Manual Nanotrap® Wastewater Protocol using Promega Maxwell® HT Environmental TNA 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 Promega Maxwell® HT Environmental TNA 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. Promega Maxwell® HT Environmental TNA Kit (Promega Cat# AX9190)
   a. Wash Buffer
   b. Resin
   c. Tris-HCl (pH 8.0)
5. 5.5M Guanidine Thiocyanate (Sigma Cat# G9277-100G)
6. Molecular Grade Water / 1x PBS without Ca²⁺ or Mg²⁺ (PBS)
7. Isopropanol
8. 50% Ethanol
9. Dilute Wash Solution (9 parts Wash Buffer/1 part 50% ethanol) (i.e. 1 mL = 900 µL Wash Buffer and 100 µL 50% ethanol)
10. Magnetic Separator for 15 mL tube, such as Invitrogen™ DynaMag™-15 Magnet (ThermoFisher Cat# 12301D)
11. Magnetic Separator for 2 mL tube, such as Invitrogen™ DynaMag™-2 Magnet (ThermoFisher Cat# 12321D)
12. Micropipettes and tips
13. 15 mL polypropylene centrifuge tubes with caps
14. Mini Vortex Mixer
15. Mini Centrifuge (Max 6000 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 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 300 µL 5.5 M Guanidine Thiocyanate solution 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 300 µL of supernatant to a fresh collection tube and discard the pellet.

16. Add 400 µL of isopropanol and 35 µL of resin to the sample. Incubate at room temperature for 20 minutes with constant rotation.

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 by re-suspending it with 1 mL of Dilute Wash Solution.

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. Repeat the pellet wash with 1 mL of Dilute Wash Solution.

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 by re-suspending it with 450 µL 50% 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 22 and 23.

25. Spin samples in the 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 Tris-HCl (pH 8.0) and incubate at room temperature for 7 minutes with agitation.

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.