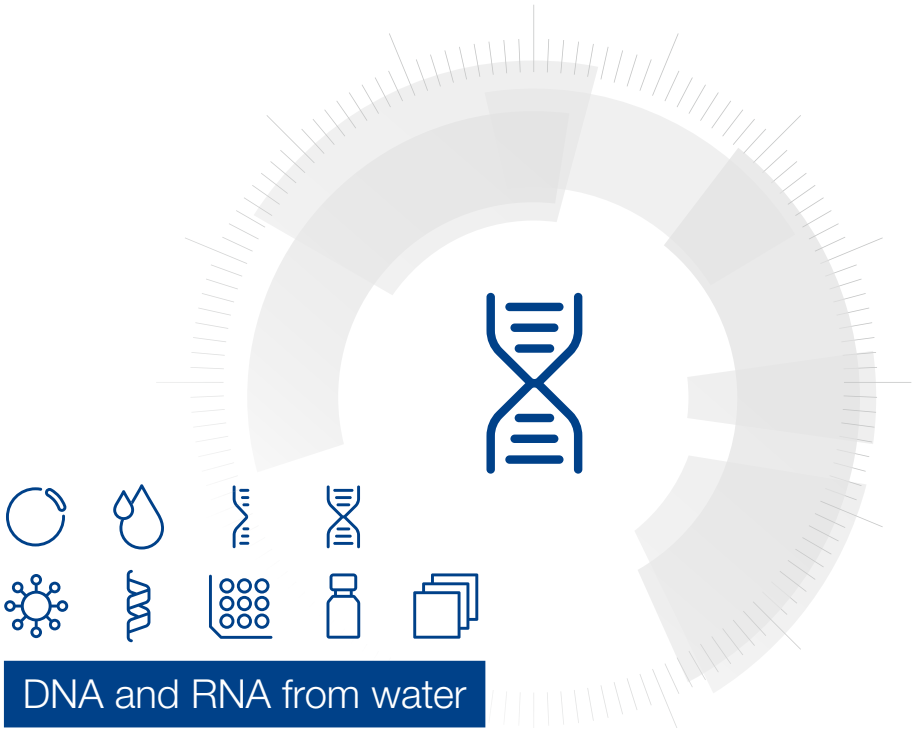
Bioanalysis

MACHEREY-NAGEL

User manual



DNA and RNA from water

■ NucleoMag® DNA/RNA Water

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1 Components

1.1 Kit contents

	NucleoMag® DNA/RNA Water	
REF	**1 × 96 preps 744220.1**	**4 × 96 preps 744220.4**
NucleoMag® B-Beads	2 × 1.5 mL	12 mL
Lysis Buffer MWA1	125 mL	500 mL
Binding Buffer MWA2	80 mL	400 mL
Wash Buffer MWA3	300 mL	3 × 300 mL
Wash Buffer MWA4	250 mL	2 × 250 mL
RNase-free H_2O	13 mL	125 mL
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1.2 Reagents, consumables and equipment to be supplied by user

Consumables

- MN Bead Tubes Type A for 25 mm filters
- MN Bead Tubes 5 mL Type A for 45 – 50 mm filters
- Personal protection equipment (e.g., lab coat, gloves, goggles)

Product	REF	Pack of
Magnet for magnetic beads separation e.g. NucleoMag® SEP	744900	1
NucleoMag® SEP Mini	744901	1
NucleoMag® SEP Maxi	744902	1
NucleoMag® SEP 24	744903	1
Separation plate for magnetic beads separation, e.g. Square-well Block (96-well block with 2.1 mL square-wells)	740481	4
	740481.24	24
MN Bead Tubes Type A e.g., bead tubes containing 0.6 – 0.8 mm ceramic beads; suitable in conjunction with MN Bead Tube Holder or mixer mill*	740786.50	50

*If using a bead mill, respect warnings, in the MN Bead Tubes user manual!

Product	REF	Pack of
MN Bead Tube Holder e.g., Tube Holder for Vortex-Genie® instrument and a 3-inch platform in order to house up to 12 bead tubes	740469	1
MN Bead Tubes 5 mL Type A e.g., 5 mL Bead Tubes containing 0.6 – 0.8 mm ceramic beads; suitable in conjunction with MN Bead Tube Holder 5 mL or mixer mill*	740799.50	50
MN Bead Tube Holder 5 mL e.g., 5 mL Tube Holder for Vortex-Genie® instrument and a 3-inch platform in order to house up to 5 bead tubes 5 mL	740459	1
rDNase Set e.g., RNase-free rDNase (lyophilized) and Reaction Buffer for rDNase for isolation of RNA	740963	1 set
Reaction Buffer for rDNase	740834.60	60 mL
Elution plate for collecting purified nucleic acids, e.g., Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)	740486.24	24
For use of kit on KingFisher® instruments: e.g., 96-well Accessory Kit A for KingFisher® (Square-well Blocks, Deep-well tip combs, Plates for 4 × 96 NucleoMag® DNA/RNA Water preps using KingFisher® 96/ Flex platform)	744950	1 set

1.3 About this user manual

It is strongly recommended that first time users of the NucleoMag® DNA/RNA Water 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.

*If using a bead mill, respect warnings, in the MN Bead Tubes user manual!

2 Product description

2.1 The basic principle

The **NucleoMag® DNA/RNA Water** kit is designed for the isolation of microbial DNA/RNA from water or air samples concentrated on a filter membrane. The procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis can be achieved by mechanical treatment of the filter membrane using MN Bead Tubes or other bead beating tubes. Lysis mixtures can be cleared by centrifugation or filtration in order to remove contaminants and residual cellular debris. For binding of nucleic acids to the paramagnetic beads, Binding Buffer MWA2 and the NucleoMag® B-Beads are added to the transferred lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers MWA3 and MWA4. Residual ethanol from previous wash steps is removed by air drying. Next, highly purified DNA/RNA is eluted with RNase-free water and can be used directly for downstream applications. The **NucleoMag® DNA/RNA Water** kit can be used either manually, or automated on standard liquid handling instruments and automated magnetic separators.

2.1.1 Kit specifications

NucleoMag® DNA/RNA Water is recommended for the rapid manual and automated isolation of total DNA and RNA from filtered water or air samples. DNA and RNA from bacteria, fungi and algae are isolated simultaneously using magnetic bead based technology and can be directly subjected for subsequent downstream applications like real-time PCR, next generation sequencing etc.

NucleoMag® DNA/RNA Water 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/RNA Water
Technology	Magnetic bead technology
Format	Highly reactive superparamagnetic beads
Sample material	Water or air samples
Sample amount	10 – 1000 mL per preparation
Typical yield	Sample dependent
Elution volume	50 – 200 µL
Use	For research use only

2.2 Storage of water samples

The most convenient way is the storage of filtered water samples. Therefore, filter the water sample through a membrane filter and store the filter directly within the appropriate amount of lysis buffer in the respective bead tube.

2.3 Water samples

2.3.1 Volume

The maximum volume of water sample that can be processed varies depending on the sample (e.g. source and quality) and filter membrane (e.g., type, diameter, pore size).

2.3.2 Clear water samples

The turbidity of water samples can vary from clear to highly turbid, due to different concentrations of particulates. In general, high volumes of clear or potable drinking water can be processed, as there is a lower chance of filter clogging. A volume of 100 mL up to several liters might be processed.

2.3.3 Turbid water samples

Turbid water samples containing high levels of sediments or suspended particles, such as clay, silt, or other inorganic or organic matter, may lead to filter clogging. The use of a 0.45 µm filter is recommended for these samples types. Furthermore, it is advantageous to let samples sediment over time. In case a sedimentation is not desired or does not occur, a pre-filter on top of the membrane filter is recommended. MACHEREY-NAGEL offers a broad range of filter paper suitable for pre-filtration of large debris.

Suitable pre-filter	Properties	Particle retention	Diameter	REF
MN 619 G	slow filtration	approx. 2 – 7 µm	55 mm	440005
MN 616 G	medium filtration	approx. 4 – 12 µm	55 mm	483005
MN 617 G	fast filtration	approx. 7 – 12 µm	55 mm	494005

In cases neither a sedimentation nor pre-filtration is desired, a specific support protocol for the isolation of DNA from highly turbid water samples is described in section 8.1. For this application additional Buffer SL1, SL3, and FOB are necessary (see ordering information). Three bottles of SL1, one bottle of SL3 and one bottle of FOB are suitable for approx. 96 preparations in conjunction with the NucleoMag® DNA/RNA Water kit.

2.4 Wastewater samples

Wastewater surveillance used as an non-invasive epidemiological tool or surveillance of microbial communities in sewage plants is a major challenge due to large and diverse amount of inhibitors. Further challenges are the low concentration of viral particles in larger volumes and the selection of the appropriate concentration method Since the start of the Corona Pandemic in 2020, wastewater surveillance has become a popular tool, allowing outbreak detection on a communal level.

The following wastewater concentration methods have been tested and are compatible with the **NucleoMag® DNA/RNA Water** Kit.

Concentration method	Typical volume used	Reference
Filtration (electronegative membrane)	40 – 50 mL	Ahmed et al. 2020
Ultrafiltration	40 – 50 mL	Ahmed et al. 2020; Mederna et al. 2020
PEG Precipitation	40 – 50 mL	Sapula et al. 2021 https://doi.org/10.1016/j.scitotenv.2021.147270
InnovaPrep Sample Concentration	40 – 100 mL (up to 5 L); 20 – 40 mL	Fores et al., 2020 https://doi.org/10.1016/j.scitotenv.2020.144786
Nanotrap® Magnetic Virus Particles	10 mL	Karthikeyan et al, 2021 https://doi.org/10.1101/2021.06.18.21259162

For more information or recommendations on concentration methods contact our techsupport (Bio-tech@mn.com).

2.5 Filter membranes

NucleoMag® DNA/RNA Water is suitable for the nucleic acid extraction from both 25 mm and 47 mm filter membranes of different composition or origin. Disposable filter funnel units as well as reusable filter funnels are compatible and can be used with different filter membrane types.

MACHEREY-NAGEL offers a broad range of different membrane filter types and sizes.

Type	Diameter	Pore size	Product	REF
Cellulose acetate (CA)	25 mm	0.2 µm	PORAFIL® CA	68000020025
		0.45 µm	PORAFIL® CA	68000045025
	47 mm	0.2 µm	PORAFIL® CA	68000020047
		0.45 µm	PORAFIL® CA	68000045047
Cellulose mixed esters (CM)	25 mm	0.45 µm	PORAFIL® CM	65100045025
	47 mm (sterile)	0.45 µm	PORAFIL® CM	65300045047

Type	Diameter	Pore size	Product	REF
Cellulose nitrate (NC)	25 mm	0.2 µm	PORAFIL® NC	6570020025
		0.45 µm	PORAFIL® NC	6570045025
	47 mm	0.2 µm	PORAFIL® NC	6570020047
		0.45 µm	PORAFIL® NC	6570045047
Polycarbonate (PC)	25 mm	0.40 µm	PORAFIL® PC	676040025
Polyethersulfone (PES)	-	-		-

2.6 Filter units

The **NucleoMag® DNA/RNA Water** kit can be adapted to the isolation of nucleic acids from filter units, such as the Sterivex™ filter units (Millipore®) using the respective support protocol (see section 9.1). For isolation of DNA/RNA from Sterivex™ filter units, additional Buffer C1 (REF 740930) is necessary. It is suited for approx. 50 preparations in conjunction with the NucleoMag® DNA/RNA Water Kit.

Make sure to seal both ends of the filter units properly. The outlet of Sterivex™ filter units (Millipore SVGPL10RC) possess a male Luer-Lok™, which can be easily closed a Luer-Lok™ cap. The inlet can be sealed with parafilm, wax or alternative plugs.

WARNING: ! It is the responsibility of the user to perform initial tests regarding sealing in order to prevent any leakage, for the used Sterivex™ filter units under the conditions used!

Alternatively, the standard protocol can be used if the filter unit plastic housing is opened, the filter membrane removed and cut into pieces before placement into the bead tube. The filter unit plastic housing can be opened by using a PVC tube cutter as described in Cruaud et al. 2017*.

2.7 Lysis of sample material

In order to obtain optimal yield of nucleic acids from concentrated microorganisms, e.g., bacteria, algae, protozoans or fungi, a complete disruption of the cells is necessary. Sample disruption efficiency depends on the following parameters and can be achieved by following the suggestions outlined in the subsequent sections.

* Cruaud, P. , Vigneron, A. , Fradette, M. , Charette, S. J., Rodriguez, M. J., Dorea, C. C. and Culley, A. I. (2017), Open the SterivexTM casing: An easy and effective way to improve DNA extraction yields. Limnol. Oceanogr. Methods, 15: 1015 – 1020. doi:10.1002/lom^3.10221

2.8 Disruption device

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

- MN Bead Tube Holder (for 2 mL bead tubes) or MN Bead Tube Holder 5 mL (for 5 mL bead tubes) in combination with the Vortex-Genie® 2 (recommended).
- Mixer Mill MM200, MM300, MM400 (Retsch®) (suitable for 2 mL bead tubes).

If other disruption devices are intended to be used, consider section 2.10 including the WARNING note!

2.9 Type of MN Bead Tube

The choice of a suitable bead tube type highly depends on the filter membrane size.

- 25 mm filter membranes: MN Bead Tube Type A (0.6 – 0.8 mm ceramic beads)
- 47 mm filter membranes: MN Bead Tube 5 mL Type A (0.6 – 0.8 mm ceramic beads)

2.10 Time and frequency of disruption

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

- 25 mm filter membranes: MN Bead Tube Type A – 4 min
- 47 mm filter membranes: MN Bead Tube 5 mL Type A – 10 min (only MN Bead Tube Holder 5 mL)

The disruption time can be increased to up to 12 minutes in order to increase disruption efficiency for difficult to lyse cells.

Note: Performance and stability testing has been conducted on the MN Bead Tubes Type A (MN Bead Tube 5 mL Type A has not been evaluated), on a Retsch® Mixer Mill MM300 at highest frequency (30 Hertz) for up to 15 minutes for optimal sample disruption, avoidance of DNA fragmentation, and tube durability. Other disruption devices (see section 2.8) 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.

2.11 Magnetic separation systems

For use of **NucleoMag® DNA/RNA Water**, 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
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.

2.12 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.

2.13 Handling of beads

Distribution of beads

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well 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. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads/binding buffer mixture from the reservoir is recommended to keep the beads 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 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 beads

Washing the 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

2.14 Isolation of DNA

If RNA-free DNA is crucial for downstream applications RNase digest may be performed, after mechanical lysis. Therefore, add 10 µL RNase A (12 mg/mL stock solution, see section 12.2, see ordering information) per 500 µL lysis buffer, mix well, and incubate at RT (18 – 25 °C) for 15 – 20 min. Proceed with the centrifugation step of the protocol.

For this application, additional RNase A (REF 740505.30) is necessary. It is suitable for approx. 96 preparations in conjunction with the NucleoMag® DNA/RNA Water kit.

Prepare RNase stock solution by adding 2.5 mL water to each vial. Dissolved RNase A solution can be stored at 4 °C for up to 3 months. For longer storage, the RNase A solution should be divided into small aliquots and stored at -20 °C.

2.15 Isolation of RNA

Isolation of pure RNA can be achieved by following the respective support protocol in section 10. For this application additional rDNase Set and additional Reaction Buffer for rDNase are necessary (see section 12.2, ordering information). It is suitable for approx. 96 preparations in conjunction with the NucleoMag® DNA/RNA Water kit.

The preparation of the rDNase reaction mixture consists out of two main steps: 1) Reconstitution of lyophilized rDNase and 2) Dilution of reconstituted rDNase in the appropriate reaction buffer volume.

- **Reconstitution of lyophilized rDNase:** Add 4 mL of RNase-free H_2O to the rDNase vial and incubate for 2 – 3 min at room temperature. Gently swirl the vials, to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation.
- **Dilution of rDNase:** Transfer the reconstituted rDNase to a suitable tube and add 28 mL of Reaction Buffer for rDNase. Gently swirl the tube. The resulting rDNase reaction will be sufficient for 96 samples. Prepare a smaller amount (e.g. ,1 mL reconstituted rDNase and 7 mL of Reaction Buffer for rDNase for 32 reactions), when performing less reactions. For each isolation combine 37.5 µL reconstituted rDNase and 262,5 µL Reaction Buffer for rDNase. If not used completely the working solution can be stored at -20 °C for at least 6 months. Do not freeze / thaw the rDNase working solution more than three times.

2.16 Elution procedures

Purified DNA/RNA can be eluted directly with the supplied RNase-free H_2O. Elution can be carried out in a volume of ≥ 50 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.

3 Storage conditions and preparation of working solutions

Attention: *Buffers MWA1 and MWA3 contain chaotropic salt! Wear gloves and goggles!*

All components of the **NucleoMag® DNA/RNA Water** kit should be stored at 15 – 25 °C and are stable until: see package label.

All buffers are delivered ready to use.

3.1 Preparation of rDNase solutions

Note: rDNase and the Reaction Buffer for rDNase are not included in the kit. An additional rDNase set and reaction buffer need to be purchased. (see section 1.2)

- **rDNase working solution:** Add 4 mL of RNase-free H_2O to each rDNase vial and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. If not used completely this working solution can be stored at -20 °C for at least 6 months. Do not freeze / thaw the rDNase working solution more than three times.
- **rDNase reaction mixture:** Transfer the reconstituted rDNase to a suitable tube and add 28 mL of Reaction Buffer for rDNase. Gently swirl the tube. The resulting rDNase reaction will be sufficient for 96 samples. Prepare a smaller amount (e.g., 1 mL reconstituted rDNase and 7 mL of Reaction Buffer for rDNase for 32 reactions), when performing less reactions. For each isolation combine 37.5 µL reconstituted rDNase and 262,5 µL Reaction Buffer for rDNase. If not used completely the working solution can be stored at -20 °C for at least 6 months. Do not freeze / thaw the rDNase working solution more than three times.

4 Safety instructions

When working with the **NucleoMag® DNA/RNA Water** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***).

Caution: Guanidine hydrochloride in buffer MWA3 and guanidinium thiocyanate in buffer MWA1 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® DNA/RNA Water** 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 overview

The NucleoMag® DNA/RNA kit offers a high flexibility allowing the simultaneous isolation of DNA and RNA, the isolation of pure DNA and the isolation of pure RNA. The following table serves as overview for the different protocols.

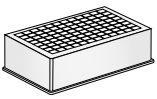
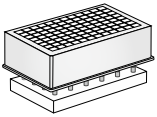
<table>
<tr><th>Isolation of DNA/RNA</th><th>Isolation of DNA</th><th>Isolation of RNA</th></tr>
<tr><td colspan="3">Filtration of water samples</td></tr>
<tr><td>Lysis</td><td>Lysis including RNase-treatment</td><td>Lysis</td></tr>
<tr><td colspan="3">Bind nucleic acid to NucleoMag® B-Beads</td></tr>
<tr><td colspan="3">1st washing step</td></tr>
<tr><td colspan="3">2nd washing step</td></tr>
<tr><td colspan="2" rowspan="2">3rd washing step</td><td>Digest DNA and rebind RNA</td></tr>
<tr><td>3rd washing step</td></tr>
<tr><td colspan="3">Air dry</td></tr>
<tr><td colspan="3">Elution</td></tr>
</table>

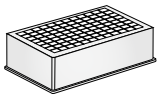
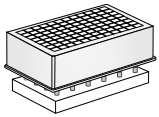
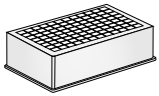
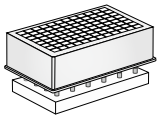
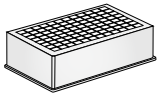
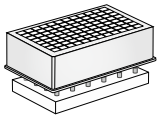
6 Protocol for isolation of DNA/RNA from water samples (25 mm filter membrane)

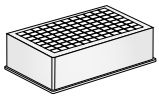
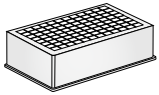
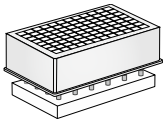
6.1 Protocol at a glance

For additional equipment and hardware requirements, refer to section 1.2 and sections 2.8 2.11, respectively.

For detailed information on each step, see page section 6.2.

1	**Filter water sample**	**Filter 100 – 1000 mL of a water sample through a filter membrane (25 mm)**	
2	**Lyse sample**	**Insert filter membrane into a MN Bead Tube Type A** **500 µL MWA1** **Agitate on a swing mill or similar device 0.5 – 15 min** **11,000 x *g*, 30 s**	
3	**Bind** DNA/RNA to NucleoMag® B-Beads	**Up to 450 µL lysate** **25 µL NucleoMag® B-Beads** **475 µL MWA2**	
		Mix by shaking for 5 min at RT ***(Optional: Mix by pipetting up and down)***	
		Remove supernatant after 5 min separation	

4	**Wash** with MWA3	Remove Square-well Block from NucleoMag® SEP **850 µL MWA3**	
		Resuspend: Shake 2 min at RT ***(Optional: Mix by pipetting up and down)***	⟷
		Remove supernatant after 2 min separation	
5	**Wash** with MWA3	Remove Square-well Block from NucleoMag® SEP **850 µL MWA3**	
		Resuspend: Shake 2 min at RT ***(Optional: Mix by pipetting up and down)***	⟷
		Remove supernatant after 2 min separation	
6	**Wash** with MWA4	Remove Square-well Block on NucleoMag® SEP **850 µL MWA4**	
		Resuspend: Shake 2 min at RT ***(Optional: Mix by pipetting up and down)***	⟷
		Remove supernatant after 2 min separation	

7	**Dry** the beads	**10 – 15 min at RT**	
8	**Elute** DNA	Remove Square-well Block from NucleoMag® SEP	
		50-100 µL RNase-free H2O ***(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	

6.2 Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.11). It is recommended to use a Square-well Block for separation (see section 1.2). 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 sections 2.8 2.11.

1 Filter water samples

Filter up to 1000 mL of water sample through an appropriate 25 mm filter membrane (not provided, see ordering information) using a suitable filtration device. (See section 2.5 for filter membrane recommendations).

2 Lyse sample

Remove the filter membrane from the filtration device by using sterile forceps, roll the membrane into a cylinder (top side facing inwards) and insert into a MN Bead Tube Type A or another bead beating tube suitable for 25 mm filter membranes.

Add **500 µL** of **Lysis Buffer MWA1** and close the tube.

Agitate the MN Bead Tube Type A 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: The MN Bead Tube Type A in combination with the MN Bead Tube Holder are recommended for filter membranes with the size of 25 mm.

Note: Optimal agitation duration, speed / frequency depends on the device used. For the MN Bead Tube Holder it is approximately 4 min; in a mixer mill MM200, MM300, MM400 (Retsch®), e.g., 0.5 – 5 min at maximal frequency (30 Hertz) is suitable (see section 2.10). 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 the warning in section 2.10 if other devices are intended to be used!

Optional: If RNA-free DNA is crucial for downstream applications an RNase digest may be performed: After mechanical lysis, add 10 µL RNase A (12 mg/mL stock solution, not provided, for further information see section 12.2) per 500 µL lysis buffer, mix well, and incubate at RT (18 – 25 °C) for 15 – 20 min. Proceed with the centrifugation step of the protocol.

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

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

3 Bind DNA/RNA to NucleoMag® B-Beads

Transfer **up to 450 µL lysate to a Square-well block**. Add **25 µL of NucleoMag® B-Beads** and **475 µL of Binding Buffer MWA2**. 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.

Note: NucleoMag® B-Beads and Buffer MWA2 can be premixed.

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.

4 Wash with MWA3

Remove the Square-well Block from the NucleoMag® SEP Magnetic Separator.

Add **850 µL Buffer MWA3** 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.

5 Wash with MWA3

Remove the Square-well Block from the NucleoMag® SEP Magnetic Separator.

Add **850 µL Buffer MWA3** 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.

6 Wash with MWA4

Remove the Square-well Block from the NucleoMag® SEP Magnetic Separator.

Add **850 µL MWA4** 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.

Optional: If DNA-free RNA is crucial for downstream applications follow the protocol in section 10.

7 Air dry magnetic beads

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

8 Elute DNA

Remove the Square-well Block from the NucleoMag® SEP Magnetic Separator.

Add desired volume of **RNase-free H_2O (50 – 100 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.

7 Support protocol for isolation of DNA/RNA from water samples (47 mm filter membrane)

7.1 Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.11). It is recommended to use a Square-well Block for separation (see section 1.2). 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 sections 2.8 2.11.

1 Filter water samples

Filter up to 1000 mL of water sample through an appropriate 47 mm filter membrane (not provided, see ordering information) using a suitable filtration device. (See section 2.5 for filter membrane recommendations).

2 Lyse sample

Remove the filter membrane from the filtration device by using sterile forceps, roll the membrane into a cylinder (top side facing inwards) and insert into a MN Bead Tubes 5 mL Type A or another bead beating tube suitable for 47 mm filter membranes. Make sure to tighten the screw caps of the MN Bead Tubes 5 mL Type A in order to avoid any leakage of chaotropic buffers.

Add **900 µL** of **Lysis Buffer MWA1** and close the tube.

Agitate the MN Bead Tube on the MN Bead Tube Holder 5 mL in conjunction with Vortex-Genie® for 10 min.

Note: Please refer to section 2.10 for advice and instructions regarding optimal agitation duration, speed / frequency and devices. 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 the warning in section 2.10 if other devices are intended to be used!

Optional: If RNA-free DNA is crucial for downstream applications an RNase digest may be performed: After mechanical lysis, add 18 µL RNase A (12 mg/mL stock solution, not provided, for further information see section 12.2) per 900 µL lysis buffer, mix well, and incubate at RT (18 – 25 °C) for 15 – 20 min. Proceed with the centrifugation step of the protocol.

Note: The MN Bead Tubes 5 mL Type A are recommended for filter membranes with the size of 45 – 50 mm.

Centrifuge the MN Bead Tube 5 mL Type A for **30 s** at **1,000 x *g*** to clean the lid

Attention: Do neither centrifuge at higher g-force, nor for longer than 30 s because this might damage the MN Bead Tube 5 mL.

3 Bind DNA/RNA to NucleoMag® B-Beads

Transfer **up to 450 µL lysate to a Square-well block** and proceed with step 3 of the standard protocol for 25 mm filter membranes (see section 6.2).

8 Support protocol for isolation of DNA from highly turbid water samples (25 mm filter membrane)

8.1 Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.11). It is recommended to use a Square-well Block for separation (see section 1.2). 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 sections 2.8 2.11.
- For general advices regarding handling of turbid water samples, refer to section 2.3.3.
- For this application additional Buffer SL1 (REF 740781.30), SL3 (REF 740783.50) and FOB (REF 740841.100) are necessary (see ordering informations). Three bottles of SL1, one bottle of SL3 and one bottle of FOB are suitable for approx. 96 preparations in conjunction with the NucleoMag® DNA/RNA Water kit.

Before starting the preparation:

- Check Lysis Buffer SL1 for precipitated SDS. Dissolve any precipitate by incubating the buffer at 30 – 40 °C for 10 min and shaking the bottle every 2 min.
- Buffers SL1, SL3 and FOB have to be ordered separately.

1 Filter water samples

Filter up to 1000 mL of water or air sample through an appropriate 25 mm filter membrane using a suitable filtration device. (See section 2.5 for filter membrane recommendations).

2 Lyse sample

Remove the filter membrane from the filtration device by using sterile forceps, roll the membrane into a cylinder (top side facing inwards) and insert into a MN Bead Tube Type A or another bead beating tube suitable for 25 mm filter membranes.

Add **700 µL** of **Lysis Buffer SL1** and close the tube.

Agitate the MN Bead Tube on the MN Bead Tube Holder in conjunction with Vortex-Genie®, swing mill or similar device for 10 min.

Centrifuge the MN Bead Tube Type A for **2 min** at **11,000 x *g*** to eliminate the foam caused by the detergent.

Attention: Do not centrifuge at higher g-force, nor longer because this might damage the NB Bead Tubes.

3 Precipitate contaminants

Transfer up to **500 µL** of the cleared supernatant to a 1.5 or 2 mL reaction tube.

Add **150 µL Buffer SL3**, close the lid and vortex for 5 s.

Incubate for **10 min** at **0 – 4 °C**.

Centrifuge for **2 min** at **11,000 x *g***.

4 Bind DNA/RNA to NucleoMag® B-Beads

Carefully transfer **up to 450 µL of cleared lysate to a Square-well block**. Add **25 µL of NucleoMag® B-Beads** and **475 µL of Binding Buffer FOB**. 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.

Note: NucleoMag® B-Beads and Buffer FOB can be premixed.

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 the supernatant from the opposite side of the well.

5 Proceed with step 4 of the **standard protocol** for 25 mm filter membranes (see section 6.2)

9 Support protocol for the isolation of DNA/RNA from Sterivex™ filter units

9.1 Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.11). It is recommended to use a Square-well Block for separation (see section 1.2). 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 sections 2.8 2.11.
- For general advice on using Sterivex™ filter units, refer to section 2.6.
- Alternatively open the filter unit plastic housing, remove the filter membrane and cut into pieces before placement into the bead tube. Proceed with step 2 of the support protocol for 47 mm filters (see section 7.1).
- For isolation of DNA/RNA from Sterivex™ filter units, additional Buffer C1 (REF 740930) is necessary. It is suited for approx. 50 preparations in conjunction with the NucleoMag® DNA/RNA Water Kit

Important notice:

- Make sure to seal or close the inlet and outlet of the Sterivex™ filter unit properly. See section 2.6 for further information.

1 Filter water samples

Filter up to 100 mL of water or air sample through a Sterivex™ filter unit according to manufacturer's instruction.

2 Lyse sample

Add 900 µL of **Buffer C1** through the inlet of the filter unit by inserting the pipette tip into the inlet.

Seal both ends properly. The outlets of Sterivex™ filter units (Millipore SVGPL10RC) possess a male Luer-LokTM, which can be easily closed a Luer-Lok™ cap. The inlet can be sealed with parafilm, wax or alternative plugs.

Note: Make sure that both ends of filter unit are sealed properly in order to prevent leakage.

Attach the sealed filter unit to a vortexer, MN Bead Tube Holder 5 mL or special adapter, for example, by using tape. Agitate the closed filter unit for 5 min at maximum speed.

Incubate for **5 min** at **70 °C**.

Note: Place filter units in a 50 mL conical tube and incubate in a heat shaker or water bath.

Attach the sealed filter unit to a vortexer, MN Bead Tube Holder 5 mL or special adapter, for example, by using tape. Agitate the closed filter unit for 5 min at maximum speed.

3 Remove lysate

Remove as much lysate as possible by attaching a suitable syringe to the inlet of the filter unit.

Optional: Transfer lysate to a MN Bead Tube Type A and agitate the MN Bead Tube Type A on the MN Bead Tube Holder in conjunction with Vortex-Genie®, swing mill or similar device for 4 min.

Optional: If RNA-free DNA is crucial for downstream applications an RNase digest may be performed: After mechanical lysis, add 18 µL RNase A (12 mg/mL stock solution, not provided, see ordering information) per 900 µL lysis buffer, mix well, and incubate at RT (18 – 25 °C) for 15 – 20 min. Proceed with the centrifugation step of the protocol.

Optional: Centrifuge the MN Bead Tube Type A for 30 s at 11,000 x g to clean the lid and to reduce foaming.

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

4 Bind DNA/RNA to NucleoMag® B-Beads

Transfer **up to 450 µL lysate to a Square-well block** and proceed with step 3 of the standard protocol for 25 mm filter membranes (see section 2.7).

10 Support protocol for the isolation of RNA from water samples (25 mm filter membrane)

10.1 Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.11). It is recommended to use a Square-well Block for separation (see section 1.2). Alternatively, isolation of RNA 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 sections 2.8 2.11.
- For isolating pure RNA additional rDNase Set (REF: 740963) and Reaction buffer for rDNase are necessary (740834.60). It is suited for approx. 96 preparations in conjunction with the NucleoMag® DNA/RNA Water kit.

Before starting the preparation:

- Check that rDNase reaction mixture was prepared according to section 3.1

1 Filter water samples

Filter up to 1000 mL of water sample through an appropriate 25 mm filter membrane (not provided, see ordering information) using a suitable filtration device. (See section 2.5. for filter membrane recommendations).

2 Lyse sample

Remove the filter membrane from the filtration device by using sterile forceps, roll the membrane into a cylinder (top side facing inwards) and insert into a MN Bead Tube Type A or another bead beating tube suitable for 25 mm filter membranes.

Add **500 µL** of **Lysis Buffer MWA1** and close the tube.

Agitate the MN Bead Tube Type A 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: The MN Bead Tube Type A in combination with the MN Bead Tube holder are recommended for filter membranes with the size of 25 mm.

Note: Please refer to section 2.10 for advice and instructions regarding optimal agitation duration, speed / frequency and devices. 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 the warning in section 2.10 if other devices are intended to be used!

Centrifuge the MN Bead Tube Type A 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 because this might damage the MN Bead Tube.

3 Bind RNA to NucleoMag® B-Beads

Transfer **up to 450 µL lysate to a Square-well block**. Add **25 µL of NucleoMag® B-Beads** and **475 µL of Binding Buffer MWA2**. 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.

Note: NucleoMag® B-Beads and Buffer MWA2 can be premixed.

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.

4 Wash with MWA3

Remove the Square-well Block from the NucleoMag® SEP Magnetic Separator.

Add **850 µL Buffer MWA3** 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.

5 Wash with MWA3

Remove the Square-well Block from the NucleoMag® SEP Magnetic Separator.

Add **850 µL Buffer MWA3** 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.

6 Digest DNA

Air dry the magnetic beads for 5 min at room temperature. Keep the Square-well Block on the NucleoMag® SEP Magnetic Separator for the drying step.

Remove the Square-well Block from the NucleoMag® SEP Magnetic Separator. Add 300 µL rDNase reaction mixture and resuspend the beads by pipetting up and down. Incubate for 15 min at room temperature

7 Rebind

Add 350 µL Buffer MWA2 to each sample. Mix by shaking for 5 min at room temperature or by pipetting up and down 6 times and a subsequent incubation for 5 min at room temperature.

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 Wash with MWA4

Remove the Square-well Block from the NucleoMag® SEP Magnetic Separator.

Add **850 µL MWA4** 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.

9 **Air dry** magnetic beads.

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

10 Elute RNA

Remove the Square-well Block from the NucleoMag® SEP Magnetic Separator.

Add desired volume of **RNase-free H_2O (50 – 100 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 RNA to either elution plates or tube stripes (see ordering information).

11 Wastewater sample concentration methods

The following protocols describe different sample concentration methods, which have been successfully tested with the **NucleoMag® DNA/RNA Water** kit.

For general recommendations for wastewater samples, refer to section, X-Y or to our webpage: ***www.mn-net.com/de/wastewater***

11.1 Ultrafiltration procedure

To learn more about waste water concentration by ultrafiltration follow this link: ***www.mn-net.com/de/wastewater-sampleconcentration-ultrafiltration***

The following step by step protocol describes the procedure for a 40 mL wastewater sample. The ultrafiltration method can be adapted to larger volumes. However, an additional pre-filtration step using a 3.5 µm filter is recommended. For larger samples volumes (> 1 Liter), tangential flow filtration is advantageous.

1	**Pellet larger particles** Centrifuge wastewater at 4600 – 4700 x *g* for 30 min at 4 °C to pellet larger particles or debris. Transfer the supernatant to a fresh tube or containe
2	**Filtration** Filter the cleared wastewater sample through a 0.2 µm filter unit or syringe filter to remove residual cellular debris
3	**Concentration** Concentrate the cleared and filtered wastewater sample using a suitable ultracentrifugation unit (e.g. Centricon® Plus-70 centrifugal ultrafilter units 10 kDa, Millipore; Cat. No.: UFC701008) and recover the wastewater concentrate according to the manufacturer's instructions.
4	**Lysis** Transfer 200 µL of the wastewater concentrate, add 200 µL MWA1 and 10 µL Liquid Proteinase K. Incubate at 56 °C for 10 min.
5	**Binding** Add 25 µL NucleoMag® B-Beads and 475 µL Binding Buffer MWA2 and proceed with step 3 of the standard protocol.

11.2 Precipitation procedure

To learn more about waste water concentration by precipitation follow this link: ***www.mn-net.com/de/wastewater-sampleconcentration-precipitation***

The following step by step protocol describes the procedure for a 40 mL wastewater sample. The precipitation method can be adapted to larger volumes.

1	**Remove particulates** Centrifuge wastewater at 4600 – 4700 x *g* for 30 min at 4 °C to pellet larger particles or debris. Transfer the supernatant to a fresh tube or container
2	**Filtration** Filter the cleared wastewater sample through a 0.2 µm filter unit or syringe filter to remove residual cellular debris.
3	**Precipitate viral particles** Dissolve 4 g of PEG 8000 and 0.9 NaCl per 40 mL cleared wastewater sample by gently mixing.Centrifuge the sample at 12.000 x *g* for 120 min at 4 °C. *Note: Use suitable centrifuge containers. Mark the side of the tube on which the pellet would be expected, as it may not be visible. The use of molecular biology grade chemicals is recommended.*
4	**Transfer the lysate** Remove the supernatant carefully without disturbing the pellet during aspiration. Perform a brief centrifugation step for 5 min at 4.000 x *g* and remove the residual liquid by pipetting.
5	**Resuspension** Add 200 µL sterile H_2O and 200 µL MWA1 to the pellet and resuspend by vortexing
6	**Lysis** Transfer the solution to a fresh reaction vessel, add 10 µL Liquid Proteinase K and incubate at 56 °C for 10 min.
7	**Binding** Add 25 µL NucleoMag® B-Beads and 475 µL Binding Buffer MWA2 and proceed with step 3 of the standard protocol.

11.3 INNOVAPREP Concentrating Pipette procedure

To learn more about waste water concentration by using the INNOVAPREP CP-Select follow this link: ***www.mn-net.com/de/wastewater-sampleconcentration-INNOVAPREP***

The following step by step protocol describes the procedure for a 40 mL wastewater sample using the INNOVAPREP Concentrating Pipette Select. The method can be adapted to larger volumes. However, an additional pre-filtration step using a 3.5 µm filter is recommended. For larger samples volumes (> 1 Liter), tangential flow filtration is advantageous

1	**Pellet larger particles** Centrifuge wastewater at 4600 – 4700 x *g* for 30 min at 4 °C to pellet larger particles or debris. Transfer the supernatant to a fresh tube or container
2	**Filtration** Filter the cleared wastewater sample through a 0.2 µm filter unit or syringe filter to remove residual cellular debris.
3	**Concentration** Concentrate the cleared and filtered wastewater sample via the INNOVAPREP CP-Selec™ sample concentration device . For a detailed protocol click here: (https://uploads-ssl.webflow.com/57aa3257c3e841c509f276e2/609185b05af782b67b70e4f2_Protocol%20-%20SARS%20CoV%202%20in%20Wastewater%20-%20Revision%20C.%20docx.pdf)
4	**Lysis** Transfer 200 µL of the wastewater concentrate, add 200 µL MWA1 and 10 µL Liquid Proteinase K. Incubate at 56 °C for 10 min.
5	**Binding** Add 25 µL NucleoMag® B-Beads and 475 µL Binding Buffer MWA2 and proceed with step 3 of the standard protocol.

11.4 Nanotrap® Magnetic Virus Particles procedure

To learn more about waste water concentration by Nanotrap Magenetic Virus Particles follow this link: ***www.mn-net.com/de/wastewater-sampleconcentration-NANOTRAP***

The following step by step protocol describes the procedure for a 10 mL wastewater sample. The method can be adapted to lower or larger volumes. The NanoTrap® Magnetic Virus Particles from Ceres Nanosciences can be used in an automated or manual manner.

1	**Capture and Concentrate viral particles** Add 150 µL of Nanotrap® Magnetic Virus Particles to 10 mL sample. Invert sample 5 times to mix and incubate for 10 minutes at room temperature
2	**Collect Nanotrap® particles** Place samples on magnetic rack and allow the Nanotrap® particles to come out of solution.
3	**Wash** Remove the supernatant and wash particles with 1 mL of 0.05 % Tween-20 in PCR-grade water. Transfer sample to fresh 1.5 mL tube.
4	**Lysis** Pellet Nanotrap® particles. Remove the supernatant. Resuspend pellet in 500 µL Lysis Buffer MWA 1. Incubate at room temperature for 10 minutes.
5	**Extraction** Pull Nanotrap® particles out of solution with a magnetic rack. Proceed with step 3 of the **NucleoMag® DNA/RNA Water** protocol using 450 µL of lysate.

12 Appendix

12.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA/RNA yield	*Insufficient mechanical treatment of filter membrane* • For most sample sources, we recommend mechanical disruption with ceramic beads for 4 min on the MN Bead Tube Holder or with commercial bead mills, mixers or homogenizers. See sections 2.8 2.10 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 10 µL RNase A solution (12 mg/mL) to the lysis buffer following mechanical lysis. If this is not successful, add the enzyme to the cleared lysate and incubate for 30 min at 37 °C. *Insufficient Elution buffer volume* • Bead pellet must be covered completely with 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 / 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 MWA4 *Filtration of the sample not sufficient* • Please refer to sections 2.2 2.7 for advice regarding sample handling and filtration.
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 80 % ethanol. *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. *Insufficient removal of inhibitor/brownish eluates* • Use the support protocol for highly turbid water sample in order to efficiently remove humic substances and inhibitors.

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.
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.

12.2 Ordering information

Product	REF	Pack of
NucleoMag® DNA/RNA Water	744220.1	1 × 96 preps
	744220.4	4 × 96 preps
MN Bead Tube Type A	740786.50	50
MN Bead Tube Holder	740469	1
MN Bead Tube 5 mL Type A	740799.50	50
MN Bead Tube Holder 5 mL	740459	1
Buffer C1	740930	60 mL
Buffer SL1	740781.30	30 mL
Buffer SL3	740783.50	50 mL
Buffer FOB	740841.100	100 mL
RNase A	740505.30	30 mg
rDNase Set	740963	1 set
Reaction Buffer for rDNase	740834.60	60 mL
NucleoMag® SEP	744900	1
NucleoMag® SEP Mini	744901	1

Product	REF	Pack of
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
96-well Accessory Kit A for KingFisher® (set consists of Square-well Blocks, Deep-well tip combs, Elution Plates; for 4 × 96 NucleoMag® DNA/RNA Water preps using KingFisher® 96/Flex platform)	744950	1 set
Filter Papers Circles MN 619 G	440005	100 pieces
Filter Papers Circles MN 616 G	486005	100 pieces
Filter Papers Circles MN 617 G	494005	100 pieces
PORAFIL® Membrane filters CA	68000020025	100 pieces
PORAFIL® Membrane filters CA	68000045025	100 pieces
PORAFIL® Membrane filters CA	68000020047	100 pieces
PORAFIL® Membrane filters CA	68000045047	100 pieces
PORAFIL® Membrane filters CM	65100045025	100 pieces
PORAFIL® Membrane filters CM	65300045047	100 pieces
PORAFIL® Membrane filters NC	6570020025	100 pieces
PORAFIL® Membrane filters NC	6570045025	100 pieces
PORAFIL® Membrane filters NC	6570045047	100 pieces
PORAFIL® Membrane filters NC	676040025	100 pieces
NucleoSpin® Soil, Mini kit for DNA from soil	740780.50 / 250	50 / 250 preps

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

12.3 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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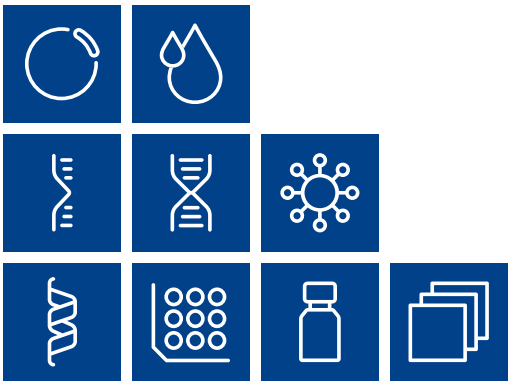
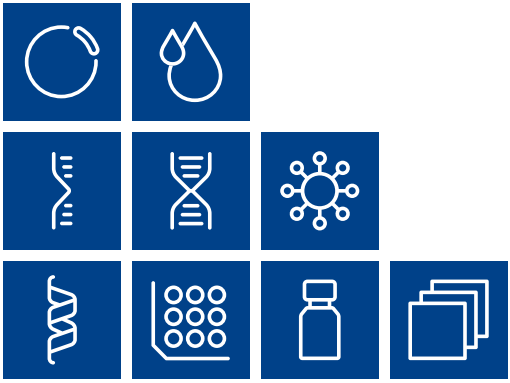


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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