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NEB #T4010 Monarch Mag Viral DNA/ RNA Extraction Kit Protocol for KingFisher Flex Automated Isolation of Viral DNA/RNA from Wastewater Samples Following Ceres Nanotrap Enrichment V.1

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We use this protocol and it's working

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Abstract

The Monarch Mag Viral DNA/RNA Extraction Kit provides a rapid and reliable magnetic bead-based process for extracting viral nucleic acids from saliva and respiratory swab samples. The kit combines the efficiency of silica-based nucleic acid purification with the ease of use of magnetic beads. Manual and automated workflows allow samples to be processed in microfuge tubes or 96-well plates. Kit sizes align to 96-well formats (100 preps, 600 preps, and 1800 preps), and the protocol is compatible with high throughput automation on a variety of platforms, including the KingFisher Flex magnetic particle processor and Agilent Bravo and MGISP liquid handler platforms.

Materials

Reagents and Materials Supplied by User:

- 100% ethanol
- 100 % isopropanol
- Nuclease-free water
- RNase-free tips, tubes, and plastics.
- Adhesive seals for 96-well plates (KingFisher Flex automation protocol)

Required Equipment For the Automation Protocol

- Vortex mixer
- KingFisher Flex or liquid handler (configured to align with the protocol)
- Automation platform-compatible plastics (e.g., 96-well deep well plates, 96-well microplates)
  Thermal mixer containing block for 96-well plates or plate shaker (may be required, depending on the automation platform)

Required plastics

- KingFisher 96-deep well plates, v-bottom, (2.0 ml), Catalog # 95040450
- KingFisher 96 microplate (200 µl), Catalog # 9700254
- KingFisher 96 deep-well tip comb and plate, Catalog # 97002820

Troubleshooting

Before start

Important Notes Before You Begin

- Review Reagent Preparation section in the manual on NEB.com.
- Store Proteinase K at –20°C upon receipt.
- Prepare Monarch Carrier RNA based on kit size used: Add 125 µl (NEB #T4010S) or 750 µl (NEB #T4010L/X) nuclease-free water, invert or pipette to mix, and transfer to an RNase-free microfuge tube. Keep on ice. Prepare single-use aliquots and store at –20°C. Avoid multiple freeze-thaw cycles.
- Prepare 80% ethanol: 80% ethanol should be prepared fresh using 100% ethanol (user supplied) and nuclease-free water (user supplied). Prepare 1 ml of 80% ethanol per reaction and add overage.
- Perform all steps at room temperature unless directed otherwise.

Required plastics

- KingFisher 96-deep well plates, v-bottom, (2.0 ml), Catalog # 95040450
- KingFisher 96 microplate (200 µl), Catalog # 97002540
- KingFisher 96 deep-well tip comb and plate, Catalog # 97002820

Starting Material Notes

This protocol has been optimized for use with wastewater samples that have been pre-processed with Ceres Nanotrap Particles.

Part I. Prepare the KingFisher Flex Instrument

1 Ensure the instrument is equipped with the KingFisher Flex 96 Deep Well head and the KingFisher Flex 96 heating block.
IMPORTANT: The heat block must be compatible with the KingFisher 96 microplate (200 µl).

2 Ensure the MagMAX Pathogen RNA/DNA (High Volume) program is loaded onto the instrument's connected computer and that the program has been modified to perform three 500 µl wash steps, a 2-minute bead drying step, and a 33–100 µl elution.

3 Enter sample, wash, and elution volumes into the program.

4 Select plate sizes for the run: KingFisher 96-deep well plates (2.0 ml) for sample and wash plates; KingFisher 96 microplate (200 µl) for elution.

Part II. Buffer Preparation

5 Prepare fresh Viral DNA/RNA Wash Buffer in a user-supplied tube or bottle (free of nucleases) according to the table.
Add components in order, as listed. Prepare up to 15% excess to ensure a sufficient volume is available for each reaction.

6 Prepare Lysis Buffer Bead Mix immediately before use, according to the table.
a. Vortex magnetic beads to form a homogeneous solution before use.
b. Add components in order, as listed.
c. For a master mix, prepare up to 15% excess to ensure a sufficient volume of buffer/bead mix is available for each reaction.
d. Store Lysis Buffer Bead Mix at room temperature. Periodically invert or vortex to keep beads in suspension.

Viral DNA/RNA Wash Buffer:

	A	B
		Volume per Reaction
	a. Combine the following:	
	Monarch Buffer BX	167 µl
	Nuclease-free Water	83 µl
	b. Vortex to mix and then add:	

	A	B
	Isopropanol	250 µl
	c. Vortex to mix	
	Total Volume	**500 µl**

Lysis Buffer Bead Mix

	A	B
		Volume per Reaction
	a. Combine the following	
	Monarch StabiLyse DNA/RNA Buffer	200 µl
	Monarch Carrier RNA	1 µl
	b. Vortex to mix and then add:	
	Isopropanol	200 µl
	c. Vortex to mix and then add:	
	Monarch Mag Beads M1	20 µl
	d. Gently vortex to mix	
	Total Volume	**421 µl**

Part III. Prepare Wash and Elution Plates

7 Aliquot 500 µl Viral DNA/RNA Wash buffer to wells in a 96-well deep well plate.

8 Aliquot 500 µl 80% ethanol to wells in each of two 96-well deep well plates.

9 Aliquot 33–100 µl nuclease-free Water to wells in a 96-well microplate.

10 Seal plates with an adhesive film until ready to use.

	A	B	C	D	E	F	G
	Plate Position	1	2	3	4	5	6
	Plate type	96 deep well	96 deep well	96 deep well	96 deep well	96-well microplate	Tip comb in 96-well microplate
	Plate Identification	Sample plate	Wash plate 1	Wash plate 2	Wash plate 3	Elution plate	N/A
	Plate Contents	Sample/Lysis Buffer Bead Mix (approx. 621 µl)	Viral DNA/RNA Wash Buffer (500 µl per well)	80% ethanol (500 µl per well)	80% ethanol (500 µl per well)	Nuclease-free water (33-100 µl per well)	N/A

Part IV. Elute target microbes from the Nanotrap pellet

11 *These steps will begin at the end of enrichment, specifically where Ceres protocol adds a Lysis Buffer. For example, for Protocol APP-042, you would follow the Nanotrap protocol till Step 11 and perform the steps below instead of Step 12.*

Add 100 µl Monarch StabiLyse DNA/RNA Buffer to the Nanotrap Particle pellet, pipette to resuspend the pellet.

12 Incubate for 10 mins at room temperature.

13 Use a magnetic rack that is compatible with sample tubes to separate Nanotrap Particles from the sample.

14 Transfer the supernatant to a deepwell plate compatible with processing on KingFisher.

15 **Sample Lysis (Sample Plate)**

Add 100 µl nuclease-free water to the Nanotrap enriched sample from Step 14 above.

16 111 Add 5 ul Proteinase K.

Note

Instead of individually performing Step 15 and 16, a master mix of nuclease water and Proteinase K can be made if desired.

17 Pipette mix or use a thermomixer for 30 seconds after sealing the plate.

18 Incubate for 15 min at room temperature.

19 Gently vortex the prepared Lysis Buffer Bead Mix and add 421 µl to each sample well.

20 Seal plate with adhesive film until ready to load onto the KingFisher Flex instrument.

Part V. Viral Nucleic Acid Purification (Bind, Wash, Elute)

21 Carefully remove adhesive film from sample, wash, and elution plates.

22 Load sample, wash, elution plates, tip comb and plate, into the appropriate positions on the KingFisher Flex worktable.

23 Run the modified MagMAX program.

24 Upon completion of the run, seal the elution plate with adhesive film and place on ice for immediate use or freeze for storage.