Manual Nanotrap® Wastewater Protocol using QIAGEN AllPrep® PowerViral® DNA/RNA 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 AllPrep® PowerViral® DNA/RNA Kit. This method has been verified with SARS-CoV-2 viral samples.

Materials and equipment:

1. Wastewater sample
2. Nanotrap® Enhancement Reagent 1 (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 AllPrep® PowerViral® DNA/RNA Kit (QIAGEN Cat# 28000-50)
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
11. β-Mercaptoethanol (ThermoFisher Cat# 21985023)

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 200 µL of molecular grade water, 600 µL of QIAGEN PM-1 Buffer, and 6 µL of β-mercaptoethanol to Nanotrap® Magnetic Virus Particles pellet.
14. Resuspend the Nanotrap® Magnetic Virus Particles pellet by vortexing the tube for 30 seconds.
15. Incubate the Nanotrap® Magnetic Virus Particles sample at room temperature for 10 minutes.
1. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample.
16. Collect the supernatant in a 1.5 mL microcentrifuge tube and discard the pellet using a pipette.

C. RNA Isolation/PCR Reaction Preparation:

17. Add 150 µL of QIAGEN Solution IRS and vortex briefly to mix. Incubate at 4ºC for 5 minutes.
18. Centrifuge at 13,000 *g* for 1 minute. Transfer 700 µL of supernatant to the new 2 mL tube.
19. Add 600 µL of PM-3 to sample.
20. Add 600 µL of PM-4 to sample and vortex briefly.
21. Load 625 µL of sample onto MB Spin Column. Centrifuge at 13,000 *g* for 1 minute. Repeat 2x.
22. Mix PM-5 and then add 600 µL to the column. Centrifuge at 13,000 *g* for 1 minute. Discard flowthrough.
23. Add 600 µL of PM-4. Centrifuge at 13,000 *g* for 1 minute.
24. Discard flowthrough and centrifuge at 13,000 *g* for an additional 2 minutes. Discard flowthrough.
25. Place column in clean collection tube.
26. Add 50 µL of QIAGEN Water to column and incubate for 3 minutes at room temperature.
27. Centrifuge at 13,000 *g* for 1 minute. RNA is now ready for RT-PCR or can be stored at -80ºC.
28. Follow preferred RT-PCR kit instructions to set up the reaction.
29. Add AllPrep Power DNA/RNA Kit-processed samples to RT-PCR reaction.

Attachments: 1. AllPrep® PowerViral® DNA/RNA Kit Handbook, April 2018.