

NucleoMag® DNA/RNA Water

Automated purification of DNA and RNA from water samples on the Hamilton NIMBUS® Presto workstation



Introduction

Molecular analytical techniques are valuable tools for the evaluation of the microbial quality of water.

Consistent with this, the reliable isolation of nucleic acids from water samples becomes increasingly important in the field of water microbiomics research, drinking water safety or pathogen surveillance in sewage. Typical applications range from genetic analysis (e.g. species identification / microbiomics), the detection of waterborne pathogens, identification of contaminants or GMO-testing.

Two of the major challenges in nucleic acid isolation from water samples are the low titer of microorganisms / viruses, such that large sample volumes must be processed in order to obtain a sufficient level of sensitivity. Further, drinking and environmental water samples contain a diverse array of contaminants (PCR inhibitors such as humic acids) that can interfere with molecular testing techniques, especially when large volumes of water are concentrated to the small volumes needed for effective molecular analysis.

The MACHEREY-NAGEL NucleoMag® DNA/RNA Water kit contains specialized buffers for sample lysis and removal of inhibitory substances, enabling efficient purification of highly pure DNA and RNA from clear to turbid water samples. Further, NucleoMag® DNA/RNA is compatible with various concentration methods (e.g., Ultrafiltration, PEG precipitation or INNOVAPREP Concentrating Pipette Select) and with a variety of filters, including conventional round filters (e.g. 25 mm to 47 mm) as well as with cartridge filters (such as Sterivex™).

The Hamilton NIMBUS Presto workstation combines the utility of automated liquid handling with the high-speed processing of the KingFisher™ Presto unit.



The NIMBUS Presto workstation combines liquid handling and magnetic rod processing for fully automated, high-throughput nucleic acid extractions.

NIMBUS Presto workstation

Technology	Automated liquid handling platform (Hamilton NIMBUS) with integrated magnetic rod processing unit (KingFisher™ Presto)
Capacity	1–96 samples (≤ 200 µL sample volume)
Processable volume	50–5000 µL
Footprint	L 1359 mm W 709 mm H 889 mm

Material and Methods

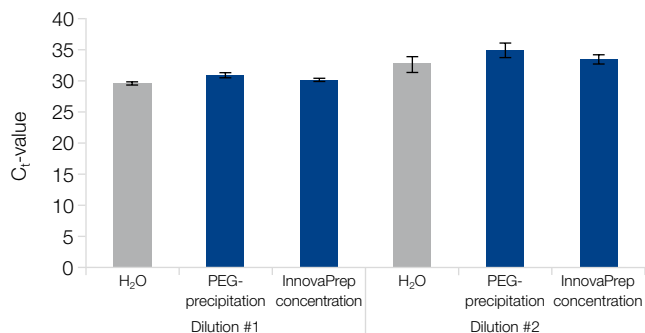
In this Application Note, we demonstrate the automated purification workflow using the MACHEREY-NAGEL NucleoMag® DNA/RNA Water and the Hamilton NIMBUS Presto system exemplarily for viral nucleic acid purification from wastewater (A) and microbial DNA isolation from water samples (B).

(A) Synthetic wastewater was prepared in accordance with ASTM standard D5905. The wastewater samples (20–40 mL) were either precipitated using PEG/NaCl or pre-filtered through a 0.2 µm filter unit and then concentrated via the INNOVAPREP CP-Select™ sample concentration device. For more information on sample concentration using the INNOVAPREP Concentrating Pipette Select, please visit www.innovaprep.com. After sample concentration, nucleic acids were isolated with NucleoMag® DNA/RNA Water. First, 10 µL of Liquid Proteinase K (see ordering information) was mixed with 200 µL of the wastewater concentrate and the indicated volumes of lysis buffer. Samples were incubated at 56 °C for 10 min and processed according to the standard protocols. (B) For microbial DNA isolation, water samples were processed according to the standard protocol using 50 mm CM filters (0.45 µm).

NucleoMag® DNA/RNA Water

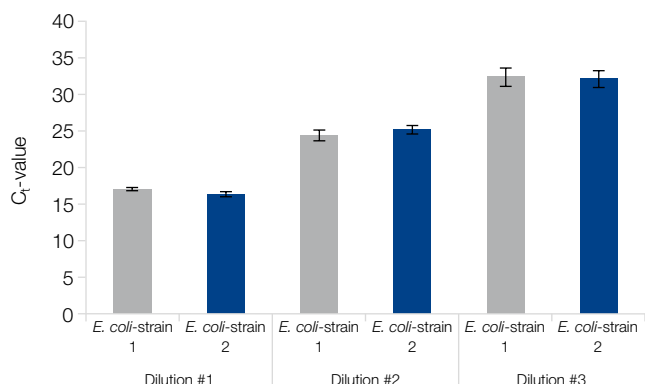
Technology	Magnetic beads
Sample material	Filtered water or air samples; ultrafiltrated or precipitated water samples; samples concentrated with INNOVAPREP Concentrating Pipette Select
Max. amount of starting material	10–1000 mL
Elution volume	50–200 µL
Typical DNA yield	depending on sample type and quality
Preparation time	Approx. 70 min (excl. lysis and filtration) / 96 samples

Application data



Armored Enterovirus RNA recovery from synthetic wastewater

NucleoMag® RNA/DNA Water was used to isolate RNA from synthetic wastewater, either concentrated via PEG-precipitation or using the InnovaPrep CP-Select™ sample concentration device. Concentrates were spiked with different amounts of armored Enterovirus RNA (Asuragen®). qRT-PCR analysis was performed using the Sigma SYBR Green Quantitative RT-PCR Kit on BioRad CFX 96 Cycler. Recovered RNA was compared to the respective RNA spiked into pure water samples (H₂O). qPCR results demonstrate the reliable detection of RNA recovery independent from pretreatment methods.



Reliable *E. coli* DNA recovery from water samples

100 mL water samples were spiked with 10² (dilution #1), 10⁴ (dilution #2) or 10⁶ (dilution #3) cells of two different *E. coli* strains (*E. coli* Top10, strain 1; *E. coli* DH5alpha, strain 2). Samples were filtered using 50 mm CM Filter (0.45 µm) and processed according to the standard protocol. qPCR analysis of eluted DNA was performed with Biorline SensiFast™ SYBR Lo-ROC Kit using *E. coli* specific primers (uidA β-glucuronidase locus). Bacterial DNA was detected consistently and reliably over a range of a dilution series.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® DNA/RNA Water	Magnetic bead-based kit for the isolation of DNA and / or RNA from water and air samples; including NucleoMag® B-Beads, buffers	1 x 96 preps 4 x 96 preps	744220.1 744220.4
Liquid Proteinase K	Ready-to-use Liquid Proteinase K, recombinant	5 mL 30 mL	740396 740396.30
NIMBUS Presto	Automated liquid handling platform with 4 pipetting channels, a CO-RE Gripper, barcode scanner, and many additional features		Hamilton*

NucleoMag® is a registered trademark of MACHERY-NAGEL; Hamilton® and NIMBUS® are registered trademarks of Hamilton Company; KingFisher™ is a trademark of Thermo Fisher Scientific; SensiFast™ is a trademark of Biorline Reagents; Taqman® is a registered trademark of Roche Diagnostics; CP-Select™ is a trademark of INNOvarep**

* For more detailed information, please visit www.hamiltoncompany.com/robotics. To find a Hamilton subsidiary or distributor in your area, please visit <http://www.hamiltoncompany.com/contacts>.

**For more detailed information, please visit <https://innovaprep.com/>.

A rapid, fully automated solution for RNA purification from various sample materials

MACHERY-NAGEL and Hamilton deliver a tailored solution for your high-throughput DNA and RNA extraction needs from clear to turbid water samples. We have adapted the NucleoMag® DNA/RNA Water procedure on the NIMBUS Presto workstation to meet the expectations of water microbiomic research and applied environmental testing laboratories.

Here, we demonstrate the successful use of the NucleoMag® DNA/RNA Water kit for nucleic acid isolation from water samples and downstream qPCR assays.

The powerful combination of the NucleoMag® technology and the NIMBUS Presto workstation has several advantages over standard nucleic acid purification procedures:

- Save hands-on time by using automated plate-prefilling and plate-handling performed by the NIMBUS workstation
- Benefit from the high-speed extraction procedure of the integrated KingFisher™ Presto unit
- Minimized downstream inhibition for reliable results and downstream assay performance
- Compatible with multiple filtration systems, ultracentrifuged or precipitated water samples