Manual Nanotrap® Wastewater Protocol using QIAGEN QIAamp® Viral RNA Mini Kit

Objective: This protocol uses Nanotrap® Magnetic Virus Particles and Nanotrap® Enhancement Reagent 2 to capture and concentrate viruses in wastewater samples. It is optimized for viral capture from 10 mL samples of wastewater and is compatible with QIAGEN QIAamp® Viral RNA Mini Kit. This method has been verified with SARS-CoV-2 viral samples.

Materials and equipment:

1. Wastewater sample
2. Nanotrap® Enhancement Reagent 2 (ER2) (Ceres Nanosciences SKU 10112)
    a. Using ER2 improves viral detection by 1-2 Ct values when used with Nanotrap® Magnetic Virus Particles, but it is ok to skip addition of ER2 in this protocol if it is not available.
3. Nanotrap® Magnetic Virus Particles (Ceres Nanosciences SKU 44202)
4. QIAGEN QIAamp® Viral RNA Mini Kit (QIAGEN Cat# 52906)
5. Mini Vortex Mixer
6. 15 mL polypropylene centrifuge tubes with caps
7. Microcentrifuge tubes (1.5 mL)
8. Magnetic Separator for 15 mL tube, such as Invitrogen™ DynaMag™-15 Magnet (ThermoFisher Cat# 12301D)
9. Magnetic Separator for 1.5 mL tube, such as Invitrogen™ DynaMag™-2 Magnet (ThermoFisher Cat# 12321D)
10. Molecular Grade Water / 1x PBS without Ca^{2+} or Mg^{2+} (PBS)

Procedure:

A. Capture:

1. Shake the wastewater bottle to mix. After shaking, let it sit for 45 seconds at room temperature.
2. Pipette 10 mL of wastewater sample into a 15 mL conical tube.
3. To each sample add 100 µL of Nanotrap® Enhancement Reagent 2 (ER2), then vortex for several seconds to mix thoroughly.
    a. Using ER2 improves viral detection by 1-2 Ct values when used with Nanotrap® Magnetic Virus Particles, but it is ok to skip addition of ER2 and proceed to the next step.
4. Add 150 µL of Nanotrap® Magnetic Virus Particles to the sample. Put the cap onto the tube and invert 2-3 times to mix the particles.
5. Incubate samples with Nanotrap® Magnetic Virus Particles at room temperature for 10 minutes. Invert 2-3 times to mix the particles at the 5-minute mark.
6. Use the magnetic rack that is compatible with the 15 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample.
7. Using a pipette, discard the supernatant carefully without disturbing the Nanotrap® Magnetic Virus Particles pellet.
8. Add 1 mL of molecular grade water to the tube and re-suspend the Nanotrap® Magnetic Virus Particles pellet using a pipette.
9. Transfer the Nanotrap® Magnetic Virus Particles and the molecular grade water to a clean 1.5 mL microcentrifuge tube for easier handling.
10. Use the magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample.

11. Using a pipette, discard the supernatant carefully without disturbing the pellet.
12. If any small amount of liquid is still present, use a smaller pipette to remove all the supernatant from the bottom of the tube.

B. Extraction:

13. Add 150 µL of 1x PBS and 560 µL of QIAGEN Virus Lysis Buffer (Buffer AVL) from Viral RNA Mini Kit to Nanotrap® Magnetic Virus Particles pellet.
14. Resuspend the Nanotrap® Magnetic Virus Particles pellet particle with a P1000 pipette, then vortex the tube for 30 seconds to homogenize the sample.
15. Incubate the Nanotrap® Magnetic Virus Particles sample at room temperature for 10 minutes.
16. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap® Magnetic Virus Particles from the resulting lysate solution.
17. Using a pipette, collect the supernatant in a new 1.5 mL microcentrifuge tube and discard the pellet. Be careful not to transfer particles into this new tube.

C. RNA Isolation/PCR Reaction Preparation:

18. Add 560 µL 100% ethanol to lysate samples, vortex to ensure the sample is properly mixed.
19. Follow QIAamp® Viral RNA Mini Kit instructions (QIAamp® Viral RNA Mini Handbook, Page 28, Step-6 -12) to process eluted samples through the column.
20. Follow preferred RT-PCR kit instructions to set up the reaction.
21. Add QIAamp® Viral RNA Mini Kit processed samples to RT-PCR reaction.

Attachments: 1. QIAamp Viral RNA Mini Handbook, July 2020.