



Isolating SARS-CoV-2 from wastewater

Protocols for wastewater sample processing with the MagMAX Wastewater Ultra Nucleic Acid Isolation Kits

Wastewater surveillance has played an important role in protecting public health since the 1940s, when it was used to detect the polio virus. And today, with the spread of SARS-CoV-2, wastewater surveillance is more important than ever.

Wastewater surveillance testing is superior to other tests such as saliva or swabs because SARS-CoV-2 is shed through feces into the wastewater collection system up to 2 weeks before any symptoms might appear.* This allows for additional testing in a defined area where viral load is seen to be increasing in wastewater, since this assay can be scaled easily to test at various population levels, from single school dormitories to entire cities.

Applied Biosystems™ MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kits offer a whole host of advantages over traditional wastewater surveillance methods (next page). And they're easy to use: you can isolate nucleic acids from concentrated wastewater in as little as 45 minutes. Our viral enrichment protocol using magnetic beads enables concentration and nucleic acid isolation in less than 2 hours.

For samples that require a concentration step, Applied Biosystems™ Dynabeads™ Wastewater Virus Enrichment beads are included in the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment. The kit also isolates nucleic acids from Gram-positive and -negative viral and bacterial targets other than SARS-CoV-2, including influenza A, norovirus, adenovirus, *E. coli*, *bacteroides*, and *bifidobacterium*.



* "Rapid, Large-Scale Wastewater Surveillance and Automated Reporting System Enable Early Detection of Nearly 85% of COVID-19 Cases on a University Campus," *mSystems*, <https://journals.asm.org/doi/10.1128/mSystems.00793-21>.

Challenges with traditional wastewater surveillance protocols

- Starting sample from wastewater is very dilute
- Only semi-established workflows exist at the moment, but there is still no complete workflow
- Inherent day-to-day variability results in highly heterogeneous samples
- Labs often receive a variety of sample sizes that require different processing approaches

Advantages of the MagMAX Wastewater kit

- **Optional virus enrichment workflow**—concentrate samples with Dynabeads Wastewater Virus Enrichment beads
- **Full workflow solution**—get tools for every step of the process, from sample collection to analysis
- **Manual and automated protocols available**—choose the right protocol for your samples and your lab
- **Established technology**—leverage established protocols from Thermo Scientific™ KingFisher™ sample purification systems for extracting RNA for SARS-CoV-2 testing

Enjoy workflow flexibility

Automated protocol for 10 mL wastewater samples with the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment:



Manual protocol for 10 mL wastewater samples processed using Dynabeads Wastewater Virus Enrichment beads:



Protocols included with the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit:

	Wastewater starting volume	Sample details	Kit reagents sufficient for	KingFisher scripts available	Protocol time	Purchase this kit	Cat. No.
1	10 mL	Concentration of 10 mL wastewater using Dynabeads beads followed by nucleic acid isolation	100 preps	Flex, Duo Prime, Apex instruments	90 minutes	MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment	A52610
2	200 µL	200 µL wastewater pre-concentrated using a preferred method, e.g., ultracentrifugation	100 preps	Flex, Duo Prime, Apex instruments	45 minutes	MagMAX Wastewater Ultra Nucleic Acid Isolation Kit	A52606
3	1 mL	<ul style="list-style-type: none"> 1 mL non-concentrated wastewater Wastewater pre-concentrated to 1 mL volume using a preferred method, e.g., precipitation 	20 preps	Flex, Duo Prime, Apex instruments	45 minutes		
				Flex, Duo Prime, Apex instruments	45 minutes		
4	50 mL or more	Concentration of 50 mL or more wastewater using filtration followed by nucleic acid isolation		Flex, Duo Prime, Apex instruments	90 minutes		
5	Sludge	200 mg sludge		Flex, Duo Prime, Apex instruments	45 minutes		

Sample data

Results from SARS-CoV-2 nucleic acid extraction using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment:

SARS-CoV-2 nucleic acid extraction from 10 mL wastewater samples after automated pre-processing with magnetic beads

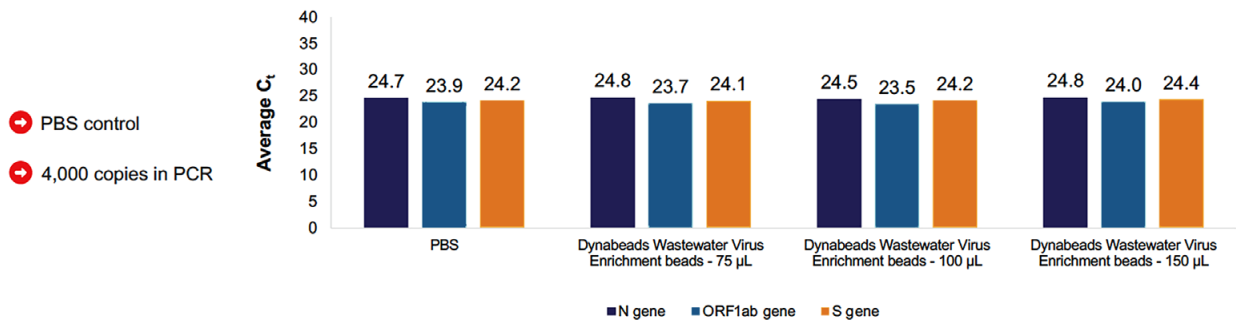


Figure 1. Efficient virus enrichment and isolation of SARS-CoV-2 RNA from 10 mL wastewater. Wastewater samples spiked with SARS-CoV-2 were processed with different volumes of Dynabeads Wastewater Virus Enrichment beads to bind the virus, followed by lysis and RNA isolation using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit and qRT-PCR analysis. C_t values of the PBS control matched those of the wastewater samples for the N, ORF1ab, and S genes of SARS-CoV-2, indicating efficient virus binding and isolation.

The MagMAX Wastewater Ultra Nucleic Acid Isolation Kit can also isolate viruses other than SARS-CoV-2:

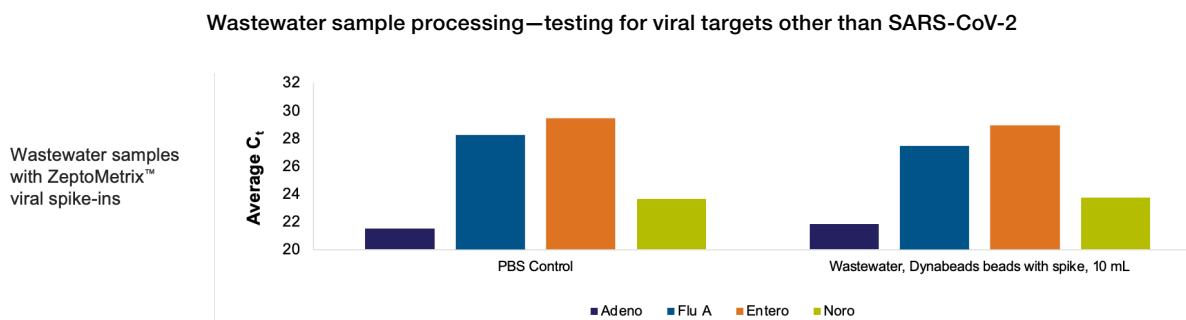


Figure 2. Successful isolation and detection of viral targets beyond SARS-CoV-2. Wastewater samples with ZeptoMetrix viral spike-ins were processed with Dynabeads Wastewater Virus Enrichment beads to bind the virus, followed by lysis and RNA isolation using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit and qRT-PCR analysis. C_t values of the PBS control matched those of the wastewater samples for the adenovirus, influenza A, enterovirus, and norovirus targets, indicating successful binding and isolation of viral targets beyond SARS-CoV-2.

Choose the following products for an end-to-end solution

- Invitrogen™ DynaMag™ magnet (find the most suitable magnet for your sample volumes at thermofisher.com/magnets)
- Thermo Scientific™ KingFisher™ Flex Purification System (Cat. No. 5400640 and 5400630)
- Invitrogen™ HulaMixer™ Sample Mixer (Cat. No. 15920D)
- Invitrogen™ Proteinase K Solution (Cat. No. 4333793)
- Hach™ AS950 Portable Compact Sampler sold through Fisher Scientific™ (Cat. No. 02-938-0013)

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

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