Manual Nanotrap® Wastewater Protocol using IDEXX Water DNA/RNA Magnetic Bead 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 IDEXX Water DNA/RNA Magnetic Bead Kit and RT-PCR 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. IDEXX Water DNA/RNA Magnetic Bead Kit (IDEXX Cat# 98-0014719-00)
    a. Binding Solution
    b. Beads
    c. Wash Buffer 1
    d. Wash Buffer 2
    e. Elution Buffer
5. Buffer AVL (Qiagen Cat# 19073)
6. Magnetic Separator for 15 mL tube, such as Invitrogen™ DynaMag™-15 Magnet (ThermoFisher Cat# 12301D)
7. Magnetic Separator for 2 mL tube, such as Invitrogen™ DynaMag™-2 Magnet (ThermoFisher Cat# 12321D)
8. Molecular Grade Water / 1x PBS without Ca^{2+} or Mg^{2+} (PBS)
9. Micropipettes and tips
10. 15 mL polypropylene centrifuge tubes with caps
11. Mini Vortex Mixer
12. Mini Centrifuge (Max 6,000 RPM that fits 1.5/2 mL 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 2 mL microcentrifuge tube for easier handling.
10. Use the magnetic rack that is compatible with the 2 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. Resuspend the Nanotrap® MagneticVirus Particles pellet particle with a P1000 pipette in 40 µL of PBS and 160 µL of Qiagen AVL Buffer
14. Incubate the Nanotrap® Magnetic Virus Particles sample at room temperature for 10 minutes.
15. Use a magnetic rack that is compatible with the 2 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample.
16. Using a pipette, collect the supernatant in a new 2 mL microcentrifuge tube and discard the pellet. Be careful not to transfer particles into this new tube.
17. Add 500 µL of IDEXX Binding Buffer and 20 µL of IDEXX Beads to sample. Incubate at 58ºC for 10 minutes.
18. Use a magnetic rack that is compatible with the 2 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample and discard the supernatant by using a pipette.
19. Using a pipette, wash the pellet with 500 µL of IDEXX Wash Buffer 1.
20. Use a magnetic rack that is compatible with the 2 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample and discard the supernatant by using a pipette
21. Using a pipette, wash the pellet with 500 µL of IDEXX Wash Buffer 2.
22. Use a magnetic rack that is compatible with the 2 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample and discard the supernatant by using a pipette
23. Repeat Steps 20 and 21.
24. Spin samples in a mini centrifuge for 10 seconds.
25. Using a pipette, remove excess IDEXX Wash Buffer 2.
26. Allow samples to air dry at room temperature for 10 minutes.
27. Using a pipette, re-suspend the pellet in 50 µL of IDEXX Elution Buffer and incubate at room temperature for 10 minutes. Vortex every 2 minutes to ensure particles don't fall out of solution.
28. Use a magnetic rack that is compatible with the 2 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample and collect the supernatant in a fresh tube.
29. Sample is ready for RT-PCR.

Attachments:

None.