Manual Nanotrap® Wastewater Protocol using NucleoMag Kit

Objective: This protocol uses Nanotrap® Magnetic Virus Particles and Nanotrap® Enhancement Reagent 1 to capture and concentrate viruses in wastewater samples. It is optimized for viral capture from 10 mL samples of wastewater and is compatible with the NucleoMag® DNA/RNA Water extraction kit. This method has been verified with SARS-CoV-2 viral samples.

Materials and equipment:

1. Wastewater sample
2. Nanotrap® Enhancement Reagent 1 (ER1) (Ceres Nanosciences SKU 10111)
    a. Using ER1 improves viral detection by 1-2 Ct values when used with Nanotrap® Magnetic Virus Particles, but it is ok to skip addition of ER1 in this protocol if it is not available.
3. Nanotrap® Magnetic Virus Particles (Ceres Nanosciences SKU 44202)
4. NucleoMag® DNA/RNA Water Kit (MACHEREY-NAGEL Cat# 744220.1)
    a. Buffer 1 (MWA1 lysis buffer)
    b. Buffer 2 (MWA2 binding buffer)
    c. Buffer 3 (MWA3 wash buffer)
    d. Buffer 4 (MWA4 wash buffer)
    e. RNase-free water
    f. B-Beads
5. 15 mL polypropylene centrifuge tubes with caps
6. Microcentrifuge tubes (1.5 mL)
7. Magnetic Separator for 15 mL tube, such as Invitrogen™ DynaMag™-15 Magnet (ThermoFisher Cat# 12301D)
8. Magnetic Separator for 1.5 mL tube, such as Invitrogen™ DynaMag™-2 Magnet (ThermoFisher Cat# 12321D)
9. Micropipettes and tips
10. Mini Vortex Mixer
11. 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 on a flat surface for 45 seconds at room temperature.
2. Pipette 10 mL of wastewater sample from the wastewater bottle into a clean 15 mL conical tube.
3. To each sample add 100 µL of Nanotrap® Enhancement Reagent 1 (ER1), then vortex for several seconds to mix thoroughly.
    a. Note: Using ER1 improves viral detection by 1-2 Ct values when used with Nanotrap® Magnetic Virus Particles, but it is ok to skip addition of ER1 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:

1. Using a pipette, re-suspend Nanotrap® Magnetic Virus Particles pellet in 500 µL of MACHEREY-NAGEL Buffer 1 (MWA1 Lysis Buffer).
2. Incubate Nanotrap® Magnetic Virus Particles in Lysis Buffer solution at room temperature for 10 minutes.
3. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap® Magnetic Virus Particles from the sample.
4. Transfer 450 µL of Lysis Buffer Solution supernatant to a fresh collection tube and discard the Nanotrap® Magnetic Virus Particles pellet.
5. Add 475 µL of MACHEREY-NAGEL Buffer 2 (MWA2 Binding Buffer) to the sample.
6. Add 25 µL of MACHERY-NAGEL magnetic B-beads to the sample. Vortex to mix. Incubate at room temperature for 10 minutes.
7. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the magnetic particles from the sample and discard the supernatant using a pipette.
8. Using a pipette, wash the pellet by re-suspending it with 850 µL of MACHEREY-NAGEL Buffer 3 (MWA3 Wash Buffer).
9. Use a magnetic rack to separate the magnetic particles from the sample and discard the supernatant by using a pipette.
10. Repeat the pellet wash with 850 µL of MACHEREY-NAGEL Buffer 3 (MWA3 Wash Buffer).
11. Use a magnetic rack to separate the magnetic particles from the sample and discard the supernatant by using a pipette.
12. Using a pipette, wash the pellet by re-suspending it with 850 µL of MACHEREY-NAGEL Buffer 4 (MWA4 Wash Buffer).
13. Use a magnetic rack to separate the magnetic particles from the sample and discard the supernatant by using a pipette.
14. Spin the samples in the mini centrifuge for 10 seconds.
15. Using a pipette, remove excess MACHEREY-NAGEL Buffer 4.
16. Allow samples to air dry at room temperature for 10 minutes.
17. Using a pipette, resuspend the pellet in 50 µL of MACHEREY-NAGEL Water (Elution Buffer) and incubate at 56ºC for 5 minutes.

18. Use a magnetic rack to separate the magnetic particles from the sample and collect the supernatant in a fresh tube.
19. The sample is ready for RT-PCR.

Attachments:

None.