube. • Bacteria: Pellet up to 25 mg (wet weight) of gram-negative or up to 35 mg (wet weight) of gram-positive bacteria in a 2 mL tube. • Yeast: Pellet up to 30 mg (wet weight) of yeast cells in a 2 mL tube.

2 Lyse samples

Add **600 µL Lysis Buffer HM1 to the sample in the 2 mL tube and gently resuspend the sample material if necessary.**

Add **25 µL of Liquid Proteinase K**, gently agitate by inversion or mix and incubate for **30 – 150 min at 56 °C**. Please check the following recommendation for the different sample types:

- Cells: 60 min
- Solid tissue: 150 min
- Plant material: 30 min
- Bacteria: Incubate for at least 30 min with 25 µL lysozyme (100 mg/mL stock solution) at 37 °C prior to the addition of Liquid Proteinase K. Add Liquid Proteinase K and incubate for 30 min at 56 °C.
- Yeast: Incubate for at least 60 min with 10 µL zymolyase (50 U) at 37 °C prior the addition of Liquid Proteinase K. Add Liquid Proteinase K and incubate for 30 min at 56 °C.

Continuous careful agitation (e.g. at 900 rpm) of the lysate is advantageous, but not necessary. Mix lysates by inversion or slow rotation from time to time.

Note: See section 2.2 for more information about the amount of starting material and the recommended lysis procedure.

3 Precipitate debris

Centrifuge for **1 min** at **11,000 x *g***.

Transfer up to 500 µL carefully to a fresh 2 mL microcentrifuge tube (not provided).

Note: Transfer as much lysate as possible to the 2 mL microcentrifuge tube. Avoid transferring material from the pellet or material, which floats on top of the lysate. Fibers or husks in the supernatant may clog the pipette tip. Aspirate the supernatant slowly and carefully.

4 Digest RNA

Add **20 µL of RNase A to** each sample and mix by inverting the tube several times.

Incubate for 15 min at room temperature.

Briefly centrifuge the 2 mL tube to remove drops from the lids (short spin only).

5 Bind DNA to NucleoMag® B-Beads

Add **25 µL of NucleoMag® B-Beads** and **400 µL of Binding Buffer HM2**. Mix by pipetting up and down 10 times using wide bore pipette tips and incubate for 5 min at room temperature.

Note: Be sure to resuspend the NucleoMag® B-Beads before usage. Vortex storage bottle until a homogenous suspension has formed.

Note: The high viscosity of buffer HM2 can lead to a phase formation. Depending on sample type and amount, a complete bead resuspension might not be possible during the whole incubation time.

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

Alternatively, resuspend beads by shaking for **5 min** at **1000 rpm using two top parts of the NucleoMag® SEP Mini (see section 2.4).**

Note: Please check section 2.4 for recommendations regarding bead resuspension.

Place the 2 mL tubes in the NucleoMag® SEP Mini magnetic separator or move the top part of the NucleoMag® SEP Mini containing the samples from the shaker onto the base part.

Separate the magnetic beads against the side of the tube by placing the 2 mL tube on the NucleoMag® SEP Mini 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 tube.

6 Wash with HM3

Remove the top part of the NucleoMag® SEP Mini from the magnetic base part.

Add **900 µL Buffer HM3 directly onto** the magnetic bead pellet and resuspend the beads by repeated pipetting up and down 10 times using wide bore pipette tips.

Alternatively, resuspend beads by shaking for 2 min at 1000 rpm.

Separate the magnetic beads by placing the top part onto the magnetic base part of the NucleoMag® SEP Mini 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 HM4

Remove the top part of the NucleoMag® SEP Mini from the magnetic base part.

Add **900 µL Buffer HM4 directly onto** the magnetic bead pellet and resuspend the beads by repeated pipetting up and down 10 times using wide bore pipette tips.

Alternatively, resuspend beads by shaking for 2 min at 1000 rpm.

Separate the magnetic beads by placing the top part onto the magnetic base part of the NucleoMag® SEP Mini magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

8 **1st Wash with 70 % ethanol**

Remove the top part of the NucleoMag® SEP Mini from the magnetic base part.

Add **900 µL Buffer 70 % ethanol directly onto** the magnetic bead pellet and resuspend the beads by repeated pipetting up and down 10 times using wide bore pipette tips.

Alternatively, resuspend beads by shaking for 2 min at 1000 rpm.

Separate the magnetic beads by placing the top part onto the magnetic base part of the NucleoMag® SEP Mini magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

9 **2nd Wash with 70 % ethanol**

Remove the top part of the NucleoMag® SEP Mini from the magnetic base part.

Add **900 µL Buffer 70 % ethanol directly onto** the magnetic bead pellet and resuspend the beads by repeated pipetting up and down 10 times using wide bore pipette tips.

Alternatively, resuspend beads by shaking for 2 min at 1000 rpm.

Separate the magnetic beads by placing the top part onto the magnetic base part of the NucleoMag® SEP Mini magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

Note: Remove all the supernatant. Use a low volume pipette tip to remove traces of 70 % ethanol.

10 **Rinse magnetic beads**

Leave the top part of the NucleoMag® SEP Mini on the magnetic base part.

Gently add **600 µL HM5** onto the opposite wall of the magnetic bead pellet and incubate for 45 – 60 s while the beads are still attracted to the magnets. Then aspirate and discard the supernatant.

Note: Do not resuspend the beads in buffer HM5.

Alternatively, air dry the magnetic beads (with opened lid) for **10 – 15 min** at room temperature.

11 **Elute DNA**

Remove the top part of the NucleoMag® SEP Mini from the magnetic base part.

Add desired volume of **Elution Buffer HM6 (100 – 200 µL) directly onto** the magnetic bead pellet and resuspend the beads by repeated pipetting up and down 10 times using wide bore pipette tips and incubate for 5 – 10 min at room temperature.

Alternatively, resuspend beads by shaking for 5 min at 1000 rpm.

Separate the magnetic beads by placing the top part onto the magnetic base part of the NucleoMag® SEP Mini magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Transfer the supernatant containing the purified DNA to a new tube.

6 Support Protocol for sample lysis using buffer HMb and HMc

6.1 Detailed protocol

This protocol is designed for an alternative lysis procedure using the Lysis Buffers HMb and HMc. For further information please refer to section 2.3.

- Check section 2.2 and 2.3 for additional information about the amount of starting material and sample material preparation.
- Check Lysis Buffer HMb for precipitates according to section 3.

1 Prepare sample

Prepare the sample material according to step 1 of the detailed protocol in section 5.2.

Add **480 µL Lysis Buffer HMb to the sample in 2 mL tube and gently resuspend the sample material if necessary.**

Add 25 µL of Liquid Proteinase K, gently agitate by inversion or mix and incubate for 30 – 150 min at 56 °C.

Plant material: 30 min

Bacteria: Incubate for at least 30 min with 25 µL lysozyme (100 mg/mL stock solution) at 37 °C prior to the addition of Liquid Proteinase K. Add Liquid Proteinase K and incubate for 30 min at 56 °C.

Yeast: Incubate for at least 60 min with 10 µL zymolyase (50 U) at 37 °C prior the addition of Liquid Proteinase K. Add Liquid Proteinase K and incubate for 30 min at 56 °C.

Continuous careful agitation (e.g. at 900 rpm) of the lysate is advantageous, but not necessary. Mix lysates by inversion or slow rotation from time to time.

Note: See section 2.2 for more for more information about the amount of starting material and the recommended lysis procedure.

2 Precipitate contaminants

Add 120 µL of Buffer HMc to each sample, mix by inverting the tubes 3 times or **shake** for **5 min** at **room temperature**.

Incubate for 5 min at 2 – 8 °C (preferably on ice).

Note: Omitting the precipitation step can be beneficial for certain sample types, such as gram-positive bacteria. In this case, proceed with the centrifugation step in order to pellet residual debris.

Centrifuge for 1 min at 11,000 x *g*.

3 **Transfer lysate**

Transfer **up to 500 µL lysate to a fresh tube** and proceed with step 3 of detailed protocol in section 5.2.

Note: Avoid transferring material from the pellet or material, which floats on top of the lysate. Fibers or husks in the supernatant may clog the pipette tip. Aspirate the supernatant slowly and carefully.

4 Proceed with step 4 (RNA digestion) of the detailed protocol in section 5.2.

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	*Insufficient extraction of nucleic acids during lysis* • To obtain higher yields of nucleic acids, prolong the incubation time of the Proteinase K lysis (up to over night). *Precipitates in buffer HM1 or HMb* • Check buffer HM1 or HMc for precipitates prior to use. Incubate the bottles for several minutes at 30 – 40 °C and mix well until the precipitate is re-dissolved completely. *Sample contains too much RNA* • Prolong the RNase digestion to 30 min and incubate at 37 °C. If this is not successful, increase the amount of RNase. *Insufficient Elution buffer volume* • Bead pellet must be covered completely with elution buffer. Use at least 100 µL of elution buffer. *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 / Suboptimal performance of DNA in downstream applications	*Insufficient washing procedure* • Use only the appropriate combinations of separator and tube, for example, 2 mL tube in combination with NucleoMag® SEP Mini. • Make sure that beads are resuspended almost completely during the washing procedure. *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.
Low DNA integrity	*Storage of sample material* • Use the sample material as freshly as possible. Freeze in liquid nitrogen and keep frozen at all times if sample acquisition and preparation cannot be performed in a timely manner. • Do not let frozen samples thaw. Use prechilled materials for weighing the sample. • Add buffer HM1 and Liquid Proteinase K as quickly as possible to the sample. • Always use material like pipette tips and centrifuge tubes that are certified free of DNase. • When using a customer supplied resuspension buffer, take appropriate measures to prevent introduction of DNases. *Excessive pipetting* *Avoid excessive and harsh pipetting steps. Use wide bore pipette tips*

Problem	Possible cause and suggestions
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. *High viscosity of eluate* • High molecular weight DNA increases the viscosity of solutions increasing the risk of bead carry-over. Repeat the magnetic separation after the first elution step and transfer the eluate to fresh tube.
Ineffective enzymatic lysis of microbial samples	*Improper reaction buffer* • Use only the reaction buffer recommended by the supplier of the enzyme. Do not carry out enzymatic digestion in Lysis Buffer HM1 because the components of the buffer will interfere with enzymatic activity. *Cell wall not susceptible to enzyme applied* • Make sure that your organism of interest can be efficiently lysed with the enzyme that you are applying. *Enzyme inactive* • Make sure that the enzyme is not expired and has been stored according to the recommendations of the supplier. *Wrong protocol for enzymatic digestion* • Make sure to use the protocol as suggested by the supplier of the enzyme *Reaction buffer overloaded* • The reaction speed of enzymes depends on the substrate concentration. If the substrate concentration is too high (too much sample) the reaction speed is decreased. • Released high molecular weight DNA contributes to the sample viscosity. If the sample viscosity is too high the lysis efficiency drops as the distribution of the enzymes becomes inhomogeneous.

7.2 Ordering information

Product	REF	Pack of
NucleoMag® HMW DNA	744160.1	1 × 96 preps
NucleoMag® SEP Mini	744901	1
NucleoMag® SEP	744900	1
Square-well Blocks	740481 740481.24	4 24
Buffer BQ1	740923	125 mL

Visit ***www.mn-net.com*** for more detailed product information.

7.3 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.

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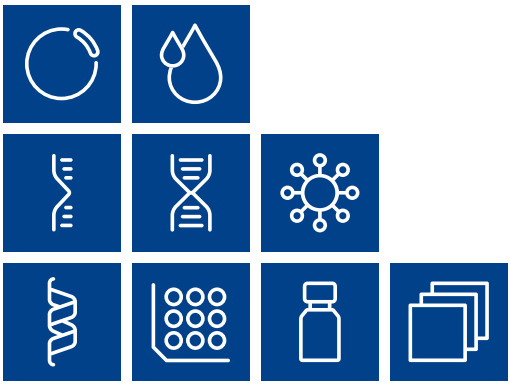
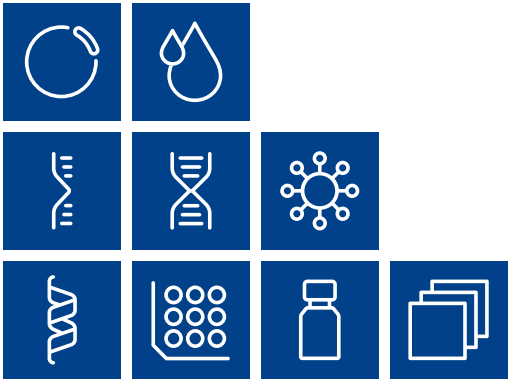


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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