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Automated Extraction of Concentrated Wastewater Samples using Promega HT Environmental TNA Kit

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Coronavirus Method De... APHL Wastewater Surve...

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We use this protocol and it's working

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Abstract

This protocol describes how to extract total nucleic acids (TNA) from viruses and bacteria concentrated from environmental samples such as wastewater, drinking water, surface and recreational waters. The resulting TNA is suitable for downstream molecular analyses. This method uses the KingFisher™ Flex automated system with the DNA/RNA extraction kit called Promega Maxwell® HT Environmental TNA Kit, AX9190.

Guidelines

For laboratories performing a concentration method prior to extraction, it is recommended to start setting up this automated extraction protocol 15 to 30 minutes before the concentration method is finished. Our laboratory's concentration protocol can be found here: **dx.doi.org/10.17504/protocols.io.261gedz7dv47/v1**.

Materials

Equipment and Supplies:

Refrigerator storage (4°C)
Pipettes and filtered pipette tips
Conical centrifuge tubes
KingFisher™ Deep Well 96 Plate, barcoded (catalog# 95040450B)
KingFisher™ 96 Tip Comb for DW Magnets (catalog# 97002534)
Thermo Scientific KingFisher™ Flex (catalog# N07669)
Round-bottom sterile 96-well plates (e.g., **Cell Treat** catalog# 229590)
Plate sealing film (e.g., **Axygen**® catalog# PCR-AS-600)
Absorbent sheets
Kimtech science Kimwipes

Reagents and Standards:

50% Ethanol solution (using 200 Proof absolute non-denatured ethanol and sterile molecular-grade water)
100% Isopropanol
Custom Promega Maxwell® HT Environmental TNA Kit, catalog# AX9190 [Includes: Alkaline Protease solution, Cell Lysis Buffer (CLD), Resin, Wash Buffer, and 25mM Tris-HCL (pH 8.0). The HT Environmental TNA kit can be purchased **here**.]
10% Bleach solution
ELIMINase™ (or another reagent that hydrolyzes DNA and RNA)
70% Ethanol solution (e.g., 200 Proof absolute non-denatured ethanol and sterile molecular-grade water)

Software:

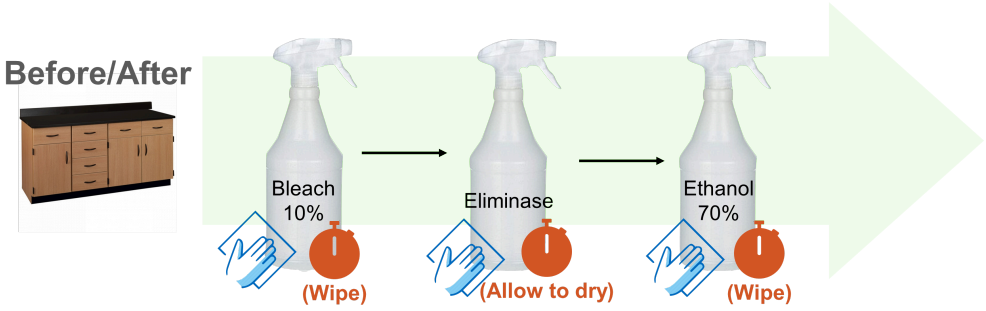
KingFisher™ Promega Maxwell HT RNA Wastewater V1: Promega_Maxwell_HT_RNA_Waste... 4KB

Protocol:

Ceres Nanosciences, Inc. has published recommendations for use of the Promega Maxwell® HT Environmental TNA Kit in **this protocol** (starting at Step 2 of the procedure). Our laboratory follows Ceres's extraction recommendations with a few exceptions: using the Thermo Scientific KingFisher™ Flex, adding isopropanol after the sample in the Lysis/Bind (Bead Binding) plate, eluting in 200µL of Tris-HCl, and using the Promega_Maxwell_HT_RNA_Wastewater_V1.bdz software program.

Before start

When using the benchtop, clean workspace area with 10% bleach, followed by ELIMINase^{TM}, and then 70% ethanol before and after use.



Obtain Concentrated Samples

5m

1 Obtain wastewater samples that have undergone a concentration method.

1.1 If concentrated samples are in storage, remove from them from the freezer and let them thaw on the benchtop.

Reagent Preparation

8m

2 Prepare single use volumes of clean reagents (that are not provided in the Promega kit) each day for a batch up to 40 samples:

2.1 Pipette 30 mL of molecular-grade 100% isopropanol into a 50mL conical centrifuge tube.

2.2 Pipette 25 mL sterile molecular-grade water followed by 25 mL of 200 proof ethanol into 50mL conical centrifuge tube. Invert the conical gently to mix the 50% ethanol solution.

Extraction Plates

30m

3 Label five KingFisher Deep Well 96 Plates "Wash 1", "Wash 2", "Ethanol" (EtOH), "Elution", and "Lysis/Bind".

Note

Plating the samples in a checkerboard pattern can help prevent well contamination if foaming occurs during the mixing steps while on the KingFisher™ Flex instrument.

4 For the Ethanol plate: add 450 µL of 50% ethanol solution to each of the sample wells as indicated on your plate map.

5 For the Wash plates: add 100 µL of 50% ethanol solution to each of the sample wells in both the Wash 1 and Wash 2 plates.

5.1 Then, add 900 µL of Wash Buffer to all sample wells in both Wash 1 and Wash 2 plates.

6 For the Elution plate: add 200 µL of 25 mM Tris-HCL to the sample wells according to your plate map.

7 Assemble the Lysis/Bind plate **in the following order**:

7.1 Add 50 µL of Alkaline Protease solution to the sample wells.

7.2 Vortex the Resin thoroughly (15-20 seconds). Then, add 35 µL of Resin to each sample well.

7.3 Transfer 300 µL of concentrated wastewater sample into its corresponding well in the Lysis/Bind plate according to the plate map. Repeat for all samples in the batch.

For the extraction blank, add 300 µL of Cell Lysis Buffer (CLD) in place of concentrated wastewater sample.

7.4 Add 400 µL of 100% isopropanol to each sample well.

Note

The isopropanol prevents foaming of the solution. If isopropanol is added before the sample, it can impact the binding of nucleic acid particles with the Resin.

Note

The volumes of the reagents in the Lysis/Bind plate can be adjusted to perform extraction following various concentration procedures. The reagent variations can be found in the table below:

Concentration Method	Volume (µL) of Concentrate/Supernatant/Sample	Volume (µL) of Cell Lysis Buffer (CLD)	Volume (µL) of Alkaline Protease	Volume (uL) of Resin	Volume (µL) of Isopropanol (add last)
Nanotraps	300	--	50	35	400
Filtration	250	250	50	35	400
Direct Extraction	125	125	50	35	400

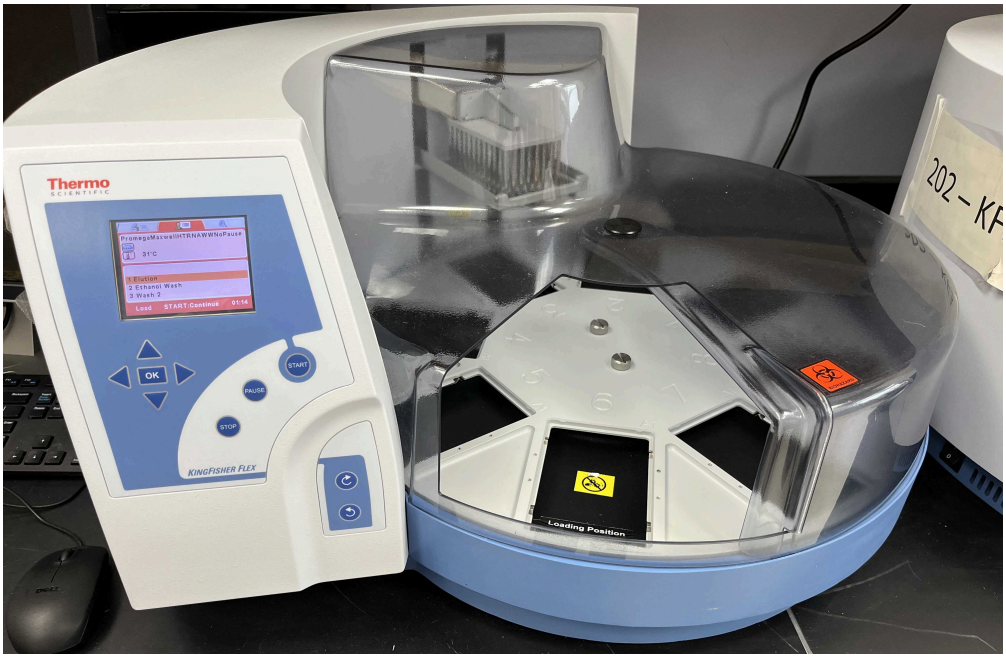
8 Insert a KingFisher 96 Tip Comb for DW Magnet into a sixth KingFisher Deep Well 96 Plate.

Loading KingFisher Flex

3m

9 Go to the menu screen on the KingFisher Flex, and select the program titled "Promega Maxwell HT RNA Wastewater V1".

9.1 Slide the door of the KingFisher Flex open, and press start to proceed.



9.2 Follow the instrument prompts to load the plates into the plate trays. Ensure all 96-well plates are loaded with A1 corner in the upper right corner of the instrument's plate tray.

9.3 Close the door and start the program. The program will run for 1 hour and 15 minutes. 1h 15m

Unloading KingFisher Flex

1m

10 When the Promega Maxwell HT RNA Wastewater V1 program is finished, remove all plates from the Kingfisher Flex as instructed by the instrument prompts.

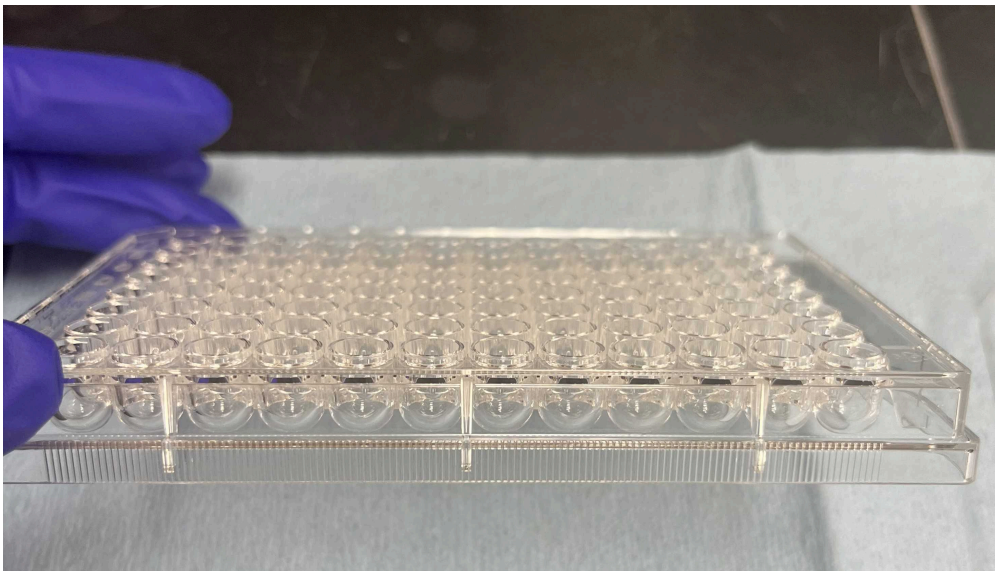
10.1 Keep the Elution plate, and set it aside to use in the next step.

10.2 Discard the Wash 1, Wash 2, Ethanol, Lysis/Bind, and Tip plates according to your biosafety protocol.

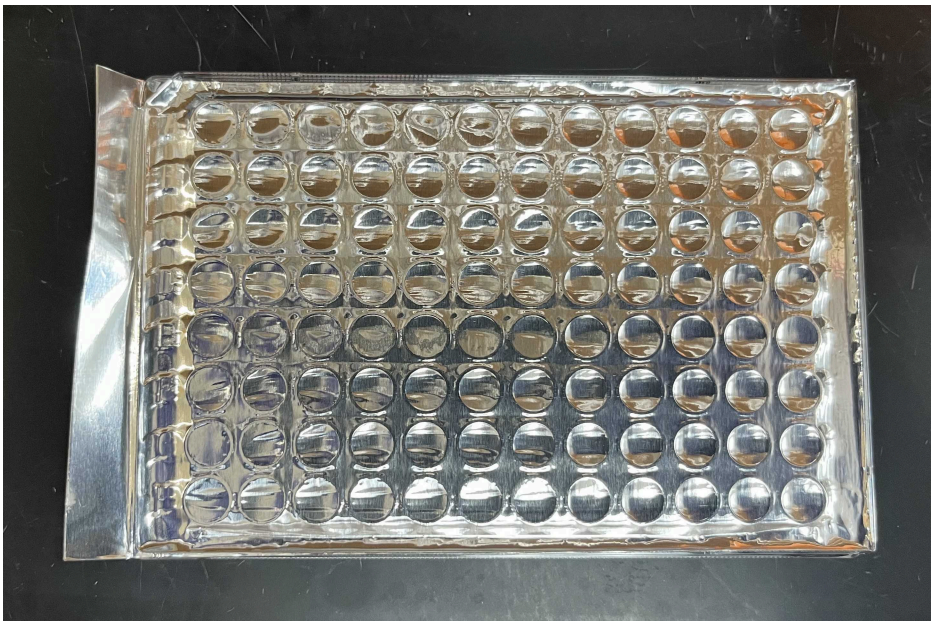
Final Plating of Total Nucleic Material

30m

11 Transfer the entire volume (around 200 µL) of extracted nucleic material from the Elution plate to a round-bottom sterile 96-well plate (pictured below). Repeat this step for each sample.



12 Cover the sterile 96-well plate with an aluminum plate sealing film.



13 Store the 96-well plate at 4 °C if the sample is going to be analyzed the same day.

13.1 If the nucleic acid material is not being analyzed that same day of extraction, store in a freezer at -70 °C or below for up to 2 years.