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MagMAX Enrichment & Extraction

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Victor Mabasa[1]

[1]National Institute for Communicable Diseases

Victor Mabasa
National Institute for Communicable Diseases

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We use this protocol and it's working

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Disclaimer

This protocol was amended from Pub. No. MAN0025695 by appliedbiosystems

Abstract

The Applied Biosystems MagMAX Wastewater Ultra Nucleic Acid Isolation Kit (Cat. No. A52610) is specifically designed for virus enrichment and total nucleic acid extraction from 10 mL wastewater samples. The purified nucleic acids suit various downstream applications, including 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
Ethanol, 100% (molecular biology grade)
MagMAX Wastewater Ultra Nucleic Acid Isolation Kit

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 vigorously 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.

Enrichment process

1 Set up and label the Sample, Lysis and Tip Comb plates outside of the instrument according to the following table:

Plate ID	Plate position	Plate type	Reagent	Vol per well
Sample plate 1	1	24 deep-well	Clarified supernatant	5 000 µL
			Dynabeads Wastewater Virus Enrichment beads	100 µL
Sample plate 2	2	24 deep-well	Clarified supernatant	5 000 µL
Lysis	3	24 deep-well	Lysis Buffer	500 µL
Tip Comb	4	24 deep-well Tip Comb		

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 nucleic acid extraction plates

Nucleic acid extraction process

5 Set up and label the Wash, Elution and Tip Comb plates outside of the instrument according to the following table:

6

Plate ID	Plate position	Plate type	Reagent	Vol per well
Wash Buffer 1	1	24 deep-well	Wash Buffer	1 000 µL
Wash Buffer 2	2	24 deep-well	80% Ethanol	1 000 µL
Elution	3	24 deep-well	Elution Solution	100 µL
Tip Comb	4	24 deep-well Tip Comb		

7 When the enrichment process is complete (approximately 20 minutes after starting the run), remove the lysis plate from the instrument and store it safely for nucleic acid extraction. Discard the remaining plates from the instrument.

8 Add 40 µL of Proteinase K to each enriched sample in the lysis plate.

9 Invert the tube of Binding Bead Mix several times to resuspend the beads, then add 520 µL of
the Binding Bead Mix to each sample

10 Load the plate, start the run on the instrument, and select the appropriate programme (Wastewater_10mL_Virus_Extraction).

11 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 final 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.