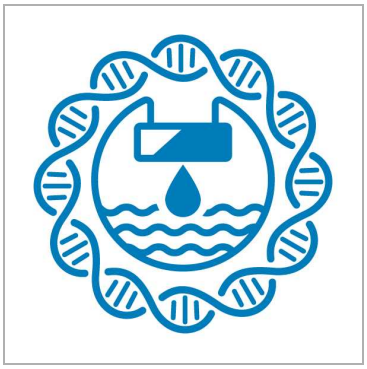

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We are still developing and optimizing this protocol

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SARS-CoV-2 RNA extraction with Ceres Nanotrap and Zymo Environ Water

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ABSTRACT

This protocol uses the Ceres Nanotrap® particle-based virus capture and concentration method for 10mL of wastewater followed by extraction with the Zymo Environ™ Water RNA extraction kit with a Zymo DNase step.

GUIDELINES

When developing this protocol, we extracted at least 2 replicates of each wastewater sample to ensure we had ample RNA for downstream processes

RNA extraction is performed at room temperature and centrifugation at 10,000-16,000 x g for 30s

Keywords: SARS-CoV-2, Ceres Nanotrap, Zymo Environ Water, RNA, wastewater

MATERIALS

1. Wastewater sample

Reagents and Kits

1. Ceres Nanosciences Nanotrap® Magnetic Viral Particles (Ceres Nanosciences: SKU 44202)
2. Ceres Nanosciences Nanotrap® Enhancement Reagent 2 (Ceres Nanosciences: SKU 10112)
3. Magnetic separator for 15 mL conical tubes, such as Invitrogen™ DynaMag™-2 Magnet (ThermoFisher Cat# 12-301-D)
4. Magnetic separator for 2mL micro centrifuge tubes, such as Invitrogen™ DynaMag™-2 Magnet (ThermoFisher Cat# 12-321-D)
5. Zymo Environ™ Water RNA Kit (Zymo Research: R2042)
6. Zymo DNA/RNA Shield™ (Zymo Research: R1100-50 or R1100-250)
7. Zymo DNase Set 1 (Zymo Research: E1010)

Equipment

1. Programable Heat Block
2. Mini vortex mixer
3. Mini Centrifuge (Max capable of 16,000 x g & fits 1.5/2mL tubes)
4. tube rotator (e.g. Fisherbrand Mini Tube Rotator Cat 88-861-05 or similar)

Consumables

1. 100% absolute ethanol
2. DNase/RNase Free Water
3. 15mL conical tubes
4. 1.5 or 2mL microcentrifuge tubes
5. 100-1000uL pipette
6. 20-200uL pipette
7. 100-1000uL filtered pipette tips
8. 20-200uL filtered pipette tips
9. serological pipetting aid
10. 10mL serological pipettes
11. 5mL serological pipettes
12. 1mL serological pipettes

SAFETY WARNINGS

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

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

BEFORE START INSTRUCTIONS

- Store the reagents separately from RNA/TNA (total nucleic acid) samples.
- Use a clean designated work area and separate pipettes for pre- and post-extraction steps to minimize the potential for cross-contamination
- Wear a lab coat and protective eyewear.
- Wear gloves and change them often.
- Prevent contamination by using aerosol-resistant pipette tips.

Before you start

1 Turn on heat block 95 °C

2 Ensure appropriate volume of DNase I is available (5µL per sample), or make new aliquots

2.1 Add 275 µL DNase/RNase-Free water to reconstitute lyophilized DNase I (1U/µL)

Note

Aliquot reconstituted DNase I in volumes appropriate for your lab's throughput (e.g., 15-20µL aliquots in 0.5mL microcentrifuge tubes) to avoid multiple freeze/thaw cycles

Viral Capture with Nanotrap® Particles

30m

3 Shake wastewater bottle to mix then let sit 00:00:45 45s

4 Using a 10mL serological pipette, carefully pipette 10 mL of wastewater into a 15mL conical tube

Note

Input volumes of 20, 30, and 40mL have also been tested. See note in step 7 for volume of nanotrap particles to add based on starting volume

5 Add 100 µL of Nanotrap® Enhancement Reagent 2 (ER2) and invert 15mL tube 2-3 times to mix

6 Re-suspend Nanotrap® particles by inverting the bottle 5 times

7 Add 150 µL Nanotrap® particles to the sample

Note

the volume of nanoparticles for different starting volumes of wastewater are as follows:

	Input Wastewater (mL)	Nanotrap Particles (µL)
	20	300
	30	450
	40	600

volume of nanoparticles for different starting volumes of wastewater

8 Incubate samples Room temperature 00:10:00 with constant rotation 10m

9 Place samples on magnetic rack to separate Magnetic Nanotrap® particles from the sample - at least 00:02:00 2m

10 After beads have settled, use a 5mL serological pipette to remove all of the supernatant without disturbing the pelleted beads

11 Add 1 mL of DNAse/RNAse Free water to the tube

11.1 Remove tube from magnet and re-suspend the pelleted beads using a 100-1000uL pipette

11.2 Transfer suspended beads to a 1.5mL microcentrifuge tube

12 Place microcentrifuge tube on the 2mL tube-compatible magnetic rack

12.1 Incubate until the beads have settled - at least 00:02:00 2m

13 Remove supernatant with a 100-1000uL pipette without disturbing the pellet. Remove any small amount of remaining supernatant with a smaller pipette tip (e.g. 2-20uL pipette)

14 Remove the tubes from the magnet and re-suspend the pellet with 375 µL Zymo DNA/RNA Shield and 125 µL Zymo DNase/RNase-Free water from the Zymo Environ Water RNA Kit

15 Incubate the samples at 95 °C for 00:05:00 5m

15.1 While samples are incubating, add 400 µL Zymo RNA Binding Buffer from the Zymo Environ Water RNA Kit to new 1.5mL tubes (one per sample)

16 Remove tubes from heat block and place on a magnetic rack and allow beads to settle until supernatant is clear - at least 00:02:00 2m

> Note
>
> Collect any liquid from caps by brief centrifugation prior to placing the tubes on the magnetic rack

16.1 Reset heat block temperature to 27 °C

17 Transfer 400 µL of supernatant to the corresponding tube prepared in step 15.1 and mix by gentle pipetting

> Note
>
> sample tubes volume = 800µL

Zymo Environ™ Water RNA Kit

5m

18 Transfer entire sample to a Zymo-Spin™ IIICG Column in a clean collection tube and centrifuge 30s
10000 x g, Room temperature, 00:00:30 and **keep the flow-through**

> Note
>
> Label both the spin column and collection tube

19 Add 800uL of ethanol (95-100%) to the flow-through in the collection tube from step 18 and mix well by gentle pipetting

> Note
>
> sample volume = 1600µL

20 Transfer 800 µL into a **new** Zymo-Spin™ IIICG Column in a clean collection tube and centrifuge 10000 x g, Room temperature, 00:00:30 and **discard the flow through** 30s

20.1 Repeat step 19 with the remaining 800 µL of sample using the same collection tube

21 Add 400 µL of RNA Prep Buffer to the column and centrifuge 30s
10000 x g, Room temperature, 00:00:30 and **discard the flow-through**

21.1 Transfer column to an RNase-Free 1.5mL microcentrifuge tube

22 Add 100 µL Zymo DNase/RNase-Free water directly to the column matrix and centrifuge 10000 x g, Room temperature, 00:00:30 and **keep the flow-through for step 24** 30s

23 Place a Zymo-Spin III-HRC Filter into a new collection tube and add 600 µL Prep Solution Centrifuge 8000 x g, Room temperature, 00:03:00 **discard the flow-through** 3m

23.1 transfer the column to an RNase-Free 1.5mL microcentrifuge tube

24 Transfer the eluted RNA from step 22 into the Zymo-Spin III-HRC filter prepared in step 23 and 16000 x g, Room temperature, 00:03:00 **keep the flow-through** 3m

25 Add 200 µL RNA Binding Buffer to the **filtrate** and mix well by gently pipetting up and down.

25.1 Add 300 µL of ethanol (95-100%) to the filtrate + RNA Binding Buffer and mix well by gently pipetting up and down.

26 Transfer the mixture into a Zymo-Spin IC column in a collection tube and 10000 x g, Room temperature, 00:00:30 **discard the flow-through** 30s

27 Add 400 µL RNA Wash Buffer to column and 10000 x g, Room temperature, 00:00:30 **Discard flow-through** 30s

28 Add 5 µL DNase I and 35 µL DNA Digestion Buffer to the column matrix

28.1 Incubate 27 °C 00:20:00 20m

29 Add 400 µL of RNA Prep Buffer to the column and 30s
10000 x g, Room temperature, 00:00:30 **discard the flow-through.**

30 Add 700 µL of RNA Wash Buffer to the column and 30s
10000 x g, Room temperature, 00:00:30 **discard the flow-through.**

31 Add 400 µL of RNA Wash Buffer to the column and 2m
10000 x g, Room temperature, 00:02:00 to ensure complete removal of the wash buffer.
Transfer the column carefully into an RNase-free tube.

32 Add 50 µL of DNase/RNase-Free Water directly to the column matrix 30s
and 10000 x g, Room temperature, 00:00:30 The eluted RNA can be used immediately or stored at -70 °C.