

[Isolate SARS-CoV-2 from wastewater using Dynabeads magnetic beads]

Protocols for wastewater sample processing with Dynabeads Intact Virus Enrichment (optimized for SARS-CoV-2)

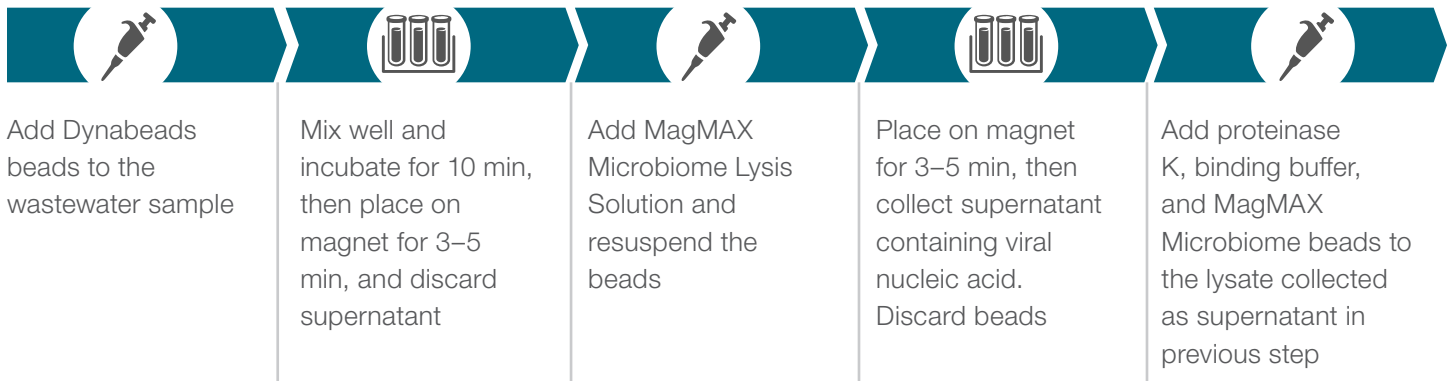
Wastewater and sewage surveillance is a useful tool for monitoring the regional spread of viral and bacterial pathogens. Surveillance at different levels of operation, such as a university dormitory or an entire city, has been shown to be a reliable way to detect SARS-CoV-2 before it becomes widespread 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 intact SARS-CoV-2 virus from wastewater and sewage samples. The method we describe here can be used for downstream nucleic acid extraction with Invitrogen™ MagMAX kits and requires just 1 hour of processing time. Using Dynabeads magnetic beads to enrich for SARS-CoV-2 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 in wastewater samples.
- Other samples: To enrich SARS-CoV-2 from cell culture medium or viral transport medium, follow the protocol described in the user guide for Invitrogen™ Dynabeads™ Intact Virus Enrichment. To enrich other viruses or process sample types other than those mentioned herein, further optimization may be required (e.g., bead quantity, incubation time).
- 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. Bead beating is only required to obtain a full microbiome profile with wastewater samples.



Manual protocol for wastewater



See full manual protocol

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

1. Add 5 mL of virus-containing wastewater to a 5 mL conical tube.
2. Pipette 50 μL of resuspended **Dynabeads Intact Virus Enrichment Beads** into the wastewater tube.
3. Mix for 10 min using a roller 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.
4. Apply the tube to the 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.
5. Apply 500 μL of Applied Biosystems™ MagMAX™ Microbiome Lysis Solution to the Dynabeads beads. Use the same lysis volume regardless of the wastewater volume.
6. Resuspend the Dynabeads beads by gently pipetting.
7. 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.

Table 1. Volume requirements for virus enrichment.

Volume of wastewater	Volume of Dynabeads magnetic beads
5 mL	50 μL
10 mL	100 μL
50 mL	750 μL

Note: For larger wastewater samples, extra care must be taken to recover the beads from both sides of the tube. For example, if an Invitrogen™ DynaMag™-15 or DynaMag™-50 Magnet is used, 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 Applied Biosystems™ MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit.

8. Add 40 μL of proteinase K, 500 μL of binding buffer, and 20 μL of the magnetic beads as described in the user manual.
9. An elution volume of 50 μL can be used for wastewater volumes of 5–50 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; 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 Microbiome Lysis Solution



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

See full protocol

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

1. From a total of 10 mL raw sewage or wastewater, add 5 mL each to 2 wells in KingFisher 24 deep-well plates (Cat. No. 95040470). No pre-filtering or centrifugation is necessary. Well A1 in plate 1 and well A1 in plate 2 should each contain 5 mL of the sample.
2. Add 100 µL of **Dynabeads Intact Virus Enrichment beads** to each sample in plate 1. **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.
3. Prepare a third 24 deep-well plate as the elution plate by adding 500 µL of MagMAX Microbiome Lysis Solution to each of the wells.
4. Load the three plates (two sample plates and one elution plate) into a Thermo Scientific™ KingFisher™ Flex Purification System fitted with a 24 deep-well head (Cat. No. 5400640).
5. Download the program for the KingFisher system for automated processing of 24 samples (**Dyna_Flex24_WastewaterEnrich**) and load it using Thermo Scientific™ BindIt™ Software (Cat. No. 5189009). Custom scripts are available upon request.
6. Downstream nucleic acid extraction can then be performed using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit and 96 deep-well plates. Follow steps 8 and 9 of the manual protocol on the previous page. An elution volume of 50 µL can be used for 5–50 mL wastewater volumes.



Required materials

- DynaMag Magnet (find the most suitable magnet for your sample volumes at [thermofisher.com/magnets](https://www.thermofisher.com/magnets))
- 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 Microbiome Ultra Nucleic Acid Isolation Kit (Cat. No. A42357 or A42358)
- MagMAX Microbiome Lysis Solution (Cat. No. A42361)
- Dynabeads Intact Virus Enrichment (optimized for SARS-CoV-2) (Cat. No. 10700D)

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)



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

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