Protocol for KingFisher Flex Automated Isolation of Viral DNA/RNA

Overview

Guidance for KingFisher Flex Automation

The KingFisher Flex is a benchtop automation instrument that enables high-throughput purification of nucleic acids. Using Thermo Scientific® BindIt® software installed on a connected computer, users download the MagMAX Pathogen RNA/DNA (High Volume) program and modify it to align with the Monarch Protocol for KingFisher Flex Automated Isolation of Viral DNA/RNA (see protocol, next page).

Users supply the required KingFisher Flex plastics, including 96-deep well plates (2.0 ml) for the bind and wash steps, and a 96 microplate (200 µl) for the elution step. A deep well tip comb is also required and serves as a cover for the magnetic rods, and a tip comb plate ensures proper positioning of the tip comb on the instrument turntable. Catalog numbers for KingFisher plates and the tip comb are listed in the protocol.

The KingFisher Flex instrument must be configured with the proper magnetic head and heat block. To align with the Monarch workflow, the instrument must be equipped with the KingFisher Flex 96 Deep Well head (compatible with KingFisher deep well plates and deep well tip comb) and the KingFisher Flex 96 heating block (compatible with the KingFisher 96 microplate). For detailed information on KingFisher Flex setup and operation, refer to the instrument user manual.

When preparing the instrument for use, users open the modified program and ensure that the proper sample, wash, and elution volumes are entered. Users should also ensure that the proper plate sizes are selected (i.e., KingFisher 96-deep well plates (2.0 ml) for sample and wash plates; 96-well microplate (200 µl) for the elution plate).

For automated processing, sample, wash, elution plates, tip comb and tip comb plate, are loaded onto the instrument in the proper positions. Users then start the program, which has a run time of approximately 30 minutes.

Important Notes Before You Begin

- Review Reagent Preparation section.
- 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 –20C. 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 200 µl saliva or a respiratory swab sample collected in viral transport media (VTM).
For samples < 200 µl, the sample volume should be adjusted to 200 µl with VTM or PBS before processing.

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

1. 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.
2. Prepare Lysis Buffer Bead Mix immediately before use, according to the table.
3. Vortex magnetic beads to form a homogeneous solution before use.
4. Add components in order, as listed.
5. For a master mix, prepare up to 15% excess to ensure a sufficient volume of buffer/bead mix is available for each reaction.
6. Store Lysis Buffer Bead Mix at room temperature. Periodically invert or vortex to keep beads in suspension.

Viral DNA/RNA Wash Buffer	
	Volume per reaction
a. Combine the following:	
Monarch Buffer BX	167 µl
Nuclease-free Water	83 µl
b. Vortex to mix and then add:	
Isopropanol	250 µl
c. Vortex to mix	
Total Volume	**500 µl**

Lysis Buffer Bead Mix	
	Volume per reaction
a. Combine the following:	

Lysis Buffer Bead Mix	
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

1. Aliquot 500 µl Viral DNA/RNA Wash buffer to wells in a 96-well deep well plate.
2. Aliquot 500 µl 80% ethanol to wells in each of two 96-well deep well plates.
3. Aliquot 33–100 µl nuclease-free Water to wells in a 96-well microplate.
4. Seal plates with an adhesive film until ready to use.

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. Sample Lysis (Sample Plate)

1. Add 5 µl Proteinase K to plate wells (KingFisher 96-deep well, 2.0 ml).
2. Add 200 µl sample (e.g., saliva or nasal swab in VTM), and pipette thoroughly to mix.
3. Seal the plate with adhesive film and incubate at room temperature for 15 minutes.
4. Carefully remove the film.
5. Gently vortex Lysis Buffer Bead Mix and add 421 µl to each well. Pipette gently but thoroughly to mix.

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

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

1. Carefully remove adhesive film from sample, wash, and elution plates.
2. Load sample, wash, elution plates, tip comb and plate, into the appropriate positions on the KingFisher Flex worktable.
3. Run the modified MagMAX program.
4. Upon completion of the run, seal the elution plate with adhesive film and place on ice for immediate use or at –80°C for storage.