



Oct 04, 2024

Polyethylene Glycol Precipitation for wastewater samples, with extraction using MagMAX Wastewater kit

DOI

<https://dx.doi.org/10.17504/protocols.io.j8nlk8pzdI5r/v1>

Shannon Fitz¹, Alex Shaw¹, michael Owusu², Dilip Abraham³

¹Imperial College London; ²Kwame Nkrumah University of Science and Technology; ³CMC Vellore

GEMS - Genomic Enviro...



Shannon Fitz

Imperial College London

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.j8nlk8pzdI5r/v1>

Protocol Citation: Shannon Fitz, Alex Shaw, michael Owusu, Dilip Abraham 2024. Polyethylene Glycol Precipitation for wastewater samples, with extraction using MagMAX Wastewater kit. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.j8nlk8pzdI5r/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: September 16, 2024

Last Modified: October 04, 2024

Protocol Integer ID: 107673

Keywords: polyethylene glycol precipitation, wastewater concentration, polyethylene glycol precipitation for wastewater sample, wastewater kit polyethylene glycol, polyethylene glycol precipitation, magmax wastewater ultra nucleic acid isolation kit, peg precipitation, wastewater sample, wastewater, nucleic acid extraction, extraction, precipitation, peg

Funders Acknowledgements:

The Gates Foundation

Grant ID: INV-049092 PA4273

Abstract

Polyethylene glycol (PEG) precipitation serves as a method of concentrating bacterial and viral pathogens contained in wastewater. PEG precipitation is used in this protocol prior to nucleic acid extraction with the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit.

Materials

⊗ NaCl (5M) Thermo Fisher Scientific Catalog #AM9760G

⊗ MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit Thermo Fisher Scientific Catalog #A52606

⊗ PEG-8000

Cost per sample: ~£5

Under £2 for PEG precipitation

Wastewater extraction kit costs roughly £3.13 per sample

Overhead costs:

Large centrifuge, serological pipet, horizontal shaker

Time required:

~2.5-3 hours (varies per number of samples)



Protocol materials

⊗ PEG-8000

⊗ NaCl (5M) **Thermo Fisher Scientific Catalog #AM9760G**

⊗ MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit **Thermo Fisher Scientific Catalog #A52606**

Polyethylene glycol precipitation, followed by extraction using MagMAX Wastewater kit

- 1 PEG precipitation for wastewater sample concentration
 - 1.1 Filter wastewater sample through a coffee filter to remove suspended solids. Transfer 40mL of filtered wastewater into a clean 50 mL tube.
 - 1.2 Add PEG8000 and NaCl. Desired final concentrations are 10% (w/v) and 0.4 M, respectively. Vortex for 10 minutes (or use horizontal shaker at high speed).

 PEG-8000

 NaCl (5M) Thermo Fisher Scientific Catalog #AM9760G
 - 1.3 Immediately centrifuge at 12,000g for 100 min at 4°C (no incubation step).
 - 1.4 Remove and discard supernatant using serological pipet. Approximately 5mL should remain in the tube.
 - 1.5 Centrifuge remaining mixture at 12,000g for 5 minutes at 4°C. Carefully remove any residual supernatant and discard.
 - 1.6 Resuspend the precipitate in 200 ul of lysis buffer from the extraction kit + 200 ul of nuclease-free water (or 5% PBS). Transfer into a clean 1.5 ml tube. Vortex at high speed for 10 seconds and centrifuge.
 - 1.7 Move into extraction using the wastewater ultra nucleic acid isolation kit.

Extraction following PEG precipitation using MagMAX Wastewater kit

- 2 Prepare Binding Bead mix:

Vortex the Binding Beads vigorously to ensure that the beads are fully resuspended.

Combine the following components for the required number of samples, plus 10% overage:



	A	B
	Component	Volume per sample
	Binding solution	500 ul
	Binding beads	20 ul
	Total	520 ul

Mix well by inversion, then store at room temperature.

2.1 Combine samples with Proteinase K and the Binding Bead Mix:

Add 40 μ L of Proteinase K to each sample.

Invert the tube of Binding Bead Mix several times to resuspend the beads, then add 520 μ L of Binding Bead Mix to each sample.

Note: Keep the Binding Bead Mix thoroughly mixed throughout the pipetting procedure. Pipet slowly to ensure the correct volume of Binding Bead Mix is added to each well. DO NOT reuse pipette tips to add the Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.

Close the samples firmly.

Shake them at 900 rpm for 5 minutes.

Place the samples on a magnetic stand for at least 5 minutes, or until all of the beads have collected.

2.2 Wash the beads:

With the samples on the magnetic stand, carefully open the tubes, then discard the supernatant.

IMPORTANT! Avoid disturbing the beads.

Remove the samples from the magnetic stand, then add 1 mL of Wash Buffer to each sample.

Close the samples, then shake at 800 rpm for 30 seconds.

Place the samples on the magnetic stand for 3 minutes, or until all of the beads have collected at the bottom of the tubes.

With the samples on the magnetic stand, carefully open the tubes, then discard the supernatant.

IMPORTANT! Avoid disturbing the beads.

Repeat wash steps using 1 mL of 80% ethanol.

2.3 Elute the nucleic acid:

Add 50–100 μ L of Elution Solution to each sample, then close the tubes.

Incubate at 75°C for 5 minutes.

Shake at 800 rpm for 5 minutes.

Place the samples on the magnetic stand for 3 minutes, or until all of the beads have collected at the bottom of the tubes.

With the samples on the magnetic stand, carefully open the tubes, then transfer the eluates to clean tubes.

IMPORTANT! Immediately close the tubes containing the eluate to prevent evaporation.

The isolated nucleic acid is ready for immediate use. Store the isolated nucleic acid at –20°C for up to 6 months or at –80°C for greater than 6 months.



Protocol references

Shotaro Torii, Wakana Oishi, Yifan Zhu, Ocean Thakali, Bikash Malla, Zaizhi Yu, Bo Zhao, Chisato Arakawa, Masaaki Kitajima, Akihiko Hata, Masaru Ihara, Shigeru Kyuwa, Daisuke Sano, Eiji Haramoto, Hiroyuki Katayama, Comparison of five polyethylene glycol precipitation procedures for the RT-qPCR based recovery of murine hepatitis virus, bacteriophage phi6, and pepper mild mottle virus as a surrogate for SARS-CoV-2 from wastewater, Science of The Total Environment, Volume 807, Part 2, 2022, 150722, ISSN 0048-9697, <https://doi.org/10.1016/j.scitotenv.2021.150722>. (<https://www.sciencedirect.com/science/article/pii/S0048969721058009>)

Thermo Fisher Scientific 2021, Sep 14-last update, MagMAXTM Wastewater Ultra Nucleic Acid Isolation Kit: Manual and automated high-throughput nucleic acid isolation from 200 µL pre-concentrated wastewater samples. Available: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0025693_MM_Wastewater_Ultra_NA_Isol_Kit_200uL_UG.pdf [2024, Sep 26].