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

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GEMS - Genomic Environ…

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We use this protocol and it's working

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Abstract

Polyethlyene 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 **Contributed by users**

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 Materials, Step 1.2

NaCl (5M) **Thermo Fisher Scientific Catalog #**AM9760G Materials, Step 1.2

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

Polyethlyene 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 **Contributed by users**

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 µL of Proteinase K to each sample.

Invert the tube of Binding Bead Mix several times to resuspend the beads, then add 520 µL 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 µL 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

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