Bioanalysis

MACHEREY-NAGEL

User manual

eDNA isolation

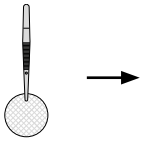
- NucleoSpin® eDNA Water

March 2023 / Rev. 04

eDNA Isolation

Protocol at a glance (Rev. 04)

NucleoSpin® eDNA Water

1	**Release eDNA from filter**	 5 mL tube	2 mL DISSOLVE 25 µL Liquid Proteinase K Mix Agitate (10 min, RT)
2	**Recover lysate**		4,500 x *w*, 2 min
3	**Remove inhibitory substances**		360 µL ACID 4,500 x *g*, 2 min
4	**Bind eDNA to the solid phase**		50 µL PREC 25 µL NucleoTrap® suspension Mix Agitate (5 min, RT) 4,500 x *g*, 3 min
5	**Wash and dry solid phase**		400 µL RESU, mix Transfer 10,000 x *g*, 1 min
			400 µL WB, mix 10,000 x *g*, 1 min
			300 µL WB, mix Transfer 10,000 x *g*, 2 min
6	**Elute eDNA**		100 µL BE, mix 1min, RT 10,000 x *g*, 1 min

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eDNA Isolation

Simplified protocol at a glance (Rev. 04)

NucleoSpin® eDNA Water

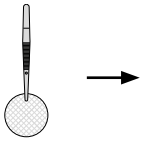
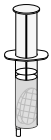
1	**Release eDNA from filter**	 5 mL tube	1.5 mL DISSOLVE 25 µL Liquid Proteinase K Mix Agitate (10 min, RT)
2	**Recover lysate**		Use of syringe recommended for adsorbent filter (e.g. glass fiber) and not required for non-adsorbent filter (e.g. cellulose acetate).
3	**Remove inhibitory substances**	  	10,000 x *g*, 1 min 270 µL ACID 10,000 x *g*, 2 min Transfer
4	**Bind eDNA to the solid phase**	 	38 µL PREC 25 µL NucleoTrap® suspension Mix Agitate (5 min, RT) 4,500 x *g*, 3 min
5	**Wash and dry solid phase**	     	400 µL RESU, mix Transfer 10,000 x *g*, 1 min 400 µL WB, mix 10,000 x *g*, 1 min 300 µL WB, mix Transfer 10,000 x *g*, 2 min
6	**Elute eDNA**	 	100 µL BE, mix 1min, RT 10,000 x *g*, 1 min

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Table of contents

1 Components

1.1 Product content

	NucleoSpin® eDNA Water	
REF	**740402.10**	**740402.50**
Buffer DISSOLVE	30 mL	125 mL
Liquid Proteinase K	600 µL	1.5 mL
Buffer ACID	6 mL	30 mL
Precipitation Buffer PREC	1.5 mL	2 x 1.5 mL
NucleoTrap® Suspension	300 µL	1.5 mL
Resuspension Buffer RESU	5 mL	25 mL
Wash Buffer WB	10 mL	50 mL
NucleoSpin® eDNA XS column (light blue rings – plus Collection Tubes)	10	50
Elution Buffer BE	13 mL	13 mL
User manual	1	1

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

Reagents and consumables:

- Filtration system - Filter. Use of an ethylene oxide treated, round Glass Fiber Filter with 45 mm diameter (REF 740564; see section 6.2 Ordering information) or a similar round filter is recommended. Alternatively, membrane-type round filter (e.g. polyester, regenerated cellulose) or cartridge filters (e.g. Sterivex™) may be used.
- NucleoSpin Filters Midi (REF 740607; see section 6.2 Ordering information) are required for extraction protocol 5.1.1.
- Collection tubes 15 mL (15 mL High-Clarity Polypropylene Conical Tube, FALCON® REF 352096) are required for extraction protocol 5.1.1.
- 5 mL tubes (e.g. Eppendorf Biopur grade, recommended, or similar) are recommended for dissolution of eDNA from round filter according to protocol 5.1.2.
- Collection Tubes (1.5 mL) for elution
- Ethanol *p.a*. for filter drying in case of filter storage
- Disposable pipet tips (aerosol barrier pipet tips are recommended)

- DNA decontamination solution, e.g. bleach or Virkon Aquatic for decontamination of reusable materials and surfaces (e.g. water collection device, lab bench, pincers).
- Optional for turbid water samples: Filter flocs MN 2101 (REF 281120) with a support filter MN 750N (REF 400750N0045) to use as prefilter for turbid water (see section 6.2 Ordering information).

Equipment:

- Water collection device (e.g. bottle, bucket, can, canister, beaker)
- Filter holder, e.g.
  - Thermo Scientific™ Nalgene™ Analytical Test Filter Funnels 250 mL, in which the 0.45 µm filter membrane can be replaced by the recommended glass fiber filter or a filter of preference.
  - Filter holder for use in the field: ADVANTEC Polypropylene Filter Holder for 47 mm Membranes (Cat. No. 43303020)
- Manual pipettors
- Vacuum device (e.g. vacuum pump) to move the water sample through the filter
- Tweezers for filter handling
- Personal protection equipment (lab coat, gloves, goggles)
- Vortex mixer
- Centrifuge for 15 mL tubes with a swing-out rotor capable of reaching 4,500 x *g* for use of extraction protocol 5.1.1
- Centrifuge for microcentrifuge tubes (1.5 mL or 2 mL)
- Tube roller for 15 mL tubes. Alternatively, 5 mL tubes and MN Bead Tube Holder 5 mL (see section 6.2 Ordering information)

1.3 About this user manual

It is strongly recommended to read the detailed protocol sections of this user manual before using the **NucleoSpin® eDNA Water** for the first time. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at www.mn-net.com.

2 Product description

2.1 The basic principle: NucleoSpin® eDNA Water – filtration method

NucleoSpin® eDNA Water is a kit intended for the isolation of eDNA from milliliter to liter water samples collected from natural freshwater and marine environments. The focus of the kit is the isolation of DNA released from diverse organisms into the water. Organisms targeted are for example populations of fish, amphibians, reptiles, crustaceans, mammals, birds, plants and other organisms, that came in contact with the sampled water.

eDNA can be described as any DNA released from organisms into the environment. With regard to water samples, eDNA can be derived from e.g. epithelial cells, excretions, decaying organisms or pieces of tissue, reproductive cells (e.g. sperms, spawn, pollen). Most eDNA is associated with particles, such as cells, organelles, or bound to grains of inorganic or organic material. For this reason, eDNA can be obtained from water samples by filtration.

After filtration of an adequate water volume, DNA from the water is retained on the filter. The filter can either be directly processed as well as being stored for later processing. DNA is released from the filter and with a special dissolving buffer (Buffer DISSOLVE) and proteinase. Subsequently, DNA is absorbed to a particular matrix (NucleoTrap®), which is captured with a NucleoSpin® XS column, washed and finally eluted with a low salt elution buffer.

2.1.1 NucleoSpin® eDNA Water – precipitation method

Alternatively, eDNA can be isolated from a smaller water volume (e.g. up to 40 mL) without the need for filtration. In this procedure an active agent and a solid matrix are added to a 40 mL water sample. eDNA it is bound to the NucleoTrap®matrix, which is then captured with a with a NucleoSpin® XS column, washed and finally eluted with low salt buffer. **For this procedure, the active agent Buffer (PREC) has to ordered separately (see section 6.2 Ordering information)**.

2.2 Product specification

- NucleoSpin® eDNA Water is designed for the isolation of eDNA from milliliter to liters of environmental water samples such as river, stream, creek, lake, pond, lagoon, bay, sea or ocean.
- NucleoSpin® eDNA Water recovers small to large eDNA fragments.
- A filtration protocol as well as a precipitation protocol is supported by the kit. For the precipitation method, extra buffer PREC has to be ordered.
- DNA yield strongly depends on the individual sample type and processed water volume. Total DNA yields of a few ng up to 3.5 µg have been observed from 1 L creek water
- Isolated DNA is suitable for diverse downstream applications such as PCR, qPCR and metabarcoding.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® eDNA Water – filtration method	NucleoSpin® eDNA Water – precipitation method
Technology	Silica beads / membrane technology	
Format	Filtration + beads + XS column	Precipitation + beads + XS column
Sample material	environmental water samples	
Sample amount	approximately 100 – 4000 mL*	up to 40 mL
Fragment size	small to large	
Typical yield	Sample dependent (ng - µg)	
Ratio $A_{260/280}$; $A_{260/230}$	typically not applicable due to low DNA content in the sample	
Elution volume	100 µL	
Filtration time	sample dependent	0 min
Preparation time	approx. 60 min per 6 samples + filtration	approx. 30 min per 6 samples
Binding capacity	not evaluated	not evaluated
Use	For research use only	

*: The volume of filterable water depends very much on the filter used as well on the water quality (turbidity, suspended matter).

2.3 Handling of sample material - Stability of eDNA in the water sample

With collection / removal of a water sample from its natural environment, changes of eDNA amount and distribution in the sample will start. Sample storage can significantly influence the stability of the eDNA. For best results, the time from sample collection to onset of eDNA isolation should not exceed several hours, if the filter is not stabilized e.g. by drying or freezing. Ideally, water samples should be filtrated in the field at the collection site. Filters can be dried with ethanol and stored / transported for later eDNA isolation. If direct filtration is not an option, water samples can be cooled to 0 °C – 4 °C for several hours before eDNA extraction. For the precipitation method (section 5.2), Buffer PREC can be added to a 40 mL water sample at the collection site / in the field in order to stabilize the eDNA within the water sample.

2.4 Stability of DNA on the filter

eDNA can be stabilized on a filter by briefly passing ethanol (5 mL) over the filter in order to dry the filter. The ethanol-wet filter can be stored for several days in a closed tube at ambient temperature avoiding a spoilage of the sample/filter.

2.5 Elution procedures

A volume of 100 µL is recommended for eDNA elution from the NucleoSpin® eDNA XS column. A smaller volume for elution is not recommended.

2.6 Size, yield and quality of eDNA obtained from water samples

DNA fragments from approximately 200 bp to >10,000 bp have been observed. Yield of total DNA can be in the range of few ng up to 3.5 µg per 1 L water sample. Average fragment length and concentration of the isolated eDNA depend on the water sample being processed.

Some types of water sample tend to cause a carryover of brownish substances, such as humic acids. For such samples a clean up with the NucleoSpin® Inhibitor Removal kit (section 6.2 Ordering information) is recommended. Typically, eDNA eluates obtained are suitable for diverse downstream analysis.

2.7 Stability of isolated DNA

Due to the low DNA content of typical water samples, the resulting low total amount of isolated DNA, fragmentation, and the absence of DNase inhibitors (the elution buffer does not contain EDTA), the eluates should be kept on ice for short term storage and frozen at -20°C or below for long term storage for optimal results.

For more information please visit our e training page: ***https://www.mn-net.com/de/ednaresearch***

Further information can be found in the technical note or the processing video.

Technical note: ***https://www.mn-net.com/media/pdf/51/4d/8e/TN-eDNA-isolation-from-aquatic-systems_Rev01_web.pdf***

Video: ***https://youtu.be/9o9qszHV4VY***

3 Storage conditions and preparation of working solutions

Attention: Resuspension Buffer RESU contains chaotropic salt! Wear gloves and goggles!

Caution: Resuspension Buffer RESU contains chaotropic salt which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- Liquid Proteinase K can be stored at room temperature 15 – 25 °C and is stable for at least one year. However, store Liquid Proteinase K at 4 °C or -20 °C after first use.
- All other kit components should be stored at 15 – 25 °C and are stable until: see package label.
- Storage / transport at lower temperatures may cause precipitation of salt in buffer. If any precipitate is visible, heat the solution to 50 °C for 30 min while mixing it, let the solution cool down to room temperature.

4 Safety instructions

When working with the **NucleoSpin® eDNA 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: Guanidinium thiocyanate in buffer RESU can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® eDNA 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 and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

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

5 Protocols

Before starting the procedure:

- Make sure to work in an environment which minimizes risk of DNA contamination: close doors and windows; prevent uncontrolled air draft; wear clean personal protection equipment; change gloves frequently. Ideally, work in a controlled positive pressure lab.
- Decontaminate your working environment (e.g. lab bench surfaces) before starting the procedure. Bleach or Virkon Aquatic can be used for decontamination (see: Quality assurance project plan – eDNA monitoring of bighead and silver carps; prepared for U.S. Fish and Wildlife Service; USFWS Midwest Region, Bloomington, MN, 2019)
- Make sure to decontaminate any reusable material (e.g. water collection container, filter holder, pincers/tweezers).
- When working with hypochlorite solutions, bleach, or Virkon Aquatic please take care to read the instructions in sections 3 and 4.
- Set up your filtration system before starting the procedure.
- Make sure to have all materials available, depending on your experimental set up (see section 1.2 for required and optional materials).
- Make sure to mix the NucleoTrap® suspension well before withdrawing an aliquot. It is important that the NucleoTrap® particles are released from the bottom of the tube and dispersed well within the liquid.

5.1 Protocol for eDNA isolation by filtration method

Water collection

Collect water in a suitable container. For good eDNA coverage, collect water subsamples from different places of the water system to be analyzed and pool them. The optimal volume of water depends on the quality of the water and the abundance of the target DNA. To start with, a target volume of 1 L is recommended. The volume of filterable water depends very much on the filter used as well on the water quality (turbidity, suspended matter).

Note: Make sure to use a DNA-free water collection container and filtering equipment.

Water filtration

As a filter we recommend to use the **MN Glass Fiber Filter** (45 mm, EO-treated, see section 6.2 Ordering information) to minimize the risk of DNA contamination. Other filter types (mixed cellulose ester, cellulose acetate, nylon, polyvinylidene fluoride, cellulose nitrate, polycarbonate) can be used but may show a lower filtration capacity or lower eDNA yield.

Filtration can be performed by either inserting the recommended Glass Fiber Filter (grid pattern facing down, side of water outlet) into an e.g. **Nalgene™ Analytical Test Filter Funnel**, 250 mL (Thermo Scientific™) in which the 0.45 µm filter membrane is replaced by the recommended filter or a similar device (see Figure 1 below).

Alternatively, the round filter can be inserted into an **ADVANTEC Polypropylene Filter Holder** for 47 mm Membranes (Cat. No. 43303020).

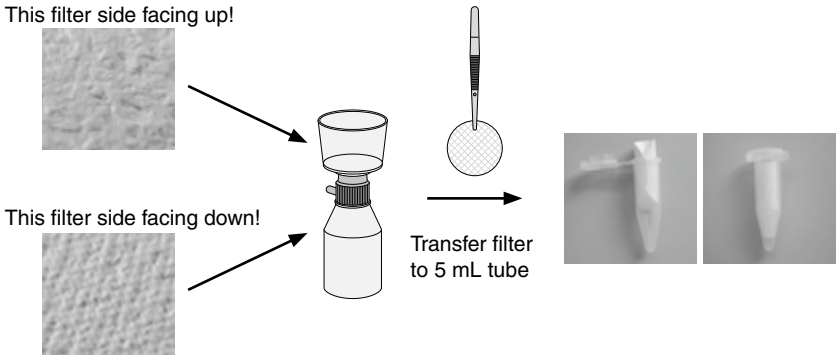


Figure 1 Usage of the recommended round Glass Fiber Filter (45 mm, EO-treated).

Alternatively, cartridge filters like **Sterivex™ Filter Units** may be used, but might show a lower filtration capacity. Cartridge filters used should not require more than approximatel 2 mL solution for eDNA detachment from the filter (i.e. inner volumen of the cartridge should not exceed approximately 5 mL).

Follow protocol 5.1.1 for processing of **Sterivex™ Filter Units**.

Alternatively, large cartridge filter can be processed.

For processing of **Smith-Root eDNA Filter Packs** follow section 5.1.3.

For processing of **large volume filter cartridges** follow section 5.1.4.

Let the water flow through the filter, e.g. by either negative pressure (vacuum) for the Nalgene™ Analytical Test Filter Funnel or by negative or positive pressure for the ADVANTEC filter holder.

If the water sample clogs the filter, the uses of Filter flocs MN 2101 and a support filter MN 750N are recommended as a prefilter. In order to do so, insert a Nalgene™ Analytical Test Filter Funnel containing the support Filter MN750N and approximately 1.6 g of filter flakes into a second Funnel which contains the GF-5 filter.

Storage / transport of filters (optional)

If storage / transport of the filter is required after filtration of water, add 5 mL of ethanol (*p.a.* quality) and let it pass through the filter. The ethanol displaces residual water from the filter and thus conserving the eDNA on the filter. Place the ethanol-wet filter into a suitable tube, e.g. a 5 mL reaction tube (e.g. Eppendorf Biopur grade).

Large filter volume cartridges might require larger amounts of ethanol due to larger filter area and dead volume.

After storage / transport of the filter, remove the filter from the tube and briefly let residual ethanol evaporate, before starting the eDNA release step.

5.1.1 Extraction protocol for round filter and small filtration cartridges

Please note: this protocol requires the use of NucleoSpin® Filter Midi, 15 mL tubes and corresponding centrifuges. If you do not have such equipment available in your workspace, please use protocol 5.1.2. instead.

1 eDNA release from filter

Place the round filter (e.g. the recommended MN Glass Fiber Filter, see ordering information) as a whole into a suitable tube, e.g. a 5 mL reaction tube (e.g. Eppendorf Biopur grade, recommended, see figure above) or a 15 mL collection tube (not provided).

Note: Do not cut the filter. Make sure that top side (the former water facing side) of the filter is now facing inward, so that the subsequently added DISSSOLVE buffer can easily contact the filtration residue.

Note: If small cartridge filter is used (e.g. Sterivex™), add DISSOLVE and Liquid Proteinase K into the cartridge.

Add **2 mL of buffer DISSOLVE** into the tube.

Add **25 µL of Liquid Proteinase K** into the buffer DISSOLVE and close the tube.

Vortex for 10 seconds. Make sure that the entire filter is wetted by the DISSOLVE buffer

Incubate the tube on a roller in a 5 mL tube (recommended) or 15 mL tube or on the MN Bead Tube Holder (for 5 mL tubes) for 10 min with moderate agitation. The filter should be surrounded by / submerged in the DISSOLVE buffer.

Note: Strong agitation / vortexing should be avoided. Some filter types (e.g. nitrocellulose, glass fiber) might disintegrate upon strong vortexing, which can subsequently interfere with the purification process.

2 Lysate recovery

Transfer the filter, the solution, and any sediment visible in the lysis tube into a NucleoSpin® Filter Midi resting in a 15 mL tube (not provided).

Centrifuge for 2 min at 4,500 xg. The lysate is recovered in the 15 ml tube.

Remove and discard the NucleoSpin® Filter Midi containing the round filter.

Note: The recommended filter type is a glass fiber filter absorbing most of buffer DISSOLVE. Other non-membrane filter likely will absorb most of the buffer, too. Therefore, the use of the NucleoSpin® Filter Midi is recommended in order to recover most of the solution. If other type of filters are used (e.g. membrane filter) the use of NucleoSpin® Filter Midi might be dispensable due to the low absorption of liquid by membrane filters. However, applying the NucleoSpin® Filter Midi is still recommended for optimum yield and ease of use.

Note: If a cartridge filter is used, remove the lysate from the cartridge and put the lysate into a 15 mL Collection Tube (not provided).

3 Removal of inhibitory substances

Add **360 µL buffer ACID** to the recovered lysate.

Invert the tube once to mix the buffer ACID into the solution.

Note: Do not mix thoroughly – pellets should not be resuspended. This step contributes to the removal of inhibitory substances, especially for humic acid rich (brownish) water samples.

Centrifuge the tube for 2 min at 4,500 x *g*.

Transfer the supernatant without any sediment into a fresh Collection Tube (15 mL, not provided).

4 eDNA binding to the solid phase

Add **50 µL buffer PREC** to the supernatant.

Add **25 µL NucleoTrap® suspension** and close the tube.

Note: Make sure to mix the suspension well before withdrawing an aliquot.

Incubate the tube at room temperature (18 – 25°C) for 5 min on a roller.

Note: Alternatively, the tube can be incubated on shaker with moderate shaking. eDNA will bind to the NucleoTrap® suspension in this step.

Centrifuge the tube for 3 min at 4,500 x *g*.

Note: The NucleoTrap® with eDNA bound to it, is sedimented in this step.

Remove and discard the supernatant.

Note: Remove the supernatant with a pipet or turn the tube and place it upside down on a paper tissue to remove residual supernatant. Some droplets may stay in the tube – this does not compromise the purification process.

5 Wash and dry solid phase

Add **400 µL Resuspension Buffer RESU** to the NucleoTrap® pellet and resuspend it by vortexing.

Transfer the suspension into a 1.5 mL tube (not provided).

Centrifuge for 1 min at 10,000 xg.

Remove and discard the supernatant.

Note: The pellet might be translucent and thus be hardly visible in this step.

Add **400 µL Wash Buffer WB** to the NucleoTrap® pellet and resuspend pellet by vortexing.

Centrifuge for 1 min at 10,000 xg.

Remove and discard the supernatant.

Note: The pellet is typically easily visible in this step.

Add **300 µL Wash Buffer WB** to the NucleoTrap® pellet and resuspend pellet by vortexing

Transfer the NucleoTrap® suspension onto a **NucleoSpin® eDNA XS column**, placed in a Collection Tube (2 mL; provided).

Drying step: Centrifuge for 2 min at 10,000 xg.

Discard the flowthrough and place the column into an elution tube (1.5 mL; not provided).

6 Elute eDNA

Add **100 µL Elution Buffer BE** onto the NucleoSpin® eDNA XS column.

Vortex for approximately 5 – 10 seconds until the NucleoTrap® material is completely resuspended.

Incubate approximately 1 min at room temperature.

Centrifuge for 1 min at 10,000 x *g*.

Discard the column and use the eDNA eluate for analysis.

5.1.2 Simplified extraction protocol for round filter and small filtration cartridges

Please note: this protocol is intended for users who do not have the option of using 15 mL tubes and corresponding centrifuges in their workspace. If you have such equipment available in your workspace, we recommend to follow the protocol 5.1.1. instead. Also note that this protocol recommends the use of the MN Bead Tube Holder for 5 mL Tubes. Please check 6.2. for ordering information. Depending on the type of filter used (high or low liquid absorbend) , a 5 mL syringe may be required for solution recovery from the filter (see ordering information).

1 eDNA release from filter

Place the round filter as a whole into a suitable tube, e.g. a 5 mL reaction tube (e.g. Eppendorf Biopur grade, recommended, see figure above).

Note: Do not cut the filter. Make sure that top side (the former water facing side) of the filter is now facing inward, so that the subsequently added DISSSOLVE buffer can easily contact the filtration residue.

Note: If a cartridge filter is used, add DISSOLVE and Proteinase into the cartridge.

Add **1.5 mL of buffer DISSOLVE** into the tube.

Add **25 µL of Liquid Proteinase K** into the buffer DISSOLVE and close the tube.

Vortex for 10 seconds. Make sure that the total filter is wetted by the DISSOLVE buffer.

Incubate the tube on the MN Bead Tube Holder (for 5 mL tubes) for 10 min with moderate agitation. The filter should be surrounded by / submerged in the DISSOLVE buffer.

Note: Strong agitation / vortexing should be avoided. Some filter types (e.g. nitrocellulose, glass fiber) might disintegrate upon strong vortexing, which can subsequently interfere with the purification process.

2 Lysate recovery

For absorbent filters (e.g. glass fiber filters), transfer the filter and the solution into a 5 mL syringe. Squeeze the liquid out of the filter by compressing it with the syringe and collect the liquid in a 2 mL tube (not provided). Discard the syringe containing the round filter.

Note: If filter material has disinterated due to excessive agitation / vortexing, the use of Syringe filter, PES, 25 mm, 5 µm (see ordering information) is recommended: pass the solution from the syringe through the syringe filter.

For non-absorbent filters (e.g. membrane filters) it is sufficient to remove the filter from the DISSOLVE buffer, let it drip-off briefly and discard the filter. Transfer the lysate into a fresh 2 mL tube (not provided). Alternatively, the filter can be pushed aside within the tube with a pipet tip in a way that the solution can be recovered directly from the tube without prior filter removal.

Note: The recommended filter type is a glass fiber filter absorbing most of buffer DISSOLVE. Other non-membrane filters are highly likely to absorb most of the buffer as well. In such cases, the use a 5 mL syringe is recommended in order to recover most of the solution. If other types of filters are used (e.g. membrane filter) the use of a syringe is dispensable due to the low absorption of liquid by membrane filters.

Note: If a cartridge filter is used, remove the lysate from the cartridge and put the lysate into a 2 mL Collection Tube (provided).

Centrifuge the recovered lysate for 1 min at 10,000 x *g*.

Note: A loose sediment might form, which can stay in the tube.

3 Removal of inhibitory substances

Add **270 µL buffer ACID** to the tube.

Invert the tube once to mix the buffer ACID into the solution.

Note: Do not mix thoroughly – pellets should not be resuspended.

Centrifuge for 2 min at 10,000 x *g* and transfer the supernatant without any sediment into a fresh Collection Tube (2 mL, not provided).

Note: Sediment might be very loose.

4 eDNA binding to the solid phase

Add **38 µL buffer PREC** to the recovered supernatant.

Add **25 µL NucleoTrap® suspension** and close the tube.

Note: Make sure to mix the suspension well before withdrawing an aliquot.

Incubate the tube at room temperature (18 – 25°C) for 5 min on a roller.

Note: Alternatively, the tube can be incubated on shaker with moderate shaking. eDNA will bind to the NucleoTrap® suspension in this step.

Centrifuge the tube for 3 min at 4,500 xg.

Note: The NucleoTrap® with eDNA bound to it is sedimented in this step.

Remove and discard the supernatant.

Note: Remove the supernatand with a pipet or turn the tube and place it upside down on a tissue to remove residual supernatant. Some droplets may stay in the tube.

5 Wash and dry solid phase

Add **400 µL Resuspension Buffer RESU** to the NucleoTrap® pellet and resuspend it by vortexing.

Centrifuge for 1 min at 10,000 x *g*.

Remove and discard the supernatant.

Note: The pellet might be translucent and thus be hardly visible in this step.

Add **400 µL Wash Buffer WB** to the NucleoTrap® pellet and resuspend pellet by vortexing.

Centrifuge for 1 min at 10,000 x *g*.

Remove and discard the supernatant.

Note: The pellet is typically easily visible in this step

Add **300 µL Wash Buffer WB** to the NucleoTrap® pellet and resuspend pellet by vortexing

Transfer the NucleoTrap® suspension onto a **NucleoSpin® eDNA XS column**, placed in a Collection Tube (2 mL; provided).

Drying step: Centrifuge for 2 min at 10,000 xg.

Discard the flowthrough and place the column into an elution tube (1.5 mL; not provided).

6 Elute eDNA

Add **100 µL Elution Buffer BE** onto the NucleoSpin® eDNA XS column.

Vortex for approximately 5 – 10 seconds until the NucleoTrap® material is completely resuspended.

Incubate approximately 1 min at room temperature.

Centrifuge for 1 min at 10,000 x *g*.

Discard the column and use the eDNA eluate for analysis.

5.1.3 Extraction protocol for Smith-Root eDNA Filter Packs

Compared to round filter paper inserted into an e.g. Nalgene™ Analyticval Test Filter Funnel as described in section 5.1., Smith-Root Filter Packs (www.smith-root.com) offer the advantage, that they are preassembled and allow easy filtration of water in the field and easy transportation of the cartridge back to the lab. Thus, the filter paper discs neither have to be handled in the field, nor has the water be transported into the lab.

Back in the lab, the filter membrane of a Smith-Root Filter Pack can easily be removed from the cartridge and further processed according to section 5.1.1 or 5.1.2. For those users who want to avoid the open handling and removal of filter discs from the Smith-Root Filter Pack, the following procedure is recommended. The dissolution of eDNA from the filter disc within the filter cartrige might yield less eDNA than the procedure of filter removal and dissolution of eDNA from the filter within a 5 mL tube. This is due to a higher eDNA release efficiency of the latter procedure.

Extraction protocol for eDNA from Smith-Root eDNA Filter Packs without remvoal of the filter disc from the cartridge

1 eDNA release from filter

After water filtration, remove excess water from the cartridge, e.g. pouring out.

Close the water outlet of the Smith Root filter cartridge. The “water out” side can be sealed by inserting a 2 mL tube into the flexible outlet.

Place the cartridge with the inserted 2 mL tube onto a shaker (e.g. Eppendorf Thermomixer).

Add **4 mL buffer DISSOLVE** and **50 µL Liquid Proteinase K** into the filter cartridge from the “water in” side and close the cartridge with parafilm.

Note: Do not use a lid or a tube for closing the “water in” side, because this will produce a pressure on the filter surface and the DISSOLVE buffer will pushed through.

Incubate the cartridge for **10 minutes** with moderate shaking (e.g. 600 rpm) on the shaker.

2 Lysate recovery

To recover the lysate from the cartridge remove the parafilm and pour the buffer from the "water in" side in a 15 mL or 5 mL tube (not provided).

Note: Injection of air (with a syringe or a 5 mL Eppendorf tube) into the "water in" opening can help to drive the lysate out of the cartridge, if pouring out of the lysate is inefficient.

Note: Typically, a volume of 3,5 mL liquid can be recovered from the cartridge, depending on the round filter type within the cartridge and residual water amount in it before addition of Buffer DISSOLVE.

Note: Some filter types will require the withdrawel of water from the "water in" and the "water out" side of the cartridge.

Centrifuge the recovered lysate for 2 min at 4500 g.

Note: A loose sediment might form, which can stay in the tube.

3 Removal of inhibitory substances

Add **720 µL of Buffer ACID** to the recovered lysate.

Invert the tube gently to mix the buffer ACID into the solution.

Note: Do not mix thoroughly - pellets do not have to be resuspend

Centrifuge for 2 min at 4.500 x *g*.

Transfer the supernatant without any sediment into a fresh collection tube (15 mL, not provided).

4 eDNA binding to the solid phase

Add **100 µL of Precipitation Buffer PREC** and **25 µL of NucleoTrap Suspension**.

Note: Make sure to mix the NucleoTrap suspension well before withdrawing an aliquot.

Incubate the tube at room temperature (18°C – 25°C) for a minimum of **5 min** on a roller.

Note: Alternatively, the tube can be incubated on a shaker with moderate shaking. The eDNA will bind to the NucleoTrap suspension in this step.

Centrifuge for 3 min at 4,500 x *g*.

Note: The NucleoTrap® suspension with eDNA bound to it, is sedimented in this step.

Remove and discard the supernatant.

5 Wash and dry solid phase

Add **400 µL Resuspension Buffer RESU** to the NucleoTrap® pellet and resuspend it by vortexing.

Transfer the suspension into a 1.5 mL tube (not provided).

Centrifuge for 1 min at 10.000 x *g*.

Remove and discard the supernatant.

Note: The pellet might be translucent an thus be hardly visible in this step.

Add **400 µL Wash Buffer WB** to the pellet and resuspend the pellet by vortexing.

Centrifuge for 1 min at 10.000 x *g*.

Remove and discard the supernatant.

Note: The pellet is typically easily visible in this step.

Add **300 µL Wash Buffer WB** to the NucleoTrap pellet and resuspend it by vortexing.

Transfer the NucleoTrap suspension onto a NucleoSpin® eDNA XS column placed in a Collection Tube (2 mL; provided).

Drying step: Centrifuge for 2 min at 10,000 x *g*.

Discard the flow through and place the column into an elution tube (1.5 mL, not provided).

6 Elute eDNA

Add **100 µL Elution Buffer BE** onto the NucleoSpin eDNA XS column.

Vortex for approximately 5 – 10 seconds until the NucleoTrap material is completely resuspended.

Incubate approximately 1 min at room temperature.

Centrifuge for 1 min at 10,000 x *g*.

Discard the column and use the eDNA eluate for analysis.

5.1.4 Extraction protocol for large volume filter cartridges

In some applications the use of large volume filter cartridges is required or desired. Examples of such filter cartridges are:

- Whatman™ Polycap GW 75 High-Capacity Capsules 0,45 µm
- AquaPrep™ 600 Capsules Groundwater sampling

There are many other large volume filter cartridges differing in membrane type, pore size, inner volume, dead volume, and filtration area. Such cartridges may enable the filtration of larger volumes of water than the recommended MN Glass Fiber Filter (45 mm, ethyleneoxide treated, see ordering information), however they might tend to clog early (despite their larger filter area), dependent on water quality, kind, and amount of suspended matter. Note that increasing the volume of water for processing also increases the total amount of inhibitors processed. We

therefore recommend to evaluate the benefits of such cartridges in comparison to procedures 5.1.1 or 5.1.3.

Most of these large volume filter cartridges require a large volume of buffer for eDNA release and flush-out. This therefore requires an adapted procedure.

Follow the procedure below for isolation of eDNA from large volume filter cartridges.

Note: Additional amounts of reagents are required for this procedure: Buffer DISSOLVE, Liquid Proteinse K, Precipitation Buffer PREC, NucleoTrap Suspension, Resuspension Buffer RESU (see section 6.2 ordering information).

1 eDNA release from filter

After water filtration, remove excess water from the cartridge, e.g. by aspiration or pouring out.

Add **40 mL buffer DISSOLVE** and **125 µL Liquid Proteinase K** into the filter cartridge from the "water in" side and close the cartridge.

Incubate the cartridge for **30 minutes** with moderate agitation (e.g. rolling). The filter area should be surrounded by / submerged in the DISSOLVE buffer.

Note: Strong agitation should be avoided. Some cartridges might tend to form plenty of foam, which can subsequently interfere with the purification process. Do not use less than 125 µL Liquid Proteinase K; a higher volume of proteinase might be beneficial. Do not incubate shorter than 30 minutes; a longer incubation might be beneficial.

2 Lysate recovery

Remove the lysate from the cartridge.

Note: Best practice for lysate removal is cartridge dependend. Injection of air into the "water in" opening can help to drive the lysate out of the cartrige, if pouring ouf of the lysate is inefficient.

Typically, a volume of 40 mL (+/- 5 mL) liquid should be recovered from the cartridge, depending on the dead volume of the cartidge and residual amount water in it before addition of Buffer DISSOLVE. Transfer the recovered liquid into a 50 mL tube (not provided).

3 Removal of inhibitory substances

Add **7 mL of Buffer ACID** to the recovered lysate.

Invert the tube gently to mix the buffer ACID into the solution.

Note: Do not mix thoroughly - pellets do not have to be resuspend

Centrifuge for 2 min at 4.500 x *g*.

Transfer the supernatant without any sediment into a fresh collection tube (15 mL, not provided).

4 eDNA binding to the solid phase

Add **100 µL of Precipitation Buffer PREC** and **75 µL of NucleoTrap Suspension**.

Note: Make sure to mix the NucleoTrap suspension well before withdrawing an aliquot.

Incubate the tube at room temperature (18 – 25°C) for a minimum of **5 min** on a roller.

Note: Alternatively, the tube can be incubated on a shaker with moderate shaking. The eDNA will bind to the NucleoTrap suspension in this step.

Centrifuge for 3 min at 4,500 x *g*.

Note: The NucleoTrap suspension with eDNA bound to it, is sedimented in this step.

Remove and discard the supernatant.

5 Wash and dry solid phase

Add **1 mL Resuspension Buffer RESU** to the NucleoTrap pellet and resuspend it by vortexing.

Transfer the suspension into a 1.5 mL tube (not provided).

Centrifuge for 1 min at 10.000 x *g*.

Remove and discard the supernatant.

Add **400 µL Wash Buffer WB** to the pellet and resuspend it by vortexing.

Centrifuge for 1 min at 10.000 x *g*.

Remove and discard the supernatand.

Add **300 µL Wash Buffer WB** to the NucleoTrap pellet and resuspend it by vortexing.

Transfer the NucleoTrap suspension onto a NucleoSpin® eDNA XS column placed in a Collection Tube (2 mL; provided).

Drying step: Centrifuge for 2 min at 10,000 x *g*.

Discard the flowthrough and place the column into an elution tube (1.5 mL, not provided).

6 Elute eDNA

Add **100 µL Elution Buffer BE** onto the NuclewoSpin® eDNA XS column.

Vortex for approximately 5 – 10 seconds until the NucleoTrap material is completely resuspended.

Incubate approximately 1 min at room temperature.

Centrifuge for 1 min at 10,000 x *g*.

Discard the column and use the eDNA eluate for analysis.

5.2 Protocol for eDNA isolation from 40 mL water by direct precipitation (without filtration)

Before starting the procedure:

- Additional precipitation buffer PREC is required for this procedure (see ordering information).
- Make sure to work in an environment which minimizes risk of DNA contamination.
- Decontaminate your working environment (e.g. lab bench surfaces) before starting the procedure.
- Make sure to decontaminate any reusable material (e.g. pincers/tweezers).
- Make sure to have all materials available, depending on your experimental set up (see section 1.2 for required and optional materials)
- Make sure to mix the NucleoTrap® suspension well before withdrawing an aliquot. It is important that the NucleoTrap® particles are released from the bottom of the tube and dispersed well within the liquid.

1 Water collection

Collect **40 mL water** in a 50 mL tube (not provided). For good eDNA coverage, collect water subsamples from different places of the water system to be analyzed and pool them. The optimal volume of water depends on the quality of the water and the abundance of the target DNA.

Note: Make sure to use a DNA-free water collection container.

Note: Water samples containing considerable amounts of particular matter (e.g. multicellular organisms, organic debris like rotten plant material) should be moderately cleared by centrifugation (min 4,500 x g) or prefiltation with by prefiltration through a sieve.

Add **800 µL PREC** to the 40 mL water sample and mix.

Note: The sample can be stored / transported for several days at room temperature (18 – 25°C) preventing sample spoilage.

Note: If water volumes smaller than 40 mL are processed, adjust the volume of PREC proportionally.

2 eDNA binding to the solid phase

Add **25 µL NucleoTrap® suspension** and close the tube.

Note: Make sure to mix the suspension well before withdrawing an aliquot.

Incubate the tube at room temperature (18 – 25°C) for 15 – 20 min on a roller.

Note: eDNA will bind to the NucleoTrap® suspension in this step.

Centrifuge the tube for 3 min at 4,500 x *g*.

Note: The NucleoTrap® is sedimented in this step.

Remove and discard the supernatant.

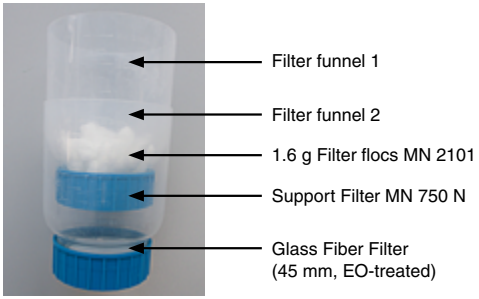
Note: Turn tube upside down to remove residual free supernatant.

3 Wash and dry solid phase

Continue the procedure as described in section 5.1 with *Wash and dry solid phase*.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Filter clogging	• Too much water used. Use less water or use MN Filter Flocs MN 2101 and Filter MN750N in a prefilter. • Water contains too much insoluble substances. Use MN Filter Flocs MN 2101 and Filter MN750N in a prefilter in order to prevent / reduce risk of filter clogging or use less water. See figure below.  **Figure 2 Example of the assembly of a prefilter with MN2101 filter flocs and support filter MN 750N.** • Membrane filter used. Membrane filter tend to clog much faster than the recommended one. Use the recommended filter.
No DNA yield	• Filter bypass. Make sure that the filter is placed correctly in the filter holder ensuring that all water passes through the filter and that there is no filter bypass. Some filter types (e.g. membrane filter) tend to have higher risks of bypasses or filter breakages. • Inappropriate DNA quantification method. Some water types contain little eDNA. Make sure your DNA quantification is sensitive enough (e.g. quantification with PicoGreen dye or by PCR).
DNA highly degraded	• Water quality: Some water samples contain predominantly highly degraded DNA while other water samples may contain larger amounts of high molecular weight DNA. • Inappropriate sample collection, storage, transport: Make sure to process the sample as soon as possible after collection. If there is a delay, it is possible to cool the water for several hours. Alternatively, filters may be conserved by ethanol treatment.

Problem	Possible cause and suggestions
Contamination of the eluate with DNA from unexpected species	• DNA contamination during sample collection or processing: Make sure to work in an environment which minimizes risk of DNA contamination: close doors and windows; prevent uncontrolled air draft; wear clean personal protection equipment; change gloves frequently; ideally work in a controlled positive pressure lab. • Contaminated reusable materials (e.g. water collection container): Make sure to only use reusable materials which has been decontaminated. • Contaminated working environment: Decontaminate your working environment (e.g. lab bench surfaces) before starting the procedure. • Run a no-sample extraction control in order to obtain information, which DNA does not originate from your sample. • Wrong or unspecific species “identification”: Make sure that your detection method is sufficient specific for organisms or your interest.

The NucleoSpin® eDNA Water kit is produced under controlled and monitored conditions with high hygienic standards. Nonetheless, it cannot be guaranteed that there is no contaminating DNA at all times of whatever source due to the omnipresence of DNA and the increasing sensitivity of DNA detection and assignment. Therefore, a no-sample extraction control (mock preparation) is recommended.

6.2 Ordering information

Product	REF	Pack of
Glass Fiber Filter (45 mm, EO-treated)	740564	50 pieces
NucleoSpin® Filter Midi	740607	50 pieces
MN Bead Tube Holder 5 mL	740459	1 piece
Filter flocs MN 2101, ashless, quantitative	281120	500 g
Filter MN 750 N (45 mm diameter)	please inquire for formats and REF	
Disposable 5 mL syringe wiht Luer tip	729101	100 pieces
Syringe filter, PES, 25 mm, 5 µm	729242	100 pieces
Buffer PREC	740568	50 mL
NucleoSpin® Inhibitor Removal	740408.10 / .50	10 / 50 preps
Liquid Proteinase K	740396	5 mL
NucleoTrap	740589	1 mL
Buffer DISSOLVE	740566	1000 mL
Buffer RESU	740565	60 mL
Buffer ACID	740567	125 mL

6.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

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

Last updated: 08/2022, Rev. 04

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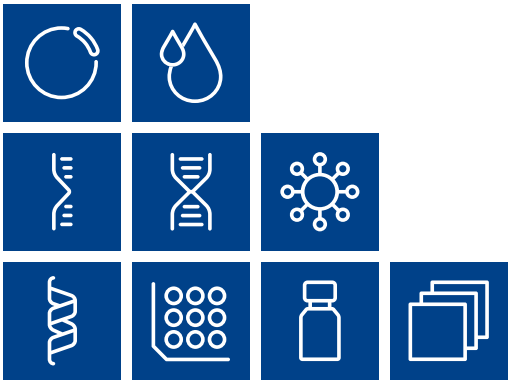
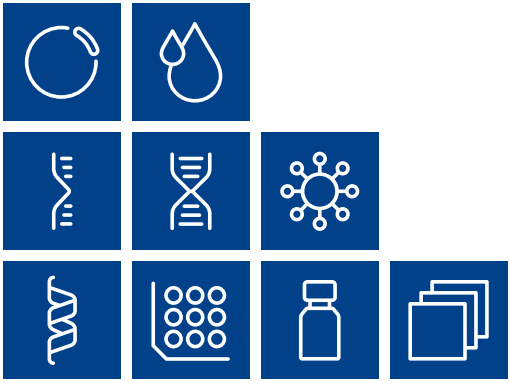


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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