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Protocol Guidance for Automated Isolation of Viral DNA/RNA in Deep Well Plates SKU T4010 V.1

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We use this protocol and it's working

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Abstract

This protocol details the automated isolation of viral DNA and RNA in deep well plates.

Guidelines

General guidance for automated viral DNA/RNA purification in 1.0 ml, 96-well deep well plates:

- A detailed KingFisher Flex automation protocol with appropriate buffer volumes is provided in the Appendices.
- Refer to the product webpage for the most up-to-date information on additional guidance and protocols for other automation/liquid handling instruments.

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 -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 320 µL 80% ethanol per reaction and add overage.
- Perform all steps at room temperature unless directed otherwise.

Automation Guidance:

The Monarch Mag Viral DNA/RNA Extraction Kit is compatible with various automated sample processing platforms (e.g., KingFisher Flex, Agilent Bravo and MGISP liquid handlers). A protocol with general guidance for automated viral DNA/RNA isolation is provided. A detailed KingFisher Flex protocol can be found in the Appendices section. Refer to the product webpage for the most up-to-date product information, automation guidance, and protocols.

General Considerations for Automated Viral DNA/RNA Isolation:

- The automation platform must be equipped with the appropriate hardware (e.g., magnet, shaker, heat block) to align with the protocol.
- An appropriate script must align with sample, wash, elution volumes and sample processing steps (e.g., sample/bead mixing, bead collection, supernatant removal, wash steps, bead drying, and heated elution).
- Plastics (e.g., 96-well deep well plates) must be compatible with the automation instrument and the workflow.

Reagent Preparation

Monarch Carrier RNA:

For reconstitution of 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.

Lysis Buffer Bead Mix:

- Prepare Lysis Buffer Bead Mix immediately before use (i.e., immediately before Sample Lysis, or during the Sample Lysis, Proteinase K incubation step).
- Prepare Lysis Buffer Bead Mix by combining Monarch StabiLyse DNA/RNA Buffer (included), Monarch Carrier RNA (included), Isopropanol (user supplied), and Monarch Mag Beads M1 (included) as described in the protocol, adding components in the order listed. Vortex magnetic beads ~20 seconds immediately before use to form a homogeneous suspension. Carefully open the bottle after vortexing to ensure the magnetic beads do not spill.
- Store Lysis Buffer Bead Mix at room temperature.
- If preparing a master mix, prepare excess to ensure there is sufficient volume of the mixture for each reaction. We recommend an overage of up to 15%.

User-Prepared Wash Buffers:

- **Viral DNA/RNA Wash Buffer:** Prepare Viral DNA/RNA Wash Buffer in a user-supplied tube or bottle (free of nucleases) by combining Monarch Buffer BX (included), nuclease-free water (included), and Isopropanol (user supplied) as described in the protocol, adding components in the order listed. The nuclease-free water included in the kit is intended to be used for preparing this wash buffer. Users are instructed to prepare an amount of wash buffer that includes up to 15% excess to ensure sufficient volume for each reaction. Prepare Viral DNA/RNA Wash Buffer fresh, as a precipitate may form upon storage.

- **80% Ethanol:** Prepare 80% fresh ethanol using 100% ethanol (user supplied) and nuclease-free water (user supplied) in a user-supplied bottle, free of nucleases. Users are instructed to prepare an amount of 80% ethanol that includes up to 15% excess to ensure sufficient volume for each reaction. To support our sustainability efforts, we have not included additional bottles and nuclease-free water for these 80% ethanol wash steps to avoid shipping excessive materials that may not apply to all users.

Materials

Buffer preparation:

Viral DNA/RNA Wash Buffer:

	A	B
		Volume per reaction
	a. Combine the following:	
	Monarch Buffer BX	53 µl
	Nuclease-free Water	27 µl
	b. Vortex to mix and then add:	
	Isopropanol	80 µl
	c. Vortex to mix	
	Total Volume	**160 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**

Materials Required & Supplied by User

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 Manual Protocol

1. Vortex mixer
2. Thermal mixer containing block for 1.5 ml tubes
3. Microcentrifuge or mini centrifuge.
4. Compatible magnetic rack for 1.5 ml tubes, e.g., DynaMag™-2 Magnet (ThermoFisher Cat# 12321D).

Equipment

DynaMag™-2 Magnet	NAME
Magnetic tube rack	TYPE
Invitrogen™	BRAND
12321D	SKU
https://www.thermofisher.com/order/catalog/product/12321D#:~:text=The%20DynaMag%E2%84%A2%2D2%20magnet%20combines%20a%20strong%20magnetic%20attraction,microcentrifuge%20tubes%20in%20numbered%20spaces	LINK

- For the Automation Protocol

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

Before start

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.

Instrument Preparation

1

Note

“Please review the important information under the “Guidelines & Warnings”.

Ensure the automation instrument is equipped with the appropriate hardware (e.g., magnet, shaker, heat block).

2 Load an appropriate script onto the instrument that aligns with sample, wash, elution volumes, and sample processing steps (e.g., sample/bead mixing, bead collection, supernatant removal, wash steps, bead drying, and heated elution).

3 Ensure instrument-compatible plastics are used and mixing speeds are compatible with liquid volumes.

Buffer Preparation

4 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.

5 Prepare lysis buffer bead mix immediately before use, according to the table.

5.1 Vortex magnetic beads to form a homogeneous solution before use.

5.2 Add components in order, as listed.

5.3 For a master mix, prepare up to 15% excess to ensure a sufficient volume of buffer/bead mix is available for each reaction.

5.4 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	53 µl
	Nuclease-free Water	27 µl
	b. Vortex to mix and then add:	
	Isopropanol	80 µl
	c. Vortex to mix	
	Total Volume	**160 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**

Sample Lysis

15m

6 Add 5 µL Proteinase K to plate wells (1.0 ml, 96-well deep well plate).

7 Add 200 µL sample (e.g., saliva or nasal swab in VTM), and pipette thoroughly to mix.

8 Seal the plate with an adhesive film and incubate at Room temperature for 00:15:00 . 15m

9 Carefully remove the film.

10 Gently vortex lysis buffer bead mix and add 421 µL to each well. Pipette gently but thoroughly to mix.

Automated Viral Nucleic Acid Purification (Bind, Wash, Elute)

24m

11 **Bind nucleic acid to beads:** 5m

Mix sample plate at ~ 1000 rpm, 00:05:00 .

11.1 Collect beads on magnet for 00:03:00 . Remove supernatant. 3m

12 **Wash beads:**

12.1 Add 160 µL viral DNA/RNA wash buffer to beads.

12.2 Mix at ~ 700 rpm, 00:02:00 . 2m

12.3 Collect beads on magnet for 00:03:00 . 3m

12.4 Remove supernatant.

12.5 Repeat wash steps 12–12.4 with 160 µL 80% ethanol.

12.6 Repeat wash steps 12–12.4 for a second wash with 160 µL 80% ethanol.

13 **Dry beads:**

Dry beads for 30 seconds – 1 minute.

14 **Elute nucleic acid:**

Add 33 µL – 100 µL nuclease-free water to beads.

14.1 Incubate plate at 65 °C with mixing for 00:05:00 . 5m

14.2 Collect beads on magnet for 00:06:00 . 6m

14.3 Transfer eluate to 96-well plate.

14.4 Place eluted nucleic acid On ice for immediate use or at -80 °C for storage.