

Viral nucleic acid isolation from water samples

General recommendation for sample preparation

General problems

Extraction of viral nucleic acids from water samples poses several challenges. The first problem is the low viral titer, resulting in high sample volume requirements for obtaining a sufficient level of detection sensitivity. Furthermore, the varying amount and size of debris suspended in the water column may cause problems within a sample as well as between different samples. A third problem is the potential presence of PCR inhibitors such as humic acids. Last but not least, the sample must be stabilized to prevent a loss of viral particles in the time span between the sample collection and purification.

Stabilization and volume reduction

Collect samples of 1 +/- 0.5 liters of effluent or other samples with an expected high content of debris and microorganisms. Remove debris by sedimentation. Alternatively, collect 50 +/- 10 liters of clear river or tap water. It is recommended to chill the water samples on ice and to isolate the viral nucleic acid within 24 hours.

Adjust the pH of the water with 2 N HCl to a value of about 3.5. Do not omit this pH-adjustment step as it is required to impart a positive net electric charge on the viral particles. The positive charge is crucial for the binding to the filter surface in the next step. Pass the pH-adjusted water sample through a 0.45 µm negatively charged filter e. g. a mixed cellulose esters membrane (e. g., Merck-Millipore HAWP09000). Use of filters with a diameter of 90 mm is recommended to prevent clogging, but the ideal filter diameter may vary in accordance with the sample volume, depending on the content of remaining debris within the sample. Viral particles will bind to the surface of the filters and can be directly isolated from there without prior elution procedures.

Nucleic acid isolation

It is recommended to use the **NucleoBond® RNA Soil Midi kit** (REF 740140.20) in combination with the DNA Set for NucleoBond® RNA Soil (REF 740141.20) for the isolation of viral nucleic acid from the filter disks.

Cut the filter disk into small pieces and transfer the fragments into a sterile 15 mL centrifuge tube. Add the ceramic beads from four MN Bead Tubes Type A, contained within the NucleoBond® RNA Soil Midi kit, to the 15 mL centrifuge tube and follow the standard kit protocol. Addition of Phenol:Chloroform:Isoamylalcohol (25:24:1) is required. This reagent is not included in the kit and not available from MACHEREY-NAGEL.

Inclusion of the DNA Set for NucleoBond® RNA Soil is necessary. Because the separation of RNA and DNA is not based on chemical properties but rather on a difference in size, large RNA genomes such as the genome of SARS-CoV-2 will elute in the DNA fraction. It is recommended to combine the RNA and DNA eluates before further analysis or to elute the complete RNA and DNA in one combined fraction with buffer EDNA only.

Isolated nucleic acid is free of PCR inhibitors due to a **patented inhibitor removal technology**.

Alternatively, the NucleoSpin® RNA Stool Kit (REF 740130.50) can be used to isolate viral nucleic acid from cut filter disks.

Put a piece of the cut filter disc into a Bead Tube Type A, that is supplied with the NucleoSpin® RNA Stool Kit and follow the instructions in the kit handbook. Use the protocol for total RNA isolation, using the larger volume of Buffer RST2 for binding and, if DNA should also be isolated, omit the optional DNA digestion step in the protocol.

If a high throughput purification for screening purposes is required, it is possible to use the **NucleoMag® DNA/RNA Water kit** (REF 744220.4), using the standard protocol, which is based on magnetic bead purification and is compatible with many liquid handling systems or automated magnetic separators. In order to process the filter disks, MN Bead Tubes Type A have to be ordered separately (REF 740812.50). Alternatively, the larger MN Bead Tubes Type A 5 mL (REF 740799.50) can be purchased for more convenient handling.

Ordering information

Kit	REF	Pack of
NucleoBond® RNA Soil	740140.20	20 preps
DNA Set for NucleoBond® RNA Soil	740140.20	20 preps
NucleoSpin® RNA Stool	740130.50	50 preps
NucleoMag® DNA/RNA Water	744220.1	96 preps
	744220.4	384 preps
MN Bead Tube Type A	740812.50	50 pieces
MN Bead Tube Type A 5 mL	740799.50	50 pieces
MN Bead Tube Holder	740469	1 piece
MN Bead Tube Holder 5 mL	740459	1 piece

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