MACHEREY-NAGEL

NucleoSpin® Soil

Protocol recommendations for DNA extraction from water

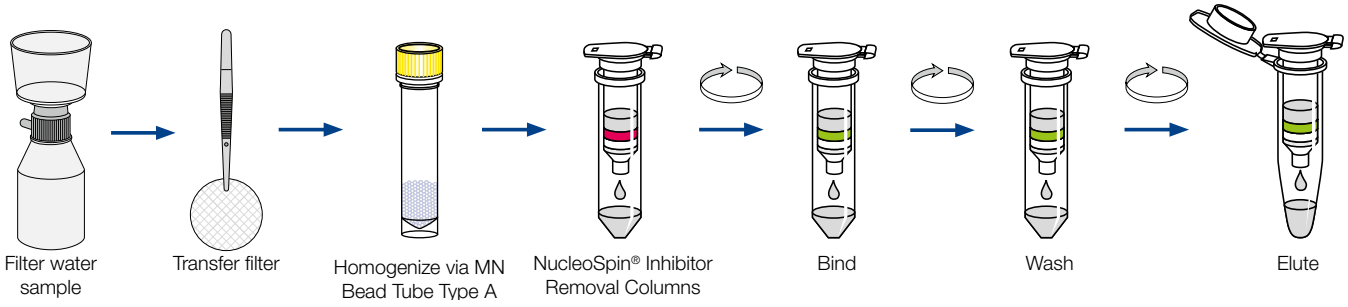
Introduction:

Here in this Tech Note we show that our NucleoSpin® Soil kit is well suited for DNA extraction from water samples. A combination of mechanical lysis with MN Bead Tubes Type A and the right lysis buffer chemistry allows reliable isolation of high-quality DNA even from hard to lyse microorganisms present in water. The superior NucleoSpin® Soil inhibitor removal technology eliminates PCR Inhibitors like humic acids. Purified DNA is suitable for PCR and other downstream applications.

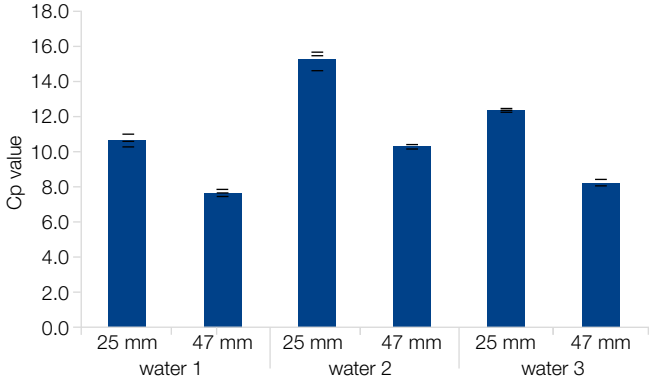
Specifications:

NucleoSpin® Soil	
Technology	Silica membrane technology, Mini prep
Sample material	Water, filter
Elution volume	50–100 µL
Fragment size	50 bp–approx. 50 kbp
Preparation time	90 min/10 preps

Procedure:



Application data:



Efficient DNA isolation from water filters

A qPCR was performed with nucleic acids isolated from round filters demonstrating reliable results across different filtration systems.

Volume of water sample:

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

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

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 prefilter on top of the membrane filter is recommended. MACHEREY-NAGEL offers a broad range of filter paper suitable for prefiltration of large debris.

Suitable prefilter	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

Storage of water samples:

Filter the water sample through a membrane filter. Roll the membrane into a cylinder (top side facing inwards) and insert it into a MN Bead Tube Type A (25 mm filters) or MN Bead Tube Type A 5 mL (47 mm filters). Store the bead tubes at -20 °C and re-warm to room temperature before starting the preparation.

Filter Membranes:

NucleoSpin® Soil 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
Cellulose nitrate (NC)	25 mm	0.2 µm	PORAFIL® CM	6570020025
		0.45 µm	PORAFIL® CM	6570045025
	47 mm	0.2 µm	PORAFIL® CM	6570020047
		0.45 µm	PORAFIL® CM	6570045047
Polycarbonate (PC)	25 mm	0.40 µm	PORAFIL® PC	676040025

Protocol for isolation of DNA from 25 mm Filters

Procedure		
1	Filter water sample	Filter water sample through an appropriate 25 mm filter membrane (not provided, see ordering information) using a suitable filtration device.
2	Mechanical lysis	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. Add 700 µL Buffer SL1 or SL2 and optionally up to 150 µL Buffer SX. Addition of Buffer SX is not recommended for water samples with a high concentration of humic acids
3	DNA extraction	Continue with step 3 of the NucleoSpin® Soil protocol.

Protocol for isolation of DNA from 47 mm Filters

Procedure		
1	Filter water sample	Filter water sample through an appropriate 47 mm filter membrane (not provided, see ordering information) using a suitable filtration device.
2	Mechanical lysis	Remove the filter membrane from the filtration device by using sterile forceps, roll the membrane into a cylinder (top side facing inwards) and insert it into a MN Bead Tube 5 mL Type A (see ordering information). Add 1400 µL Buffer SL1 or SL2 and optionally up to 300 µL Buffer SX. Addition of Buffer SX is not recommended for water samples with a high concentration of humic acids. Vortex the MN Bead Tube 5 mL Type A on the MN Bead Tube Holder 5 mL (see ordering information) horizontally in conjunction with a Vortex-Genie® for 15 min at maximum speed.
3	Centrifugation	Centrifuge the Bead Tubes at > 4,500 x g for 2 minutes. Remove and discard filter using a sterile pipette tip.
4	Precipitate contaminants	Add 300 µL Buffer SL3 and vortex for 5 s. Incubate at 0–4 °C for 5 min. Centrifuge the Bead Tubes at > 4,500 x g for 5 minutes at 4 °C.
5	Filter lysate	Load up to 650 µL supernatant into a NucleoSpin® Inhibitor Removal Column (red ring) and centrifuge at 11,000 x g for 1 min. Collect flowthrough in a clean 15 mL centrifuge tube (not supplied). Repeat this step until the remaining supernatant is filtered using fresh NucleoSpin® Inhibitor Removal Columns (not supplied, see ordering information) for each repetition.
6	Adjust binding conditions	Add 500 µL Buffer SB to the pooled flowthrough and vortex for 5 s. See ordering information for additional Buffer SB if needed.
7	Bind DNA	Load up to 550 µL sample into a NucleoSpin® Soil Column (green ring) and centrifuge at 11,000 x g for 1 min. Discard flowthrough. Repeat this step to load the remaining sample. Continue with step 8 of the NucleoSpin Soil protocol.

Ordering information

Kit	Specification	REF	Pack of
NucleoSpin® Soil	Mini spin kit for isolation of total DNA from diverse soil types, 2 ml MN Bead Tubes Type A included	740780.10/.50/.250	10 / 50 / 250
MN Bead Tubes Type A	2 mL tubes with 0.6–0.8 mm ceramic beads; for homogenization of soil, sediments, and stoo	740786.50	50 preps
MN Bead Tubes Type A 5 mL	5 mL tubes with 0.6–0.8 mm ceramic beads; for homogenization of soil, sediments, and stool	740799.50	50 preps
MN Bead Tube Holder	Rubber-foam adapter for processing MN Bead Tubes with Vortex-Genie® 2	740469	1 piece
MN Bead Tube Holder 5 mL	Rubber-foam adapter for processing MN Bead Tubes 5 mL with Vortex-Genie® 2	740459	1 piece
Buffer SB	Binding Buffer SB	740785.50	60 mL
NucleoSpin® Inhibitor Removal Columns	Columns for Inhibitor Removal	740789.50/.250	50 / 250

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