May 30, 2025

Manual Wastewater Concentration and Extraction V1.0

DOI
https://dx.doi.org/10.17504/protocols.io.n92ldndjxv5b/v1

Allison Steedman[1], Mark Zeller[1]

[1]Scripps Research

A Andersen Lab
Scripps Research

DOI: https://dx.doi.org/10.17504/protocols.io.n92ldndjxv5b/v1

Protocol Citation: Allison Steedman, Mark Zeller 2025. Manual Wastewater Concentration and Extraction V1.0. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldndjxv5b/v1

Protocol status: Working
We use this protocol and it's working

Created: May 30, 2025

Last Modified: May 30, 2025

Protocol Integer ID: 219235

Keywords: wastewater, extraction, ceres, concentration, magmax, rna, dna, manual, magnetic-bead based, dna from collected wastewater, collected wastewater, manual wastewater concentration, wastewater, throughput version of this protocol, rna, extraction, throughput version, protocol, dna

Funders Acknowledgements:
Bill & Melinda Gates Foundation
Grant ID: INV-057213

Abstract

This protocol is designed to manually extract RNA/DNA from collected wastewater.
For a high-throughput version of this protocol, please refer to this protocol: https://www.protocols.io/view/high-throughput-wastewater-sars-cov-2-detection-pi-81wgb74xyvpk/v1

Materials

Wastewater, Ceres Nanotrap Beads: SKU#44202-30, MagMax Lysis Buffer: A42361, MagMAX Viral/Pathogen Kit: A42352

Concentration

1 Invert wastewater sample to mix

2 Aliquot 10mL of wastewater into a 15mL conical tube

3 Add 150ul Ceres Nanotrap Microbiome A Particle beads into 10mLs of wastewater and invert to mix

4 Incubate at room temp for 15 minutes on slow shaker (or invert every 5 minutes)

5 Place 15mL conical on magnetic stand for 5 minutes or until beads are fully separated

6 Pipette off the supernatant without disturbing the bead pellet

7 Remove from magnet and add 500ul MagMax Lysis Buffer. Resuspend the pellet and transfer to a 1.5mL tube.

8 Incubate at room temp for 10 minutes on a slow shaker

9 Place 1.5mL tube on magnetic stand for 2 minutes or until beads are fully separated

10 Collect supernatant into new 1.5mL tube and discard bead pellet. Continue right to extraction.

Extraction

11 Prepare the following mixture in a 1.5mL conical tube:

	Reagent	Volume (ul)
	Binding Solution	500
	Beads	20
	Proteinase K	10
	Sample/Lysate	400

*Reagents (aside from sample) from MagMAX Viral/Pathogen Kit (A42352)

12 Vortex to mix

13 Incubate for 10 minutes

14 Place 1.5mL conical on magnetic rack for 2 minutes or until beads are fully separated

15 Discard supernatant

16 Take off magnetic rack and add 1000ul MagMax Wash Buffer and resuspend bead pellet

17 Place 1.5mL conical on magnetic rack for 2 minutes or until beads are fully separated

18 Discard supernatant

19 With the tube still on the magnet, add 1000 µl of 80% EtOH, incubate for 30 seconds, and remove the EtOH wash.

20 Repeat previous step for another 80% EtOH wash step

21 Leave the plate on the magnet to air dry for 3 min.

22 Remove the tube from the magnet. Add 50ul of MagMax Elution solution and pipette and vortex to resuspend

23 Incubate at room temperature for 10 minutes

24 Place the tube onto the magnet. Transfer the supernatant to the final tube. The sample is ready for downstream analysis or can be stored at -80° C

Protocol references

https://www.protocols.io/view/high-throughput-wastewater-sars-cov-2-detection-pi-81wgb74xyvpk/v1