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Automated concentration of viral particles in wastewater samples using Nanotrap Microbiome A Beads

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Protocol status: Working

We use this protocol and it's working

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Abstract

This method describes how to concentrate viral particles in water samples using magnetic beads (Nanotrap® Microbiome A Particles) to enable the subsequent quantification of microbial organisms in those samples. This method uses the KingFisher™ Apex automation system and may be used for wastewater, drinking water, surface and recreational waters.

Guidelines

Wastewater samples should be stored at 4°C throughout processing. Prevent sample bottles from staying at room temperature for an extensive amount of time (10 minutes).

Remember to shake wastewater sample bottles thoroughly (at least 25 times) before processing the samples.

Materials

Equipment and Supplies:

Refrigerator storage (4°C)
Biosafety level 2 biosafety cabinet (BSC)
Sterile pipettes and sterile pipette tips
KingFisher™ Deep Well 24 Plate, barcoded (95040470B)
KingFisher™ Deep Well 24 Tip Plate (97002610B)
Thermo Scientific KingFisher™ Apex (catalog# 5400930)
Absorbent sheets
Kimtech science Kimwipes
Freezer storage (-80°C or below)
Permagen 24-Well Ring Magnet Plate (SKU: [MSP24](#))
Sample storage tubes (e.g.: 1.5 mL microcentrifuge tubes)

Reagents and Standards:

Bovine coronavirus (BCoV) (Our laboratory uses [Zoetis Calf-Guard Bovine Rotavirus-Coronavirus Vaccine](#). We have purchased this product at different retailers including [PBS Animal Health](#) and [Blain's Farm & Fleet.](#))
10% Phosphate Buffered Saline (PBS) solution
Nanotrap® Enhancement Reagent 2 (SKU: 10112)
Nanotrap® Microbiome A Particles (SKU: 44202)
Promega Cell lysis buffer (CLD) (catalog# A1731)
10% Bleach solution
ELIMINase™ (or another reagent that hydrolyzes DNA and RNA)
70% Ethanol solution (using 200 Proof absolute non-denatured ethanol and sterile molecular-grade water)

Software:

KingFisher™ NT Classic Extend 042822 program:  Concentration_Ceres_NT Wastewate... 2KB

Protocols:

Published recommendations for use of the Nanotrap® Microbiome A Particles can be found in [this protocol](#) (Step 1 of the procedure). Our laboratory follows this protocol with a few exceptions: 5,000uL of environmental water sample is added to "Sample Plates 1 and 2" opposed to the recommended 4,875uL, and the "Lysis Plate" contains 600uL of Cell Lysis Buffer instead of the recommended 300uL. We use the KingFisher™ NT Classic Extend 042822 program instead of the Ceres-recommended program (NT_Microbiome_A_Promega_24.kfx). The Extended program runs a total of 59 minutes due to the extended bead collection time.

Protocol materials

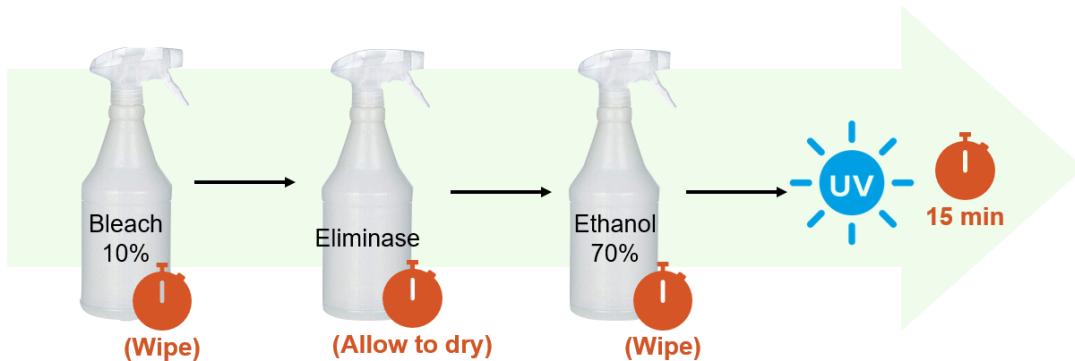
 Cell Lysis Buffer (CLD), 1400ml [Promega Catalog #A1731](#) Step 3

Safety warnings

- Raw wastewater samples should be handled inside a certified Biosafety Level 2 biosafety cabinet (BSC).

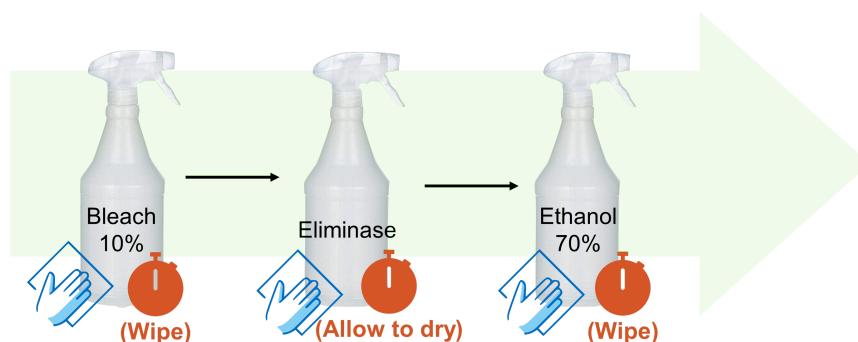
Before start

Before and after using the BSC, clean work area with 10% bleach, followed by ELIMINaseTM, and 70% ethanol. Then, turn on the BSC interior ultraviolet (UV) light for at least 15 minutes.



When using the benchtop, clean workspace area with 10% bleach, followed by ELIMINaseTM, and then 70% ethanol before and after use.

Before/After



Sample Spike Prior to Concentration

- 1 Upon receipt, the temperatures of wastewater samples are measured and recorded. Samples with temperatures less than  2 °C and greater than  10 °C are recorded and flagged as temperatures outside of this range (2°C - 10°C) may compromise the sample quality.
- 1.1 Spike wastewater samples with  20 µL of bovine coronavirus vaccine (BCoV) per 250 mL of wastewater sample to serve as a virus recovery control measure at least 20 minutes before concentration.
Separately, spike 50 mL of 10% Phosphate Buffered Saline (PBS) with  4 µL BCoV to serve as a method blank.

Sample receiving and spiking will take roughly 1 hour for 15-20 samples.
- 1.2 Store samples at  4 °C throughout processing.

Concentration Preparation

- 2 Obtain three KingFisher Deep Well 24 Plates and one KingFisher Deep Well 24 Tip Plate.
- 3 For the Elution plate: On the benchtop, add  600 µL of  Cell Lysis Buffer (CLD), 1400ml **Promega Catalog #A1731** into each well of a KingFisher Deep Well 24 Plate as indicated by your plate map. Cover Elution plate with a Kim-wipe to avoid contamination and set aside to use later.
- 4 For the Sample plates (Sample 1 and Sample 2): Inside the BSC, add  5 mL of wastewater sample into matching wells of the remaining two KingFisher Deep Well 24 Plates (10mL total are processed). Repeat this step for all samples listed on the plate map.

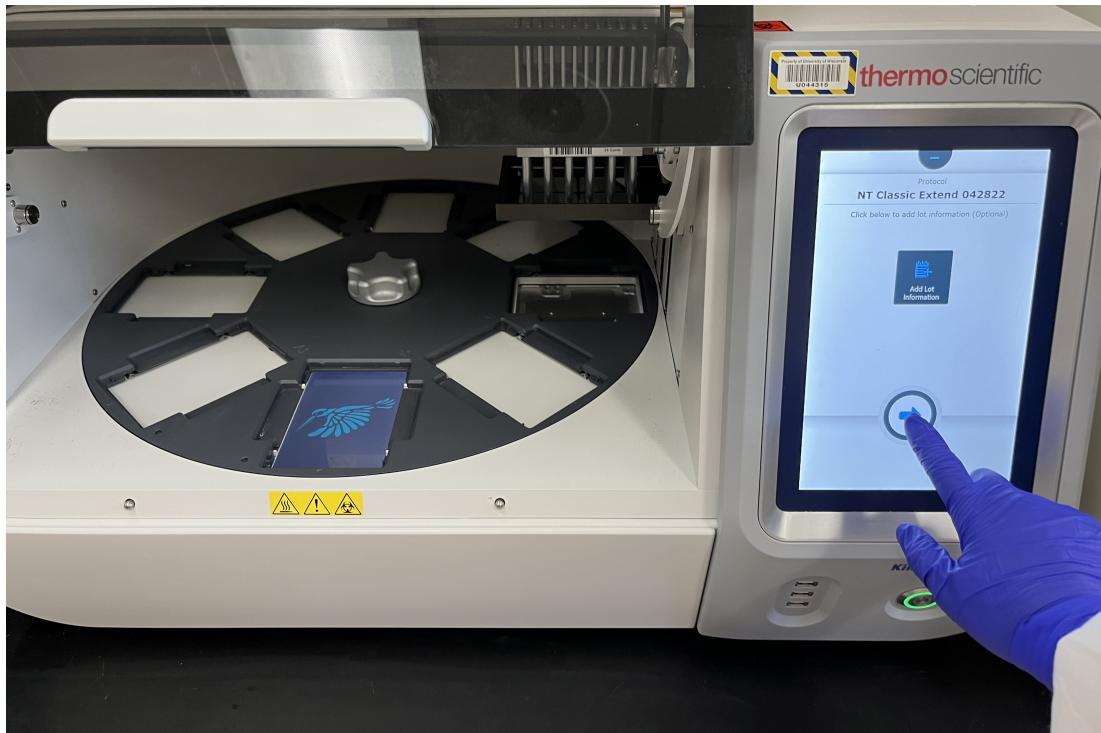


- 4.1 Add $\text{50 } \mu\text{L}$ of Nanotrap Enhancement Reagent 2 to all wells in both Sample 1 and Sample 2 plates.
- 4.2 Remove Nanotrap Microbiome A Particles from refrigerator and vortex thoroughly (10-20 seconds). Avoid leaving magnetic particles at room temperature for an extensive amount of time (5 minutes).
- 4.3 Immediately after vortexing, add $\text{75 } \mu\text{L}$ of Nanotrap Microbiome A Particles to all wells with wastewater sample in both Sample 1 and Sample 2 plates.

Concentration: Loading KingFisher Apex

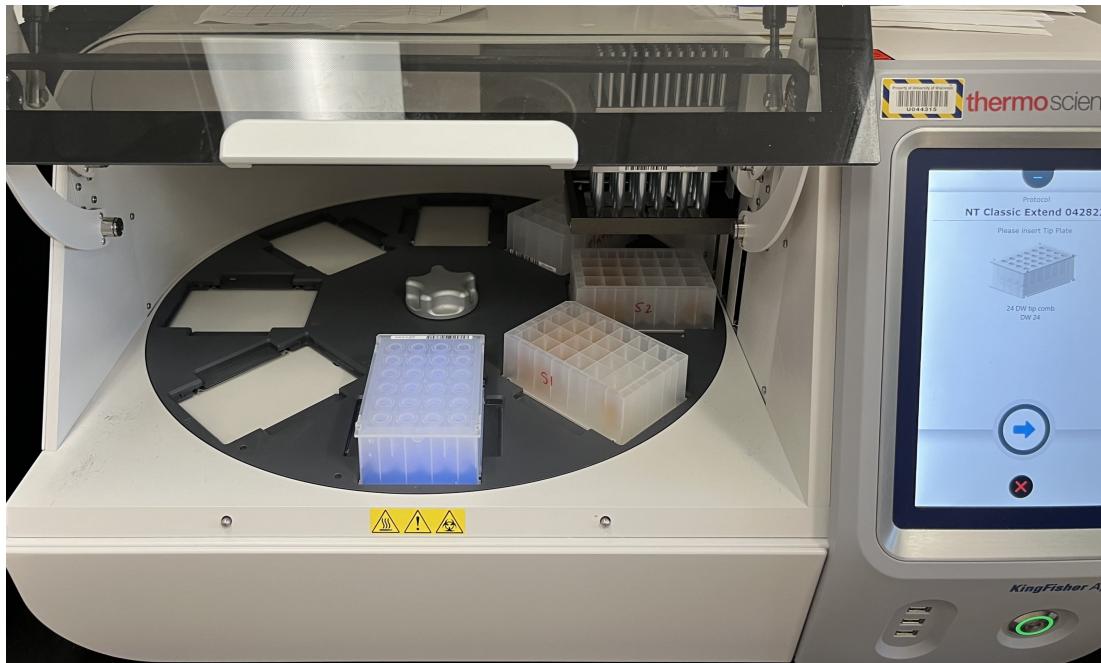
1h

- 5 Go to the menu screen on the Thermo Scientific KingFisher Apex and select the program titled "NT Classic Extend 042822".
- 5.1 Open the door of the KingFisher Apex, and click the next arrow to proceed.



- 5.2 Load the different plates according to the instructions on the screen. Ensure all 24-well plates are loaded with A1 corner in the upper right corner of the instrument's plate tray.
- 5.3 Lower the door and start the program. The program will run for 60 minutes.

1h



Note

ThermoFisher Scientific protocols provide laboratories with the option to insert the pipette tips directly into the last plate loaded into the instrument or to obtain separate Deep Well 24 Tip Plates. Our laboratory has tried both options, and we recommend using separate Deep Well 24 Tip Plates for this automated concentration protocol. We experienced issues with the KingFisher Apex's ability to accurately pick up the tips when they started in a plate that contained a large volume of liquid.

Concentration: Unloading KingFisher Apex

2m

- 6 When the NT Classic Extend 042822 program is finished remove all plates from the Kingfisher Apex as instructed by the instrument prompts.
 - 6.1 Discard Sample 1 plate, Sample 2 plate, and Tip plate.
 - 6.2 Keep the Elution plate for the next step.

Concentrated Wastewater Samples

30m

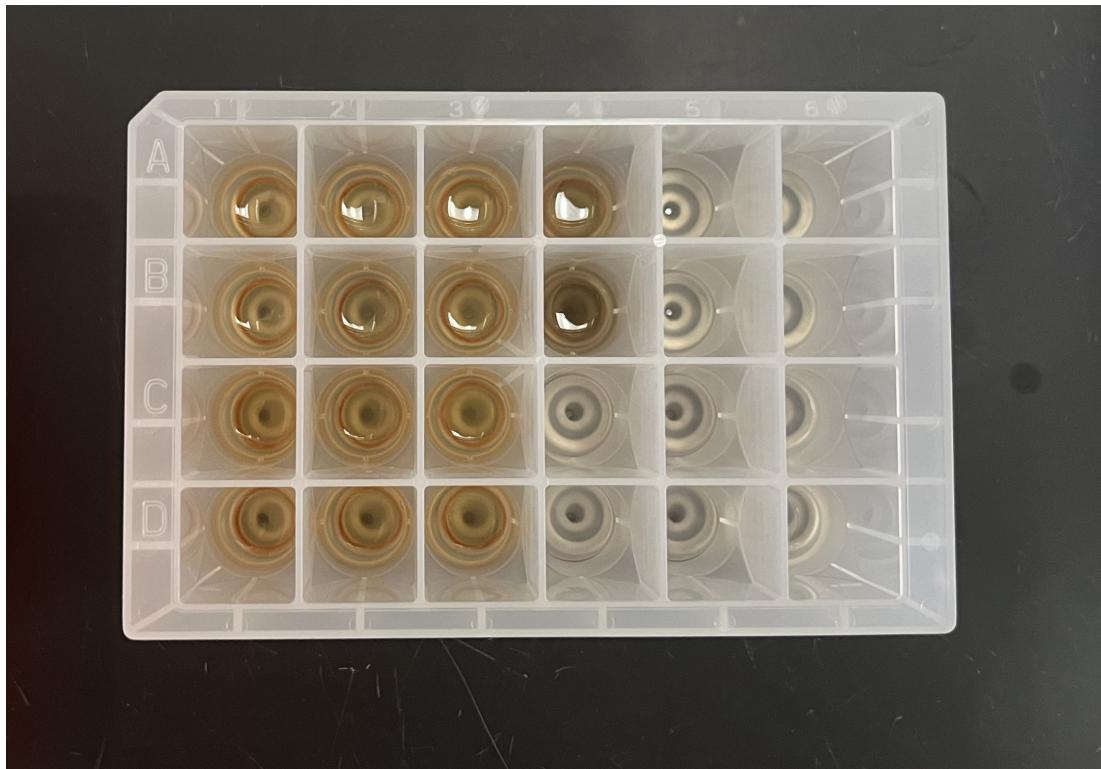
- 7 In the clean benchtop space, place the Elution plate on a Permagen 24-Well Ring Magnet Plate for 7-10 minutes to separate trace magnetic beads from the concentrated samples.



- 7.1 Ensure magnetic beads have been separated from samples by looking for a solid ring around the base of each well (see photos below). Bead rings should be visible after sitting on magnetic separation plate for 7-10 minutes.



Prior to magnetic separation, the picture above shows sample wells **without** the Nanotrap bead ring.



The picture above shows sample wells **with** the Nanotrap bead ring after sitting on the ring magnet plate for 7-10 minutes. The method blank (in well D6) will not develop a bead ring. The color of the bead rings and sample solution will vary depending on the darkness of the sample received. Well B4 is much darker in color than the rest of the samples because the sample received was much darker than the other samples in this batch.

- 7.2 Transfer \ddagger 600 μL of sample from the elution plate (still sitting on the ring magnet plate) to its corresponding labeled storage tube. Be careful not to touch the tip of the pipette to the ring of magnetic beads at the base of the well. If the magnetic beads are transferred to the archive sample, they will interfere with the extraction process.
- 7.3 Once all samples are removed, discard the Elution plate.

Extraction

- 8 Extraction of SARS-CoV-2 nucleic acid material may now be performed on the concentrated wastewater samples using any extraction method.
- 8.1 If not performing extraction on the concentrated samples, go to the next step.

Archive Storage

2m

- 9 Store the concentrated wastewater samples in a freezer at  -80 °C or below for up to 2 years.