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Ceres Nanotrap & Dynabeads Combined Wastewater Virus Enrichment & Total Nucleic Acid Extraction

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Wastewater Genomics S...

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We use this protocol and it's working

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Abstract

This protocol utilises Ceres Nanotrap beads and Dynabeads for virus enrichment from 20 mL wastewater samples. The Applied Biosystems MagMAX Wastewater Ultra Nucleic Acid Isolation Kit (Cat. No. A52610) is then employed for total nucleic acid extraction. The purified nucleic acids are suitable for various downstream applications, such as real-time PCR, digital PCR, and next-generation sequencing. Both the enrichment and extraction processes are automated on the KingFisher Flex Purification System with a 24-deep-well head, using in-house adapted programmes from MagMAX_Wastewater_10mL_Flex24.

Guidelines

General

- Perform all steps at room temperature (20–30°C), unless otherwise noted.
- Clean the work surfaces with RNaseZap to remove nucleases, then wipe the surfaces with 70% to 100% molecular biology grade ethanol to remove additional contaminants.
- Precipitates can form in the Lysis Buffer, Binding Solution, and Wash Buffer if stored below 20°C. If this occurs, warm the reagents at 37°C, then gently mix to dissolve the precipitates. Avoid creating bubbles.

Binding Bead Mix

- Vortex Binding Beads thoroughly before each use.
- Ensure that the beads stay fully mixed within the solution during pipetting.
- Avoid creating bubbles during mixing and aliquoting.
- The Binding Bead Mix is very dense so pipet carefully to ensure that the correct volume is added to the sample

Materials

Reagents

Nuclease-free water
Ceres Nanotrap Microbiome A Particles
Ceres Nanotrap Enhancement Reagent 1
100% Ethanol (molecular biology grade)
MagMAX Wastewater Ultra Nucleic Acid Isolation Kit (A52610)

Consumables

RNase-Free Microfuge Tubes, 1.5 mL
KingFisher Flex 24 Deep-Well Plate
MicroAmp Clear Adhesive Film
Conical Tubes (50 mL)

Equipment

KingFisher Flex Purification System with 24 deep-well head
Standard laboratory vortex
Pipettes

Before start

- Prepare 80% ethanol using 100% absolute ethanol and nuclease-free water, ensuring a minimum volume of 2 mL per sample.
- Vortex the Binding Beads gently to ensure that the beads are fully resuspended.
- Prepare Binding Bead Mix — Combine 500 µL of Binding Solution with 20 µL of Binding Beads per sample, preparing enough for the required number of samples plus an additional 10% overage.
- Mix well by inversion, then store at room temperature.

Automated Dynabeads enrichment process

1 Set up and label your plates according to the following table:

	Plate name	Plate type	Contents	Vol per well
	Sample plate 1	24 deep-well	Clarified supernatant	5 000 µL
			Dynabeads	100 µL
	Sample plate 2	24 deep-well	Clarified supernatant	5 000 µL
	Lysis buffer	24 deep-well	Lysis buffer	500 µL
	Tip comb	24 deep-well tip comb	Not applicable	

2 Select the appropriate program on the instrument (Wastewater_10mL_Virus_Isolation).

3 Start the run, then load the prepared sample and processing plates into position when prompted by the instrument.

4 While the instrument is running, set up the plates for Ceres Nanotrap enrichment process.

Automated Ceres Nanotrap enrichment process

5 Set up and label your plates according to the following table:

	Plate name	Plate type	Contents	Vol per well
	Sample plate 1	24 deep-well	Clarified supernatant	5 000 µL
			Microbiome A particles	75 µL
			Enhancement Reagent 1	50 µL

	Plate name	Plate type	Contents	Vol per well
	Sample plate 2	24 deep-well	Clarified supernatant	5 000 µL
			Microbiome A particles	75 µL
			Enhancement Reagent 1	50 µL
	Lysis buffer	24 deep-well	Lysis buffer	500 µL
	Tip comb	24 deep-well tip-comb		

6 Once the Dynabeads enrichment process is complete (~20 minutes after starting the run), remove the lysis plate from the instrument and store it at 4°C for nucleic acid extraction. Discard the remaining plates from the instrument.

7 Select the appropriate program (Wastewater_10mL_Virus_Isolation).

8 Start the run, then load the prepared Ceres Nanotrap enrichment plates into position when prompted by the instrument.

9 While the instrument is running, set up the nucleic acid extraction plates.

10 Once the Ceres Nanotrap enrichment process is complete (~20 minutes after starting the run), remove the lysis plate and store it at 4°C for nucleic acid extraction. Discard the remaining plates from the instrument.

Nucleic acid Extraction

11 Carefully combine 500 µL of lysed material from the Dynabeads plate with the corresponding 500 µL of lysed material from the Ceres Nanotrap plate.

12 Set up and label your plates according to the following table:

	Plate name	Plate type	Content	Vol per well
	Binding plate	24 deep-well	Lysed sample	1 000 µL
			Binding bead mix	1 040 µL
			Proteinase K	80 µL
	Wash buffer 1	24 deep-well	Wash buffer	1 000 µL
	Wash buffer 2	24 deep-well	80% Ethanol	1 000 µL
	Elution buffer	24 deep-well	Elution buffer	100 µL
	Tip comb	24 deep-well tip comb		

13 Load the Binding plate along with Wash 1, Wash 2, Elution, and a new tip comb. Start the run on the instrument and select the appropriate programme (Wastewater_10mL_Virus_Extraction)

14 Immediately remove the Elution plate from the instrument at the end of the run and cover it. Alternatively, transfer the eluate to a new tube or plate for nal storage. The isolated nucleic acid is ready for immediate use. For storage, keep it at –20°C for up to 6 months or at 80°C for longer than 6 months

Protocol references

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