Automated Nanotrap® Wastewater Protocol using MagMAX™ Kits

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 three nucleic acid extraction kits from Thermo Fisher. The automated script can process up to 24 samples at once and can be amended for the throughput in your lab. This method has been verified with SARS-CoV-2 viral samples.

Materials and equipment:

Sample	
Wastewater Sample	
Equipment	**Suggested Vendor**
3-24 well-KingFisher™ Deep Well Plates	Thermo Fisher Scientific™
1-24 well-KingFisher™ Deep Well Comb	Thermo Fisher Scientific™
3-96 well-KingFisher™ Deep Well Plates	Thermo Fisher Scientific™
1-96 well-KingFisher™ 200 µL Micro Well Plate	Thermo Fisher Scientific™
1-96 well-KingFisher™ Deep Well Comb	Thermo Fisher Scientific™
Thermo Fisher™ KingFisher™ Apex System or Flex System	Thermo Fisher Scientific™
Reagents	**Suggested Vendor**
80% Ethanol	(Decon™ Laboratories Decon Labs # 3916EA)
Nanotrap® Enhancement Reagent 1 (ER1)[1]	Ceres Nanosciences SKU# 10111
Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	**Inclusion in Kit**
Binding Buffer	Yes (Thermo Fisher™ Cat. No. A42352)
Wash Buffer	Yes (Thermo Fisher™ Cat. No. A42352)
Elution Solution	Yes (Thermo Fisher™ Cat. No. A42352)
Proteinase K	Yes (Thermo Fisher™ Cat. No. A42352)
MagMAX Beads	Yes (Thermo Fisher™ Cat. No. A42352)
Further Materials	**Suggested Vendor**
Nanotrap® Magnetic Virus Particles	Ceres Nanosciences SKU# 44202
Applied Biosystems™ MagMAX™ Microbiome Lysis Solution	Thermo Fisher™ Cat. No. A42361

[1]Using ER1 improves viral detection by 1-2 Ct values when used with Nanotrap® Magnetic Virus Particles, however, it is not a required reagent for the workflow

Precipitate can form in ER1 if stored below room temperature. Allow ER1 to return to room temperature to dissolve the precipitate.

Alternate Kits:

- Applied Biosystems™ MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit (Thermo Fisher™ Cat. No. A42357)*
- Applied Biosystems™ MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Thermo Fisher™ Cat. No. A48383)

*Applied Biosystems™ MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit includes the lysis solution in the kit

Procedure:

Capture and Concentrate Virus using Nanotrap® Particles

1. **Ceres Nanotrap® KingFisher™ Procedure - Part 1**
    a. *Prepare Sample Plates*
        i. Invert the wastewater bottle 5 times to mix. After inverting, place on a flat surface for 45 seconds at room temperature and pipette 4,875 µL of wastewater sample from wastewater bottle to one well (one well per sample) of a new KingFisher™ 24 Well Deep Well Plate.
        ii. Add another 4,875 µL of each sample to the same well on a second KingFisher™ 24 Well Deep Well Plate.
            1. For example, if you loaded a sample into well A1 of the first plate, load the second volume of that sample into well A1 of the second plate.
        iii. Add 50 µL of Nanotrap® Enhancement Reagent 1 (ER1) to each wastewater sample on the two KingFisher™ 24 Well Deep Well sample plates.
            1. Note: This step is optional.
        iv. Add 75 µL of Nanotrap® Magnetic Virus Particles to each wastewater sample on the two KingFisher™ 24 Well Deep Well sample plates.
        v. Insert the comb into one of the sample plates. This will be "Sample Plate 1" while the other plate will be "Sample Plate 2".

    b. *Prepare Lysis Plate*
        i. Add 500 µL of Applied Biosystems™ MagMAX™ Microbiome Lysis Solution to the third KingFisher™ 24 Well Deep Well Plate matching the number and location of the "Sample Plate" wells.

    c. *Run Nanotrap® KingFisher™ Script (Request file at sales@ceresnano.com)*
        i. If using a KingFisher™ Flex System, run *KF-008-WW-Nanotrap-24.bdz.* If using a KingFisher™ Apex System, run *KF-003-WW-Nanotrap-24.kfx.*
        ii. Follow the on-screen instructions loading the previously prepared plates at the appropriate time.

    d. Once the protocol is completed, the KingFisher™ plate to which lysis solution was added will contain lysate that is ready to be run on the MagMAX™ KingFisher™ extraction procedure.

2. MagMAX™ KingFisher™ Extraction Procedure -Part 2

a. *Prepare Wash Plate 1*
   i. Add 1 mL of MagMAX™ Wash Buffer to a new KingFisher™ 96 Deep Well Plate matching the number and location of the KingFisher™ 96 Deep Well Plate-Sample Plate wells.

b. *Prepare Wash Plate 2*
   i. Add 1 mL of 80% EtOH to a new KingFisher™ 96 Deep Well Plate matching the number and location of the KingFisher™ 96 Deep Well Plate-Sample Plate wells.

c. *Prepare Elution Plate*
   i. Add 50 µL of MagMAX™ Elution buffer to a new KingFisher™ 96- 200 µL plate matching the number and location of the "Sample Plate" wells.

d. *Prepare MagMAX™ Bead Binding Plate*
   i. To a new KingFisher™ 96 Deep Well Plate, add 400 µL of the lysate from each well of the lysis plate used in Part 1 of the protocol. Keep track of which well contains which sample in this new bead binding plate. There should be about 100 µL of lysate remaining in each well of the lysis plate which can be discarded.
      1. Note: Waste should be discarded in accordance with your lab's policy.
   ii. Add 530 µL of MagMAX™ Binding Solution to each well in which lysate was added.
   iii. Add 10 µL of MagMAX™ Thermo Scientific Proteinase K to each well in which lysate was added.
   iv. Add 20 µL of MagMAX™ DNA/RNA Binding Beads to each well in which lysate was added. The total final volume should be 960 µL in each sample-containing well of this plate.
   v. Insert the KingFisher™ 96 Deep Well Comb into the bead binding plate.

e. *Run MagMAX™ KingFisher™ Script (See attached file)*
   i. If using a KingFisher™ Flex System, run *KF-008-WW-MagMAX-96.bdz.* If using a KingFisher™ Apex System, run *KF-003-WW-MagMAX-96.kfx.*
   ii. Follow the on-screen instructions loading the previously prepared plates at the appropriate time.

f. Once the protocol is completed, the KingFisher™ 96 -Elution Plate will contain purified viral RNA that is ready to be loaded onto a PCR plate.

Attachments: 4

KingFisher™ Flex
1. *KF-008-WW-Nanotrap-24.bdz*
2. *KF-008-WW-MagMAX-96.bdz*

KingFisher™ Apex
1. *KF-003-WW-Nanotrap-24.kfx*
2. *KF-003-WW-MagMAX-96.kfx*