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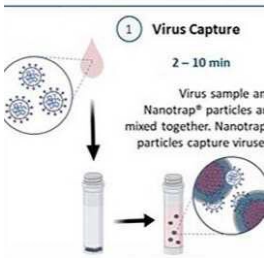
Wastewater grab sample processing with Nanotrap® Microbiome A particles (40 ml)



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Dilip Abraham[1], Venkata Raghava Mohan[2], Nirmal Kumar[1]

[1]Wellcome Trust Research Laboratory, Christian Medical College Vellore, India;
[2]Department of Community Health and Development, Christian Medical College Vellore, India

Dilip Abraham
CMC Vellore

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is used to carry out processing of wastewater using Nanotrap® Microbiome A Particles (Ceres Nanosciences). Nanotrap® particles are affinity-capture magnetic hydrogel particles that have been shown to capture and concentrate microorganisms (like SARS-CoV-2) from wastewater prior to RNA or TNA extraction. The sample is mixed with the particles, incubated, and separated with a magnet. The supernatant is removed, the pellet is spiked with extraction controls, and subjected to lysis followed by extraction. This protocol has been adapted from the online protocol available from Ceres Nanosciences.

Image Attribution

Barclay RA, Akhrymuk I, Patnaik A, et al. Hydrogel particles improve detection of SARS-CoV-2 RNA from multiple sample types. Sci Rep. 2020;10(1):22425. Published 2020 Dec 30. doi:10.1038/s41598-020-78771-8

Guidelines

Processed sample pellet needs to be extracted on the same day so as to avoid any nucleic acid degradation. The Nanotrap® A particles and the pellet obtained after sample concentration should not be frozen.

Materials

1. Nanotrap Magnetic Virus Particles (10) **Ceres Nano Catalog #**44202
2. Nanotrap Enhancer Reagent Er-2 **Ceres Nano Catalog #**SKU 10112
3. QIAamp® Fast DNA Stool Mini Kit **Qiagen Catalog #**51604 - Inhibitex buffer
4. qPCR DNA Extraction and Inhibition Control CY5-QXL670 **Eurogentec Catalog #**RT-SPCC-Q02
5. RNA MS2 from Bacteriophage MS2 **Roche Catalog #**10165948001
6. Nuclease-free water **Ambion Catalog #**AM9932
7. Finnpipette F1 100 to 1000 µL Thermo Fisher Catalog #4641100N
8. Finnpipette F1 20 to 200 µL Thermo Fisher Catalog #4641080N
9. Finnpipette F1 2 to 20 µL Thermo Fisher Catalog #4641060N
10. Finnpipette F1 0.2 to 2 µL Thermo Fisher Catalog #4641010N
11. ART Barrier Specialty Pipette tips 1000 µL Thermo Fisher Catalog #2279-05PK
12. ART Barrier Specialty Pipette tips 200 µL Thermo Fisher Catalog #2069-05PK
13. ART Barrier Specialty Pipette tips 20 µL Thermo Fisher Catalog #2149P-05PK
14. ART Barrier Specialty Pipette tips 10 µL Thermo Fisher Catalog #2139-05PK
15. 50 mL Centrifuge Magnetic Stand 6 tube PERMAGEN- SKU# MSR6X50
16. 1.5 mL Microcentrifuge Magnetic Stand 24 Tube PERMAGEN- SKU#MSR24
17. 50 ml Centrifuge Tube with flat Cap, Sterile Pfact Catalog #PFACPCT-50-B-S
18. 25 ml Serological Pipettes Nunc™ Catalog #170368N
19. 1.7 mL MaxyClear Snaplock Microcentrifuge Tube Axygen® Catalog #MCT-175-C
20. Glass beads, acid-washed 212-300 µM (50- 70 U.S. sieve) Sigma Catalog #G1277-500G
21. 2ml Screw Cap Micro Tubes Thermo Scientific™ Catalog #346911
22. 2 ml Snap Cap Low Retention Microcentrifuge Tubes Thermo Fisher Catalog #3453

Before start

Extraction control preparation

- **DNA control**

Before first use of the sample process control (SPC), prepare a 1/10 dilution of the control DNA in nuclease free water and store them at -70 °C as small aliquots to avoid freeze thaw. Add 1 µL of 1/10 diluted SPCC into particle pellet.

- **RNA control**

Prepare a 1/10 dilution of the MS2 extraction control in nuclease free water and store them at -70 °C as small aliquots to avoid freeze thaw. Add 1 µL of 1/10 diluted MS2 into particle pellet.

Wastewater grab sample processing with Nanotrap® Microbiome A particles

50m

1 Wastewater grab samples collected in 50 mL centrifuge tubes are transported to the laboratory in cold chain, maintaining a temperature of 4 °C .

2 Once received, place the sample tubes on a tube rack and leave at Room temperature for 10 minutes, allowing large aggregates to settle at the bottom of the tube. 10m

3 Using a serological pipette, carefully transfer 40 mL of clear supernatant into a new 50 ml centrifuge tube without disturbing the sediment.

4 Add 400 µL of ER2 Enhancer reagent to the supernatant, invert 2-3 times and mix throughly.

5 Add 600 µL of Nanotrap® A particles to the above and invert mix 2-3 times. Incubate at Room temperature for 00:20:00 . 20m

6 Place the tube on a 50 ml magnetic stand for 00:10:00 min and allow the Nanotrap® particles to settle. 10m

7 Carefully discard the supernatant without disturbing the pellet. If needed, use a pipette to remove all the supernatant from the tube.

8 Remove the tube containing pellet from the magnetic rack. The pellet is now ready for total nucleic acid extraction. The pellet should not be frozen, extraction is to be carried out on the same day.

9 Add 700 µL of the InhibitEX buffer along with 1 µL of 1/10 diluted SPC and 1 µL of 1/10 diluted RNA-MS2 control into each particle pellet and resuspend the pellet by vortexing the tube briefly.

10 Transfer the suspension into a 1.7 mL microcentrifuge tube. Incubate at Room temperature for 00:10:00 . 10m

11 Place the microcentrifuge tubes on a 1.5 ml magnetic separation rack until the Nanotrap® particles have settled, resulting in a clear solution.

12 Collect the supernatant into 2 mL microcentrifuge tube containing ~ 370 mg of acid-washed glass beads and discard the pellet.

Protocol references

1. https://www.ceresnano.com/_files/ugd/f7710c_c975f6fec8eb4183b3d8feb0a29041f8.pdf

2. Barclay RA, Akhrymuk I, Patnaik A, et al. Hydrogel particles improve detection of SARS-CoV-2 RNA from multiple sample types. *Sci Rep*. 2020;10(1):22425. Published 2020 Dec 30. doi:10.1038/s41598-020-78771-8