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# Nanotrap® KingFisher™ Concentration/Extraction & MagMAX KingFisher™ Extraction

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These protocols have been adapted from Ceres "Nanotrap® Wastewater Protocol using MagMAX Kits" (APP-030, Revision 0, Nov 2021).

This protocol uses Nanotrap® Magnetic Virus Particles and Nanotrap® Enhancement Reagent 1 (ER1) 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 ThermoFisher. This protocol has been used and adapted for SARS-CoV-2 viral capture at Emory University for wastewater surveillance of COVID-19.

DOI

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Nanotrap, KingFisher, Ceres, nano, magnetic virus particles, magnetic, automated, wastewater, SARS-CoV-2, COVID-19

protocol ,

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55724

Autoclave - Amsco Lab 240 Steam Sterilizer
Bovine Respiratory Syncytial Virus (BRSV, INFORCE 3, Zoetis, Parsippany, NJ)(Control)
Corning Molecular Biology Grade Water (Reference no. 46-000-CM)
Wastewater sample
Pipettes
Eppendorf Research Plus Single Channel Pipette
Eppendorf Repeater Pipette
Ceres Nanosciences Nanotrap® Enhancement Reagent 1 (SKU 10111-10)
Ceres Nanosciences Nanotrap® Magnetic Virus Particles (In Solution) (SKU #44202)
Thermo Scientific™ KingFisher Apex
Thermo Scientific™ MagMAX™ Microbiome Lysis Solution (Catalog no. A42361)
Thermo Scientific™ MagMAX Viral/Pathogen Nucleic Acid Extraction Kit (ThermoFisher Cat# A42352):

- Binding Buffer (Catalog no. A42359)
- Proteinase K (Catalog no. A42363)
- MagMAX Binding Beads (Catalog no. A42362)
- Wash Buffer (Catalog no. A42360)
- Elution Buffer (Catalog no. A42364)

80% Ethanol
Thermo Scientific™ KingFisher™ Plastics for 24 deep-well format (Catalog no. 95040470)
Thermo Scientific™ KingFisher™ Plastics for 24 deep-well tip comb and plate format (Catalog no. 97002610)
Thermo Scientific™ KingFisher™ Plastics for 96 deep-well format (Catalog no. 95040450)
Thermo Scientific™ KingFisher™ Plastics for 96 standard and PCR formats (Catalog no. 97002540)
Thermo Scientific™ KingFisher™ Plastics for 96 deep-well and tip comb format (Catalog no. 97002534)

## Ceres Nanosciences Nanotrap® KingFisher™ Procedure 10m

1 Label two KingFisher™ 24 Well Deep Well sample plates “Sample Plate 1” and “Sample Plate 2”. You will split the total sample volume to process across the two plates for each sample.

Plastics for 24 deep-well format

**KingFisher** **95040470**



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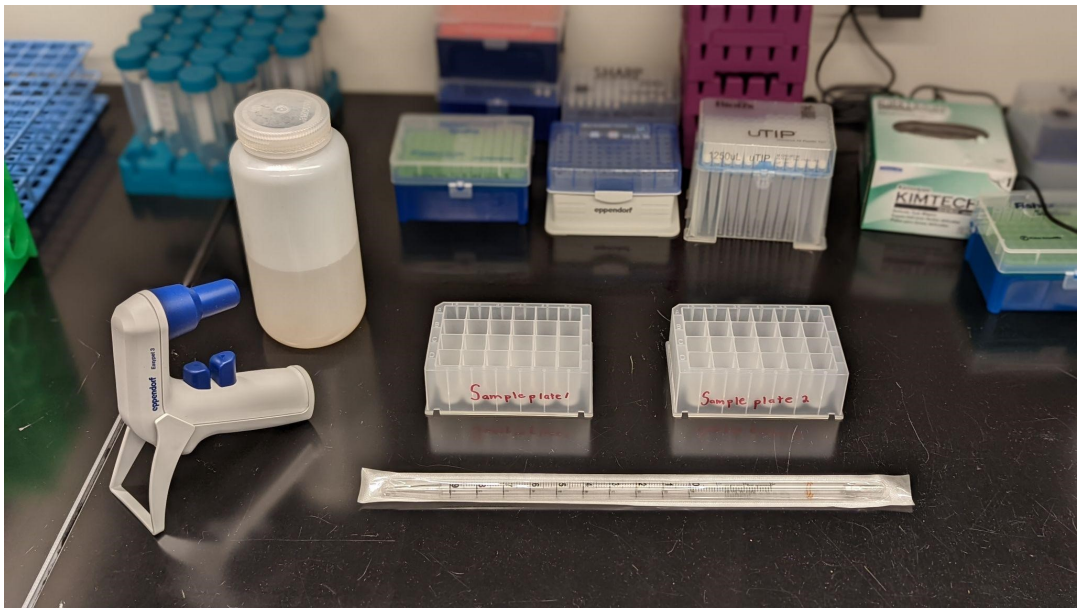


Two Labeled KingFisher™ 24 Well Deep Well sample plates

2 Aliquot **10 µL** of Bovine Respiratory Syncytial Virus (**BRSV**) INFORCE 3 Intranasal Bovine Vaccine **Zoetis Catalog #INF-00089** to every well in Sample Plate 1 that will receive a wastewater sample.

BRSV acts as a positive control and is **only** added to Sample Plate 1. Do NOT add BRSV to Sample Plate 2.

3 Aliquot **4865 µL** of wastewater sample to one well (one well per sample) of Sample Plate 1 (the new KingFisher™ 24 Well Deep Well sample plate).



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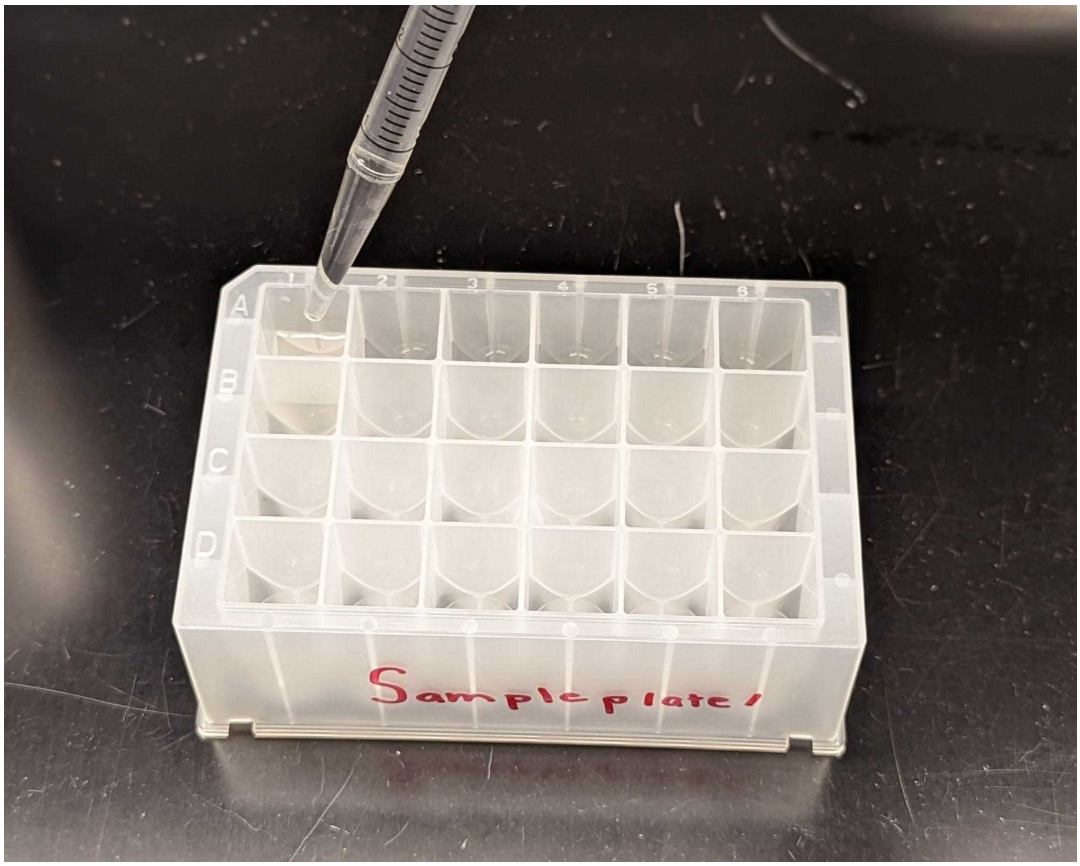


Wastewater sample is aliquoted into well A1 of Sample Plate 1.

4 Aliquot another **4865 µL** of wastewater sample to the same well on Sample Plate 2 (a second KingFisher™ 24 Well Deep Well sample plate).

4.1 For example, if you loaded a sample into well A1 of Sample Plate 1, load the second volume of that sample into well A1 of Sample Plate 2.



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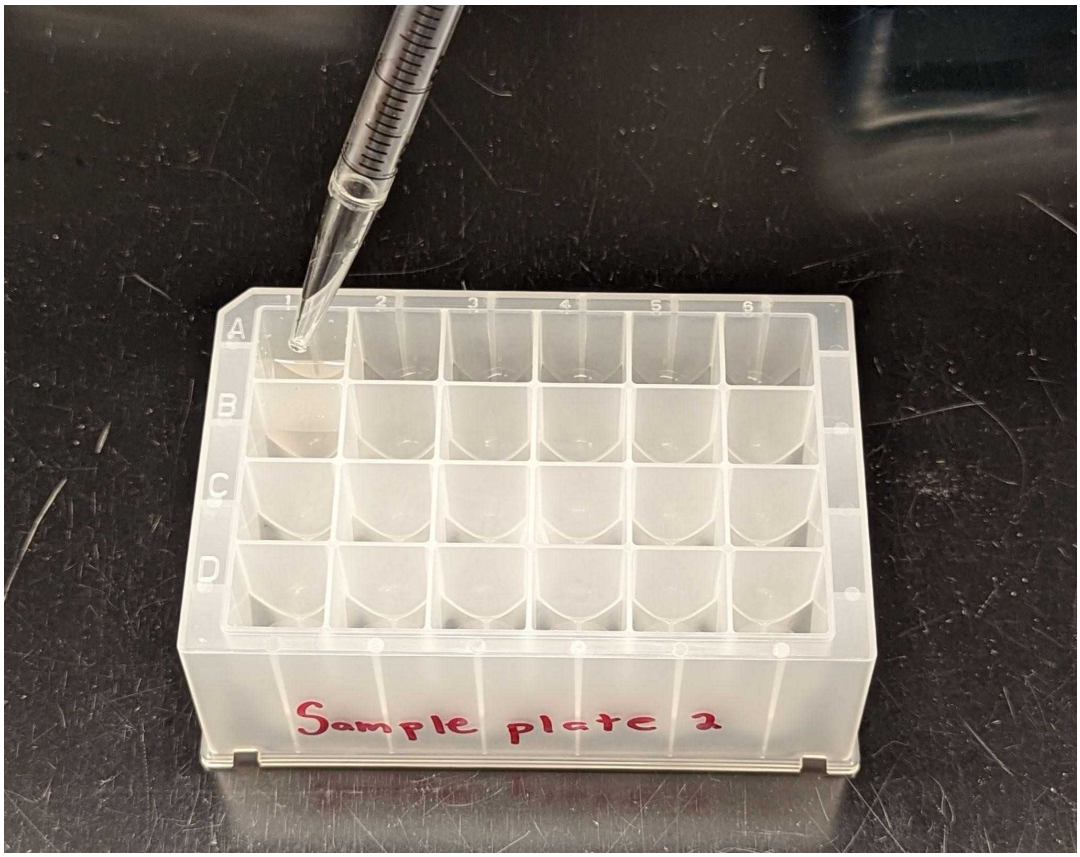


Wastewater sample is aliquoted into well A1 of Sample Plate 2.

5 Designate the same well on Sample Plate 1 and Sample Plate 2 as a negative control. Add Molecular Biology Grade **4865 µL** of Water **Corning Catalog #46-000-CM** into the designated well on both sample plates.

Do NOT introduce BRSV or wastewater into these two designated wells.

6 Enhancement Reagent 1 **Ceres**
Aliquot **50 µL** of **Nano Catalog #10111-10** to each wastewater sample well, including the negative control well, in both sample plates.

7 Incubate the sample plates for **00:10:00** at **20 °C Room temperature** 10m



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After adding Ceres Nanosciences Nanotrap® Enhancement Reagent 1, incubate both sample plates at room temperature for 10 minutes.

8 ☒Nanotrap Magnetic Virus Particles (10) **Ceres**

Aliquot **75 µL** of **Nano Catalog #44202**

to each wastewater sample well, including the negative control well, in both sample plates.

Prior to use, the product Nanotrap particles should be vortexed and thoroughly mixed.

9 Insert the KingFisher™ 24 well-KF Deep Well Comb into Sample Plate 1.

Plastics for 24 deep-well tip comb and plate format

**KingFisher** **97002610**

10 Prepare the Lysis Plate by aliquoting **500 µL** of ☒MagMAX™ Microbiome Lysis Solution **Thermo Fisher Catalog #A42361** to a third Kingfisher™ 24 Deep-Well Plate matching the number and location of the “Sample Plate” wells.



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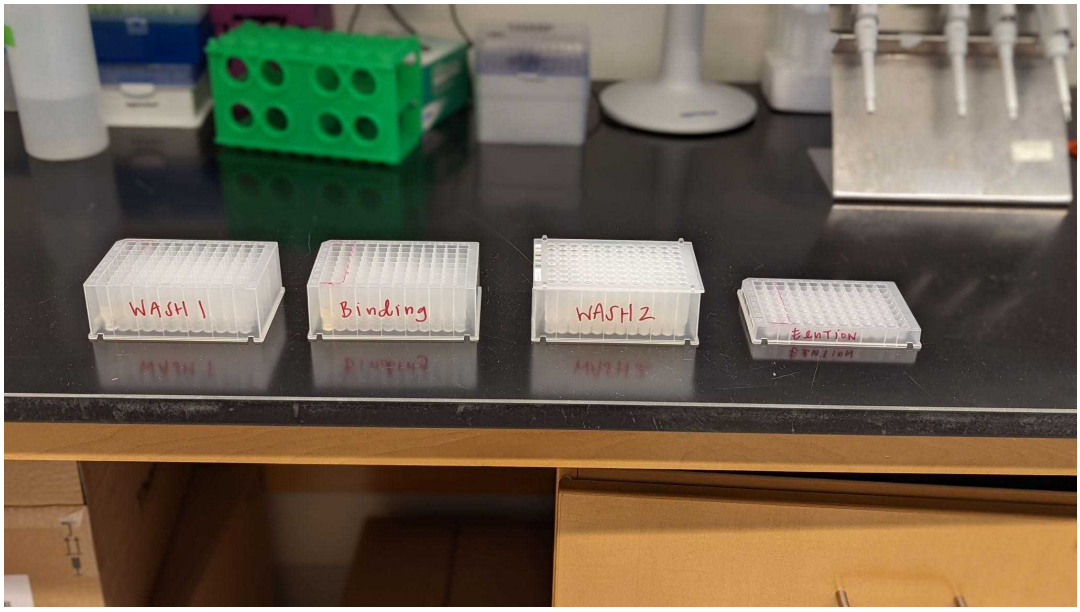
11 Run NT KingFisher™ Script

- 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*.
- Follow the on-screen instructions loading the previously prepared plates at the appropriate time.

12 Once the protocol is completed, the KingFisher™ plate to which lysis solution was added will contain **500 µL** of lysate that is ready to be run on the MagMAX KF extraction procedure.

## MagMAX KingFisher™ Extraction Procedure

13 Label four KingFisher™ 96 Deep Well Plate-Sample Plate wells "Wash 1", "Binding", "Wash 2", and "Elution".




During the MagMAX KingFisher™ Extraction Procedure, the following plates will be prepared: Wash 1, Binding, Wash 2, and Elution.

14 Prepare Wash 1 Plate by aliquoting **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.

Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit contains:

- Wash Buffer
- Binding Buffer
- Elution Buffer



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- Proteinase K
- MagMAX Beads

MagMAX™
Viral/Pathogen Nucleic Acid Isolation Kit
**Applied Biosystems™ A42352**

15 Prepare the Elution Plate by aliquoting **60 µL** of MagMAX Elution buffer to a new KingFisher™ 96 - 200 µL plate matching the number and location of the “Sample Plate” wells.

16 To prepare the Binding Plate, obtain a new KingFisher™ 96 Deep Well Plate.

17 Aliquot **10 µL** of MagMAX Proteinase K to each well in the Binding Plate in which lysate will be added.

18 Aliquot **400 µL** of the lysate from each well of the lysis plate used in Part 1 of the protocol into the Binding Plate.

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.

19 Aliquot **530 µL** of MagMAX Binding Solution into each deep well which lysate was added in the Binding Plate.

20 Vortex and thoroughly mix the MagMAX DNA/RNA Binding Beads.



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21 Aliquot **20 µL** of vortexed binding beads to each well in which lysate was added in the Binding Plate.

The total final volume should be 960 µL in each sample-containing well of this plate. If volume exceeds 1000 µL, the possibility of contamination via spillage into adjacent wells can occur.

22 Insert the KingFisher™ 96 Deep Well Comb into the Binding Plate.

23 Prepare Wash Plate 2 by aliquoting **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.

24 Run MagMAX KingFisher™ Protocol

- 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*.
- Follow the on-screen instructions loading the previously prepared plates at the appropriate time.

25 Once the protocol is completed, the KingFisher™ 96-Elution Plate will contain ~60 µL purified viral RNA that is ready to be loaded onto a PCR plate or 1.7 mL RNA tube.



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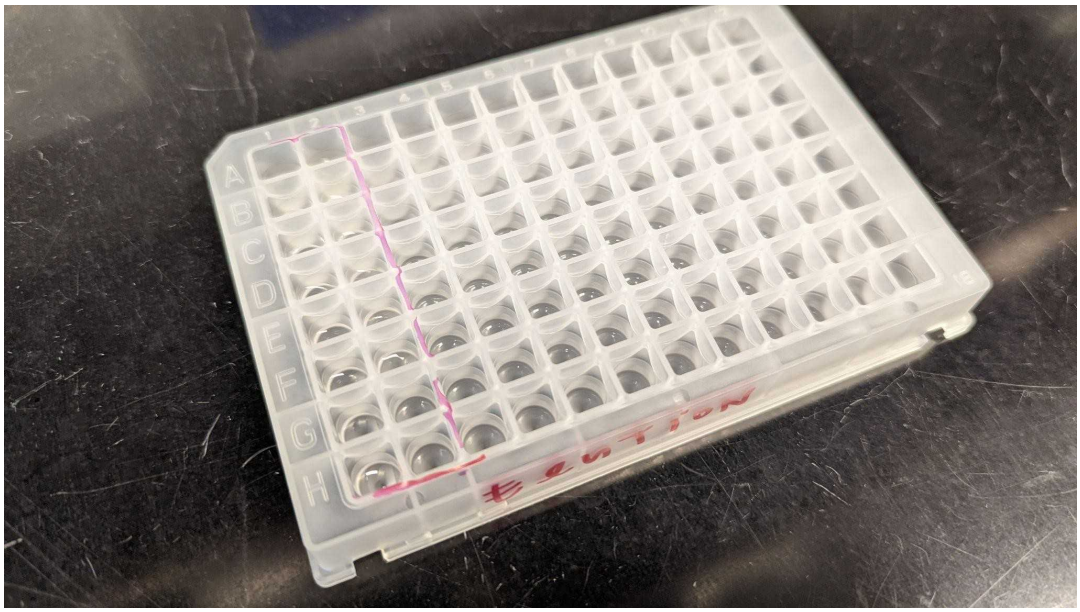


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