Manual Nanotrap® Wastewater Protocol using ZymoBIOMICS® MagBead 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 ZymoBIOMICS® MagBead DNA/RNA 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. ZymoBIOMICS® 96 MagBead DNA/RNA Kit (Zymo Cat# R2135)
    a. DNA/RNA Lysis Buffer
    b. MagBinding Beads
    c. Wash Buffer 1
    d. Wash Buffer 2
    e. DNAse/RNAse Free Water
5. Magnetic Separator for 15 mL tube, such as Invitrogen™ DynaMag™-15 Magnet (ThermoFisher Cat# 12301D)
6. Magnetic Separator for 1.5 mL tube, such as Invitrogen™ DynaMag™-2 Magnet (ThermoFisher Cat# 12321D)
7. 100% pure Ethanol
8. Micropipettes and tips
9. Microcentrifuge tubes (1.5 mL)
10. 15 mL polypropylene centrifuge tubes with caps
11. Mini Vortex Mixer
12. Mini Centrifuge (Max 6000 RPM that fits 1.5/2mL tube size)

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. Re-suspend the Nanotrap® Magnetic Virus Particles pellet in 400 µL of Zymo DNA/RNA Lysis Buffer and incubate particle samples at room temperature for 10 minutes.
14. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample.
15. Using a pipette, transfer supernatant to a fresh collection tube and discard the pellet.
16. Add 400 µL of Ethanol and 30 µL of Zymo Beads to the sample. Incubate at room temperature for 20 minutes.
17. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample and discard the supernatant using a pipette.
18. Using a pipette, wash the pellet with 500 µL of Zymo Wash Buffer 1.
19. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample and discard the supernatant using a pipette.
20. Using a pipette, wash the pellet with 500 µL of Zymo Wash Buffer 2.
21. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample and discard the supernatant using a pipette.
22. Using a pipette, wash the pellet with 500 µL 100% ethanol.
23. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample and discard the supernatant using a pipette.
24. Repeat Steps 23 and 24.
25. Spin samples in a mini centrifuge for 10 seconds.
26. Using a pipette, remove excess ethanol.
27. Allow samples to air dry at room temperature for 10 minutes.
28. Using a pipette, re-suspend the pellet in 50 µL of Zymo DNase/RNase Free Water and incubate at room temperature for 5 minutes with shaking.
29. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the magnetic particles from the sample and collect the supernatant in a fresh tube.
30. The sample is ready for RT-PCR.

Attachments:

None.