



Wastewater monitoring for infectious diseases

Protocols for wastewater sample processing with MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment

Wastewater and sewage surveillance is a useful tool for monitoring the regional spread of viral and bacterial pathogens. Surveillance at different levels of operation, such as a university dormitory or an entire city, has been shown to be a reliable way to detect SARS-CoV-2 before it becomes widespread in a population. As the world continues to be challenged by SARS-CoV-2, we have worked to develop protocols for manual or automated enrichment of SARS-CoV-2 viral RNA (both intact and all fragments >100 bp) from wastewater and sewage samples. The method we describe here can be used for downstream nucleic acid extraction after enrichment for viral/pathogen targets with Applied Biosystems™ MagMAX™ Wastewater kits and requires just 90 minutes of processing time. Using Applied Biosystems™ Dynabeads™ Wastewater Virus Enrichment beads for SARS-CoV-2 and other viral targets from wastewater samples followed by bead-based nucleic acid extraction is simple, fast, and reliable.

General guidelines and optimization

- This protocol has been optimized for the concentration of SARS-CoV-2 and other viral targets in wastewater samples since these samples are highly diluted and pre-enrichment is needed to improve the detection of pathogens.
- The procedure should be performed at room temperature unless stated otherwise.
- Proteinase K treatment is required to efficiently recover viral RNA from wastewater. However, bead beating and mechanical sample homogenization are not necessary for analysis of SARS-CoV-2 and other viral targets. Bead beating is only required to obtain a full microbiome profile with wastewater samples.



Manual protocol for wastewater



Manual processing of wastewater samples for isolation of viral and bacterial targets with pre-enrichment option

Note: The following protocol is for 5 mL wastewater samples, but it can be scaled up according to Table 1.

Pre-processing of wastewater samples

1. Take the required volume of wastewater to be processed in a 50 mL conical tube and vortex the tube on high speed for 30 sec. Note: For processing 5 mL, 10 mL should be spun down, and for processing 10 mL, 15 mL should be spun down to collect the clear supernatants, leaving 5 mL in the tube to avoid taking pelleted or floating material.
2. Spin down the conical tube at 10,000 x g for 10 min with deceleration speed set at 5 to collect the supernatant. Pipette very slowly to avoid disturbing the pellet to proceed with enrichment. **Note:** Take only the clear supernatant and try to avoid any waste material getting into the pipette by using barrier tips.
3. Pipette 50 µL resuspended Applied Biosystems™ Dynabeads™ Intact Virus Enrichment Reagent for 5 mL volume and 100 µL for 10 mL volume of pre-processed clean supernatant into the tube containing the supernatant.
4. Mix for 10 min using a nutating mixer or Invitrogen™ HulaMixer™ Sample Mixer to promote efficient viral particle capture.
Tip: While the samples are incubating with the beads, prepare the tubes or plates for viral nucleic acid extraction.
5. Apply the tube to an Invitrogen™ DynaMag™ magnet for 3–5 min, or until the beads separate, then remove and discard the supernatant. **Note:** The time required on the magnet depends on the sample and tube size.

Table 1. Volume requirements for virus enrichment.

Volume of wastewater	Volume of Dynabeads magnetic beads
5 mL	50 µL
10 mL	100 µL

6. Apply 500 µL of Applied Biosystems™ MagMAX™ Wastewater Lysis solution to the Dynabeads beads. Use the same lysis volume regardless of the wastewater volume.
7. Resuspend the Dynabeads beads by gently pipetting.
8. Apply the tube to the DynaMag magnet for 3–5 min, then harvest the supernatant and add it to a tube (or plate for automation) for downstream nucleic acid extraction. Discard the beads. **Note:** For larger wastewater samples, extra care must be taken to recover the beads from both sides of the tube. For example, with the Invitrogen™ DynaMag™-15 and DynaMag™-50 Magnets, the beads are collected on both sides of the tube. A quick spin can help facilitate collection of the beads at the bottom of the tube. The rest of the procedure follows the instructions of the MagMAX Wastewater kit.
9. Add 40 µL of proteinase K, 500 µL of binding buffer, and 20 µL of the magnetic beads as described in the user manual.
10. An elution volume of 50–100 µL can be used for wastewater volumes of 5–10 mL.

Automated protocol for wastewater processing and nucleic acid purification on a KingFisher instrument



Aliquot untreated wastewater samples into the KingFisher 24-well plate and add Dynabeads beads and buffers to one sample plate only and run the enrichment script; run the enrichment script with three 24 deep-well plates and a tip comb plate on the KingFisher instrument



Elute the virus-enriched material in MagMAX Wastewater Lysis Solution



Add the eluate, proteinase K, binding buffer, and MagMAX Wastewater beads to a fresh 96-well plate and perform nucleic acid extraction using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment

Automated processing of 5–10 mL wastewater samples for viral and bacterial targets with pre-enrichment option

This protocol is for the Thermo Scientific™ KingFisher™ Flex instrument, but the KingFisher™ Apex or KingFisher™ Duo Prime instrument may also be used. Contact us for more information.

Follow steps 1–9 for pre-processing of samples, given in the manual workflow on the previous page. Then follow the additional steps below.

1. Add 100 μ L of Applied Biosystems Dynabeads Wastewater Virus Enrichment beads to each sample in plate 1 only. **Note:** Samples in both sample plates are processed, and the concentrated viral RNA will be eluted into a single elution plate containing the lysis buffer.
2. Prepare a third 24 deep-well plate as the elution plate by adding 500 μ L of lysis solution to each of the wells.
3. Load the three plates (two sample plates and one elution plate) into a KingFisher Flex Purification System fitted with a 24 deep-well head (Cat. No. 5400640).
4. Download the program for the KingFisher system for automated processing of 24 samples (Dyna_Flex24_WastewaterEnrich_V2; Dyna_Apex24_WastewaterEnrich - for Apex; and Dyna_Duo24_WastewaterEnrich for Duo Prime) and load it using Thermo Scientific™ BindIt™ Software (Cat. No. 5189009). Custom scripts are available upon request.
5. Downstream nucleic acid extraction can then be performed using the Applied Biosystems™ MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit and 96 deep-well plates using the following scripts: MagMAX_Wastewater_Flex96_V2; MagMAX_Wastewater_Apex96; and MagMAX_Wastewater_Duo96. Follow steps 8 and 9 of the manual protocol on the previous page. An elution volume of 50–100 μ L can be used for 10 mL wastewater volumes.



Required materials

- For manual protocol: DynaMag magnet (find the most suitable magnet for your sample volumes at [thermofisher.com/magnets](https://www.thermofisher.com/magnets))
- For automated protocol: KingFisher Flex Purification System or other KingFisher instrument
- Sample mixer or roller to tilt and rotate tubes, such as the HulaMixer Sample Mixer

Buffers and solutions

- MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment

Required materials for the KingFisher instrument

- Thermo Scientific™ KingFisher™ 96 Deep-Well Head
- Thermo Scientific™ KingFisher™ 96 Deep-Well Plate, V-bottom, polypropylene (50–1,000 µL)
- Thermo Scientific™ KingFisher™ 96 Tip Comb for Deep-Well Magnets

View plate and magnetic head alternatives for 24 deep-well plates at [thermofisher.com/automation](https://www.thermofisher.com/automation)

Ordering information

Product	Cat. No.
MagMAX Wastewater Ultra Nucleic Acid Isolation Kit	A52606
MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment	A52610



Find out more at [thermofisher.com/magmaxwastewater](https://www.thermofisher.com/magmaxwastewater)