



NucleoMag[®] DNA / RNA Water

Automated DNA / RNA purification from environmental water samples on the NucleoMag[®] X32

Application benefits

The integration of the well-established NucleoMag[®] DNA / RNA Water kit, Ceres Nanotrap[®] Microbiome A particles and the NucleoMag[®] X32 provides a streamlined and efficient workflow for optimized and rapid capture and concentration of microbes, enabling the isolation of high quality nucleic acids from large volume environmental water samples.

- Rapid and optimized concentration of microbes and virus combined with automated DNA / RNA extraction
- No centrifugation or filtration required
- Simultaneous processing of up to 32 samples in parallel
- Consistent and reliable results
- No programming required: Verified and pre-installed methods available

Keywords

Waste water, sewage, microbes, viral particles, waterborne pathogens, magnetic beads, sample concentration, inhibitor removal, NucleoMag[®], Ceres Nanotrap[®], magnetic beads, magnetic rod system, NucleoMag[®] X32



Introduction

Researchers, private companies, and public health agencies widely utilize wastewater testing to monitor infections in specific communities, campus dormitories, and health centers. Molecular wastewater testing plays a crucial role in early detection and tracking of viral outbreaks or microbial contaminations in water sources. It helps evaluate the effectiveness of public health interventions and identify emerging pathogenic threats. Water testing enables non-invasive, population-level surveillance, offering cost-effective monitoring of infection rates and supporting proactive public health measures alongside individual testing.

The extraction of DNA and RNA from wastewater presents a challenge due to inhibitory substances and the need for large sample volumes. Conventional wastewater concentration methods exacerbate the issue by concentrating inhibitors along with the target pathogens. Moreover, many concentration techniques are labor-intensive and costly. This application note highlights a rapid, efficient, and cost-effective method for extracting pathogen DNA/RNA from complex wastewater samples. It involves using MACHEREY-NAGEL's NucleoMag[®] DNA/RNA Water kit in combination with Ceres Nanosciences' Nanotrap[®] Microbiome A magnetic particles and Enhancement Reagent 1 for optimized microbe and virus capture and concentration from environmental water.

This Application Note showcases the automated extraction of nucleic acids from raw environmental water samples using the NucleoMag[®] DNA/RNA Water kit on the NucleoMag[®] X32 system, which is a compact nucleic acid extraction platform based on magnetic bead technology. With the capacity to process up to 32 samples simultaneously, this user-friendly instrument offers pre-installed verified scripts and detailed protocol information, streamlining the mixing, magnetic bead transfer, washing, and elution steps and saving valuable hands-on time. For more information on the NucleoMag[®] X32 and additional application notes, please visit <https://www.mn-net.com/de/NucleoMag-X32>.

NucleoMag [®] DNA / RNA Water	
Technology	Magnetic beads for isolation of RNA and DNA
Sample material	10 – 1000 mL water sample
Elution volume	50 – 200 µL
Fragment size	300 bp – approx- 50 kbp
Max. sample number on the NucleoMag [®] X32	32 samples

NucleoMag [®] X32	
Technology	Automated magnetic rod system
Display	7 inch-color touch screen
Capacity / volume per well	1 – 32 samples / 50 µL to 1000 µL
Dimensions	417 × 410 × 426 mm
Weight	30 kg
Contamination control	UV lamp, internal filter system
Website	www.mn-net.com/de/NucleoMag-X32

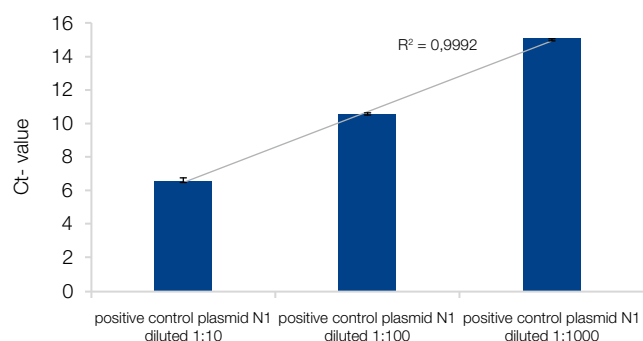
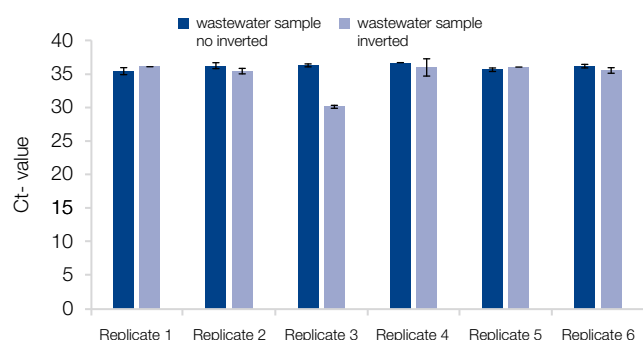
Material and Methods

The experiments followed the Ceres Nanoscience protocol APP-083** as instructed by the manufacturer. This protocol utilizes Nanotrap® Microbiome Particles and Enhancement Reagent 1 to quickly capture and concentrate microbes from raw sewage samples without the need for filtration or centrifugation. In summary, 10 mL environmental water samples were combined with 100 µL Enhancement Reagent 1 (ER1) and 150 µL Nanotrap® Microbiome A Particles to concentrate the microbes. The NucleoMag® DNA/RNA Water kit was used to purify nucleic acids from the Nanotrap® particle pellet, employing reversible adsorption of nucleic acids to paramagnetic

NucleoMag® B-Beads in the presence of buffer MWA2. Following magnetic separation, the NucleoMag® B-Beads were washed with wash buffers MWA3 and MWA4 ethanol to eliminate PCR inhibitors and other contaminants. After air drying, highly pure nucleic acids were eluted using RNase-free water.

The NucleoMag® X32 magnetic rod device was utilized for all binding, washing, and magnetic bead separation steps after Nanotrap® sample preparation. For detailed instructions on sample preparation and protocol, please refer to the Ceres nanoscience protocol APP-083**.

Application Data



Detection of SARS-Cov-2 in waste water samples

A 10 mL volume of wastewater underwent concentration using Ceres Nanosciences' Nanotrap® particles, and viral RNA was subsequently extracted using the MACHERY-NAGEL NucleoMag® DNA/RNA Water kit on the NucleoMag® X32 extraction robot. The wastewater sample was obtained from a wastewater treatment plant located in Düren, Germany (NRW) and had previously tested positive for SARS-CoV-2 RNA. The sample was divided into five equal biological replicates of 10 mL each, and some replicates were inverted while others remained non-inverted prior to processing. Viral detection was accomplished using the SensiFast™ Probe One-Step Lo-ROX kit from Biorline on an Applied Biosystems® 7500 Real-Time PCR System with N1 primers. The consistent and sensitive detection in all five replicates demonstrates the method's robustness and repeatability.

Absence of inhibitory effects in downstream analysis from carried-over inhibitors

To exclude the potential inhibition of downstream analysis caused by carry-over of inhibitors present in the wastewater sample, positive pEX-A128-nCoV_N1 control plasmids were added to the eluate at different dilution levels. The eluate was then subjected to qRT-PCR analysis using the SensiFast™ Probe One-Step Lo-ROX kit from Biorline on an Applied Biosystems® 7500 Real-Time PCR System. The N1 gene consistently and reliably showed detection across a range of dilutions with excellent linearity ($R^2 = 0.9992$), showing ideal and inhibitor-free suitability of eluates for common downstream analysis.

Ordering information

Product	Specifications	Quantity	REF
NucleoMag® DNA/RNA Water	Magnetic bead-based isolation of DNA and RNA from water and air samples; including NucleoMag® B-Beads and buffers and RNase-free water	1 × 96 preps 4 × 96 preps	744220.1 744220.4
Nanotrap® Magnetic Microbiome A Particles*	Magnetically functionalized particles to capture and concentrate microbes from raw sewage requiring no filtration or centrifugation	10 mL or 30 mL	44202*
Nanotrap® Enhancement Reagent 1*	The Enhancement Reagent in combination with the Nanotrap® particles improves the binding of microbes in raw sewage samples, further improving downstream detection of nucleic acids	10 mL 30 mL	10111 – 10 10111 – 30
NucleoMag® X32	Magnetic rod system for automated nucleic acid extraction using MACHERY-NAGEL NucleoMag® kits, parallel processing of up to 32 samples	1	747020
96 Deep-well plates	96 deep-well plates for NucleoMag® X32	25	744955
Tip combs	8-place magnetic tip combs for NucleoMag® X32	50	744960

NucleoMag® is a registered trademark of MACHERY-NAGEL; SensiFast™ is a registered trademark of Meridian Bioscience®;

Nanotrap® is a trademark of Ceres Nanosciences®

*For more detailed information, please visit www.ceresnano.com

**APP-083: www.ceresnano.com/_files/ugd/f7710c_64349c81d2d443f5acc3242f729a617c.pdf (online version; May 2023)