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Protocol for capture and concentration of viruses from wastewater samples (up to 50 mL) using Magnetic Nanotrap® particles.

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2019-nCoV Wastewater Epidemiology

Nanotrap Applications

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ABSTRACT

This protocol provides a method for Magnetic Nanotrap® particle-based capture and concentration of viruses from wastewater samples.

PROTOCOL CITATION

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KEYWORDS

Nanotrap, wastewater, surveillance, COVID, COVID-19, SARS-CoV-2, Pathogen surveillance, Easy workflow

LICENSE

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MATERIALS

NAME	CATALOG #	VENDOR
QIAamp® Viral RNA Mini	52906	Qiagen
Microcentrifuge tubes	C2170	Denville Scientific Inc.
PBS 1x without calcium & magnesium	Cat# 21-040-CVR	VWR International
MicroAmp®; Optical 96-Well Reaction Plate with Barcode & Optical Adhesive Films	4314320	Thermo Fisher
RT-PCR Grade Water	AM9935	Thermo Fisher
Mini Vortex Mixer	M10101001	
Nanotrap Magnetic Virus Particles (10)	44202	Ceres Nano
RT-PCR Kit		
DynaMag™-2 Magnet	12321D	
Sorvall™ Legend™ Micro 21R Microcentrifuge	75002447	Thermofisher

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

Follow required Biosafety level requirements.



Virus capture


35m


1 Place a 50 mL conical tube on a tube rack.


2 Add  **50 mL** of wastewater sample to the conical tube.


3 

Let the wastewater sample sit on the benchtop at  **Room temperature** for  **00:10:00** . This will allow large aggregates to settle at the bottom of the tube.

4 

Use a pipet to transfer  **40 mL** of the top clear supernatant, without disturbing the sediment pellet, into a new conical tube.

5 

Add  **600 µl** of Nanotrap particles to the sample, and invert 2-3 times to mix the particles.


6 Incubate samples with Nanotrap particles at  **Room temperature** for  **00:20:00** .

7 Use a magnetic rack to separate the Magnetic Nanotrap® particles from the sample.  **00:05:00**

8 Discard the supernatant carefully without disturbing the pellet. If required use a small pipet to remove all the supernatant from the tube.


Qiagen RNA extraction from the Magnetic Nanotrap® particles

30m

9 

Add  **140 µl** of 1X PBS to the particle pellet.

10 

Add  **560 µl** of Qiagen lysis buffer from the QIAamp Viral RNA kit to the sample and resuspend the mix by vortexing the tube briefly.

11 

Incubate the sample at  **Room temperature** for  **00:10:00** .

12 Use a magnetic rack to separate the Magnetic Nanotrap® particles from the sample.  **00:00:30**


13 

Collect the supernatant in a  **2 mL** microcentrifuge tube and discard the pellet.

14 

Add  **560 µl** of  **100 % volume** ethanol to samples, briefly vortex to ensure the sample is properly mixed.

15 

Add  **630 µl** of the sample mix onto QIAamp Mini column and close the cap.

16 

Centrifuge the sample for  **6000 x g, Room temperature , 00:01:00** .

17 Place the QIAamp Mini column into a clean 2 mL collection tube, and discard the tube containing the filtrate.

18 Add the remaining sample from step 14 onto the QIAamp Mini column and close the cap

19 

Centrifuge the sample for  **6000 x g, Room temperature , 00:01:00** .

20 Place the QIAamp Mini column into a clean 2 mL collection tube, and discard the tube containing the filtrate.

21 

Open the QIAamp Mini column and add  **500 µl** of Buffer AW 1.

22 

Centrifuge the sample for **6000 x g, Room temperature , 00:01:00** .

23 Place the QIAamp Mini column into a clean 2 mL collection tube and discard the tube containing the filtrate.

24 

Open the QIAamp Mini column and add **500 µl** of Buffer AW 2.

25 


Centrifuge the sample for **20000 x g, Room temperature , 00:03:00** .

26 Place the QIAamp Mini column into a clean 2 mL collection tube, and discard the tube containing the filtrate.

27  

Centrifuge the sample for **20000 x g, Room temperature , 00:01:00** to remove any Buffer AW2 carryover.

28 Place the QIAamp Mini column into a clean 2 mL collection tube, and discard the tube containing the filtrate.

29 

QIAamp Mini column and add **60 µl** of Buffer AVE and incubate at **Room temperature** for **00:03:00** .

30 

Centrifuge the sample for **6000 x g, Room temperature , 00:01:00** .

31 

The eluate collected in the collection tube is ready for analysis.

Detection

32  

Use any SARS-CoV-2 RT-PCR detection kit. Follow manufacturer instructions to set up the RT- PCR.



The sample elution can be used for wastewater surveillance using MINion or other sequencing platforms.

