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Wastewater Concentration Using the Ceres Nanotrap A Particles, Total Nucleic Acid Extraction, and PCR Inhibitor Removal for recovery of viral pathogens V.1

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Wastewater Surveillanc...

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We use this protocol and it's working

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Abstract

This standard operating procedure (SOP) sets out the workflow for processing wastewater samples for with the goal of recovering and detecting viral pathogens. The steps include virus concentration using the Ceres Nanoparticles A method and total nucleic acid extraction using MagMAX Wastewater Ultra Nucleic Acid Isolation Kit

The raw wastewater/sewage samples are concentrated using the Ceres Nanoparticles A method, adapted to 40 mL starting volume from the Ceres Nanotrap A 35 mL Manual Protocol with MagMAX Wastewater Ultra Nucleic Acid Isolation Kit. The samples are mixed with Nanotrap A particles and Enhancement Reagent 3, incubated on a shaker, and then pelleted on a magnetic rack. The supernatant is removed, and the beads are resuspended in Nanotrap Buffer 2, and pelleted once more. The pellet is then resuspended in lysis binding solution from the extraction kit, incubated at 56°C, and recaptured on the magnetic rack. The final lysate from the concentration step is then removed, leading into automated or manual extraction.

Safety information

Infectious agents – Wastewater contains pathogens, including viruses, bacteria and fungi. Appropriate safety precautions must be exercised when handling wastewater samples.

Guidelines

All procedures can be performed by suitably trained members of staff.

Materials

- Nanotrap® Enhancement Reagent 3 (ER3) **Ceres Nano** **Catalog #**10113-10
- Nanotrap Magnetic Virus Particles (10) **Ceres Nano** **Catalog #**44202
- Nanotrap® Buffer 2 **Ceres Nano** **Catalog #**10102-100
- MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit **Thermo Fisher Scientific** **Catalog #**A52606

Equipment

Cole-Parmer BH-250D-4 Dri-Block Digital Block Heater; Quadruple Insert; 100Deg C; 230 VAC

NAME

ScientificLabs

BRAND

BLO1042

SKU

https://www.scientificlabs.co.uk/product/block-heaters/BLO1042

LINK

Equipment

Fisherbrand™ Lab Thermometer

NAME

FisherScientific

BRAND

11597273

SKU

https://www.fishersci.co.uk/shop/products/liquid-filled-total-immersion-thermometer-1/11597273#?keyword=Thermometer

LINK

Equipment

Mini Centrifuge

NAME

FisherScientific

BRAND

16617645

SKU

https://www.fishersci.co.uk/shop/products/fisherbrand-standard-mini-centrifuge/16617645

LINK

Equipment

Thermo Scientific™ HulaMixer™ Sample Mixer

NAME

Thermo Scientific

BRAND

15920D

SKU

https://www.fishersci.co.uk/shop/products/hulamixer-sample-mixer/10548425#?keyword=15920D

LINK

Equipment

DynaMag™-2 Magnet

NAME

Invitrogen

BRAND

12321D

SKU

https://www.thermofisher.com/order/catalog/product/12321D?ef_id=Cj0KCQiAgvPKBhCxARIsAOlK_EoUALIdVrIpw8GmPVS0DIU17F62ar8NJ__tBEhstfUYr6L-k5v7jEkaAhJMEALw_wcB:G:s&s_kwcid=AL!3652!3!762546101515!!!g!!!16893189581!143415109412&cid=bid_sap_rst_r01_co_cp1362_pj

LINK

Equipment

50 ml Magnetic Separation Rack

NAME

New England Biolabs

BRAND

S1507S

SKU

https://www.neb.com/en-gb/products/s1507-50-ml-magnetic-separation-rack

LINK

Safety warnings

Experiments MUST be performed in the CL2 within the Class II BSC. Before starting the procedure, ensure that the working area (Class II BSC) and equipment e.g pipettors are thoroughly clean.

Separate dedicated Personal Protective Equipment (PPE) is to be worn in each working area.

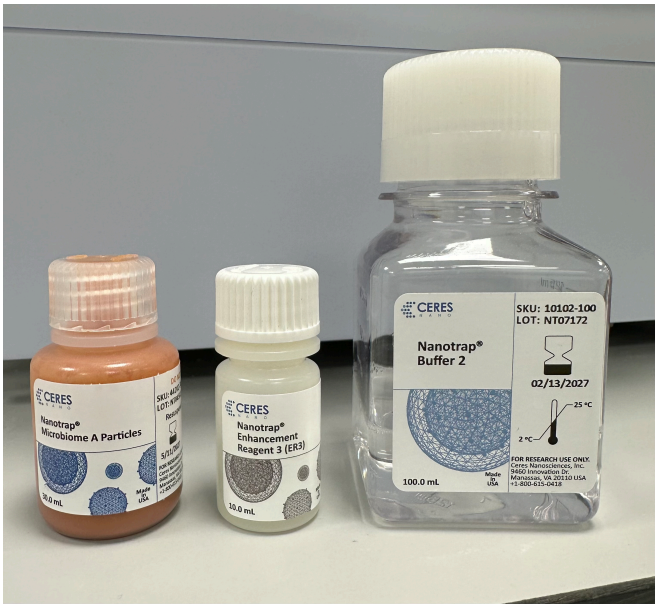
Procedure to concentrate viruses

1h 13m

1 Working inside the class II/A2 biological safety cabinet, mix the 40 mL wastewater sample thoroughly by inverting several times, then let settle (45 seconds at room temperature). 1m

2 Add 114.3 µL of Nanotrap Enhancement Reagent 3 (ER3) into the sample, close, and invert twice to mix. 1m

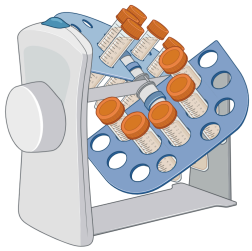
3 Add 600 µL of Nanotrap Microbiome A Particles into the sample, close, and invert twice to mix. 1m



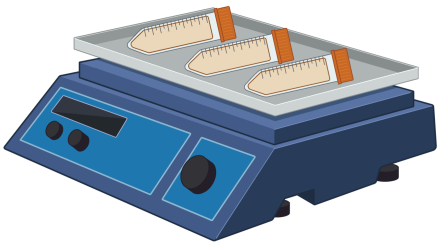
Depicted above: all reagents used in this protocol - Nanotrap A Particles (step 3), Enhancement reagent 3 (step 2), and Nanotrap Buffer 2 (step 7)

4 Incubate samples combined with Nanotrap A Particles and Enhancement Reagent 3 at room temperature for 30 minutes, inverting every few minutes (can also use a rotator or horizontal shaker). 30m

30 min



Option A: Rotator



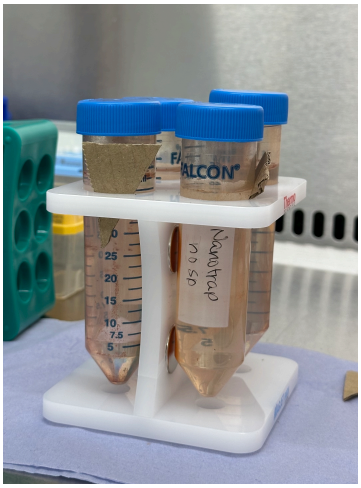
Option B: Horizontal shaker

Can use either rotator (option A) or horizontal shaker (option B). Secure samples in place with tape to avoid falling off if using horizontal shaker. For both rotator or horizontal shaker, secure lids tightly and seal tubes with parafilm prior to commencing 30 minute incubation.

Note

During this incubation, set the heating block to 56°C.

5 Place samples on the 50 mL magnetic rack for 10 minutes to pellet the Nanotrap particles. 10m



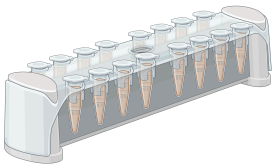
Beads pelleting against 50 mL magnetic rack

6 Once 10 minutes are up, remove and discard supernatant, taking care to avoid disturbing the pellet. If necessary, use a smaller pipette to remove residual supernatant from the sample. 3m

7 Resuspend the pelleted Nanotrap particles in 1 mL of Nanotrap Buffer 2, taking care to completely collect all particles. 5m

Transfer the suspension to a clean 2 mL microcentrifuge tube.

8 Place the sample tubes on a 2 mL magnetic rack to pellet the Nanotrap Particles for 2 minutes. 2m



9 Carefully remove and discard supernatant using a P-1000 pipette, taking care not to disturb the pellet. If necessary, use a smaller pipette to remove any residual supernatant. 3m

10 Add 500 µL of MagMAX Wastewater Lysis Solution to the pelleted Nanotrap particles, resuspending thoroughly with the pipette. 2m

11 Close tubes and incubate for 10 minutes on heating block set to 56°C. 10m

12 Replace the sample tubes on the 2 mL magnetic rack and allow samples to pellet for 2 minutes. 2m

Note

If necessary, centrifuge samples (2000g for 2-5 seconds) to remove drops from the lid prior to placing on magnet.

13 For manual extraction, transfer approximately 500 µL of lysate from each sample to a fresh 1.5-2 mL tube and discard pelleted beads. 3m

For automated extraction using the KingFisher Duo, transfer the lysate to the 96-deep-well plate (row A).

Sample is now ready for extraction following either manual or automated small volume extraction workflow.

Extraction using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit

1h 48m 50s

14 **Reagent preparation** 2m

Prepare 80% ethanol by diluting 100% (absolute) ethanol with nuclease-free water.
Prepare a minimum of 2 mL of 80% ethanol per sample.

15 **Bead Mix** 30s

> Note
>
> Prepare the bead mix on the day of use.

Vortex the Binding Beads vigorously to ensure complete resuspension.

15.1 Prepare the Binding Bead Mix by combining the required components below for the total number of samples plus an additional 10% excess. 3m

	Component	Volume per sample
	Binding Solution	500 µL
	Binding Beads	20 µL
	Total volume	520 µL

15.2 Mix the Binding Bead Mix well by inversion, then store at room temperature. 10s

16 **Automated extraction on KingFisher Duo**

Attached here is the KingFisher Duo Prime protocol for importing into your Instrument.

FILE MagMAX_Wastewater_DUO96.bdz 3.6KB

Processing plate layout

	Row ID	Plate row	Reagent	Volume per well
	Sample	A	Sample lysate + Proteinase K + Binding Bead Mix	960 µL

	Row ID	Plate row	Reagent	Volume per well
	Tip Comb	B	Place a 12 tip comb in the plate	
		C	Empty	
	Wash 1	D	Wash Buffer	1, 000 µL
	Wash 2	E	80 % Ethanol	1, 000 µL
	Elution	Separate elution strip	Elution Solution	100 µL

Note

To avoid evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp Clear Adhesive Film until they are loaded into the instrument

16.1 Transfer 500 µL of lysate to row A of a new 96-deep-well sample plate. 2m

16.2 Add 40 µL of Proteinase K to each sample well. 2m

16.3 Invert the Binding Bead Mix several times to resuspend the beads, then add 520 µL of Binding Bead Mix to each sample in row A. 3m

Note

Keep the Binding Bead Mix well mixed throughout pipetting. Pipette slowly to ensure accurate volumes, and use fresh pipette tips for each addition. Do not reuse tips due to the high viscosity of the mix.

16.4 Place a 12-tip comb into row B of the 96-deep-well plate. Leave row C empty. 10s

16.5 Add 1,000 µL of wash buffer to row D. 2m

16.6 Add 1,000 µL of 80% ethanol to row E. 2m

16.7 Add 100 µL of elution buffer into a separate elution strip tube 1m

16.8 Select the MagMAX_Wastewater_DUO96 program on the KingFisher DUO

16.9 Start the run, then load the prepared plate and elution strip tube into position when prompted by the instrument

16.10 At the end of the run (~28 minutes), immediately remove the plate from the instrument and transfer the eluate to a new tube or plate for downstream analysis or storage. 28m

> Note
>
> The isolated nucleic acid is ready for immediate use. For long-term storage, store extracted nucleic acid at −80 °C. Samples may be stored in 1.5 mL tubes.

17 **Manual Extraction**

17.1 Transfer the 500 µL lysate to labelled clean 1.5 mL tube 3m

17.2 Add 40 µL of Proteinase K to each sample tube 2m

17.3 Invert the tube of Binding Bead Mix several times to resuspend the beads, then add 520 µL of
Binding Bead Mix to each sample 3m

17.4 Transfer the sample tubes on a shaker and set to shake for 5 minutes at 900rpm. 5m

17.5 Briefly spin down and place the sample tubes on a magnetic stand for at least 5 minutes, or until all of the beads have collected. 5m

17.6 With the sample tube on the magnetic stand, carefully remove and discard the supernatant. 5m

Note

Important! Avoid disturbing the beads.

17.7 Remove the tube from the magnetic stand, then add 1 mL of Wash Buffer to each sample 2m

17.8 Close the tubes then shake at 800 rpm for 30 seconds. 30s

17.9 Place the sample tube on the magnetic stand for 3 minutes, or until all of the beads have collected on the side of the tube. 3m

17.10 With the sample tube on the magnetic stand, carefully remove and discard the supernatant. 3m

17.11 Repeat step 17.7 through step 17.10, using 1 mL of 80% ethanol. 8m 30s

17.12 Briefly centrifuge and remove any residual solution with a small volume fine-tipped pipette, without disturbing the pellet. 3m

17.13 Air-dry the beads by leaving the tube open for 2 minutes to allow residual ethanol to evaporate. 2m

Note

Avoid overdrying the beads to the point of the pellet cracking as this may lower the efficieincy of nucleic acid recovery.

17.14 Add 100 µL of Elution Solution to each sample and vortex vigorously to fully resuspend the pellet. 3m

17.15 Incubate at 75°C for 5 minutes. 5m

17.16 Shake at 800 rpm for 5 minutes. 5m

17.17 Briefly centrifuge and place the sample tube on the magnetic stand for 3 minutes, or until all of the beads have collected to the side of the tube. 3m

17.18 With the sample tube on the magnetic stand, carefully transfer the eluates to a new clean 1.5 mL tube. 2m

17.19 Keep the isolated nucleic acid on ice for immediate use or store at −80 °C until ready for further downstream analysis

Protocol references

Based on the protocol APP-082 from Ceres Nanosciences.