Monarch® Mag Viral DNA/RNA Extraction Kit

NEB #T4010S/L/X

100/600/1,800 preps

Version 1.0_01/24

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Kit Contents

Component	Application/Usage	T4010S (100 preps)	T4010L (600 preps)	T4010X (600 preps x3)	Storage temperature
Monarch StabiLyse™ DNA/RNA Buffer	Lysis buffer	24 ml	145 ml	145 ml (x3)	20–25°C
Proteinase K, Molecular Biology Grade	Digests proteins and inactivates RNase	0.55 ml	3.55 ml	3.55 ml (x3)	–20°C upon receipt
Monarch Carrier RNA	Poly(A) carrier RNA enhances recovery of low amounts of nucleic acid	125 µg (lyophilized)	750 µg (lyophilized)	750 µg (x3) (lyophilized)	20–25°C (–20°C after reconstitution)
Monarch Mag Beads M1	Silica-coated magnetic beads for nucleic acid capture	2.4 ml	14 ml	14 ml (x3)	20–25°C
Monarch Buffer BX	Wash buffer concentrate	19 ml	122 ml	122 ml (x3)	20–25°C
Nuclease-free Water	For reagent preparation and elution of viral nucleic acid	25 ml	122 ml	122 ml (x3)	20–25°C

Storage Recommendations

- Upon receipt, store Proteinase K at –20°C for long-term storage and stability.
- Store lyophilized Monarch Carrier RNA at room temperature until reconstituted. Reconstituted Monarch Carrier RNA should be aliquoted into single-use aliquots and stored at –20°C for long-term storage and stability. Avoid multiple freeze-thaw cycles.
- Always keep reagent bottles tightly closed.
- For information regarding the composition of buffers, please consult the Safety Data Sheets available at www.neb.com.

Introduction

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.

Silica-coated magnetic beads are utilized for highly sensitive nucleic acid capture from samples containing few target molecules. Silanol groups on the bead shell provide an optimal binding surface for nucleic acids, and the uniform, submicron bead size offers a high surface area with abundant binding sites. The superparamagnetic properties of the bead core result in a fast magnetic response, contributing to ease of handling during use and compatibility with automation.

An optimized viral nucleic acid extraction procedure employs a sample lysis step followed by a simple bind-wash-elute process. Samples are treated with Proteinase K and then mixed with a lysis buffer/bead mixture containing lysis buffer, carrier RNA, isopropanol, and magnetic beads, which promote binding of viral nucleic acid onto the silica-coated beads. Carrier RNA is utilized as a co-precipitant in the workflow to enhance the recovery of low amounts of viral nucleic acid. After binding viral DNA and RNA to the magnetic beads, the beads are washed to remove contaminants, and nucleic acid is eluted in nuclease-free water. Purified viral nucleic acid is suitable for downstream applications, including qPCR/RT-qPCR, ddPCR, and library prep for Next Generation Sequencing (NGS).

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 1,800 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.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Properties

Purification format	Magnetic bead
Processing format	Manual or automated
Sample purification (representative examples)	Viral DNA and RNA* from respiratory viruses (enveloped and non-enveloped, dsDNA and ssRNA)
Samples sources	Saliva, respiratory swab in VTM**
Sample input volume	Up to 200 µl***
Carrier supplied	Poly A carrier RNA****
Binding capacity	Up to 3 µg
Elution volume	33–100 µl
Tested automation platforms	KingFisher Flex; Agilent Bravo and MGISP liquid handlers
Compatible downstream applications	qPCR, RT-qPCR, ddPCR, library prep for NGS
Performance data	See product webpage

* Viral DNA and RNA are purified in parallel. Preparation of DNA-free RNA or RNA-free DNA requires further treatment with the appropriate nuclease (not supplied).
** Other sample input sources may also be compatible with this kit. See product webpage for additional information and updates.
*** The sample input volume may be scalable to accommodate larger sample volumes. Further workflow optimization may be required.
**** Use of carrier RNA is recommended for recovery of low amounts of viral nucleic acid. Carrier RNA should be omitted if the downstream application utilizes poly(A) RNA enrichment; however, viral nucleic acid recovery may be reduced.

General Guidelines & Safety Information

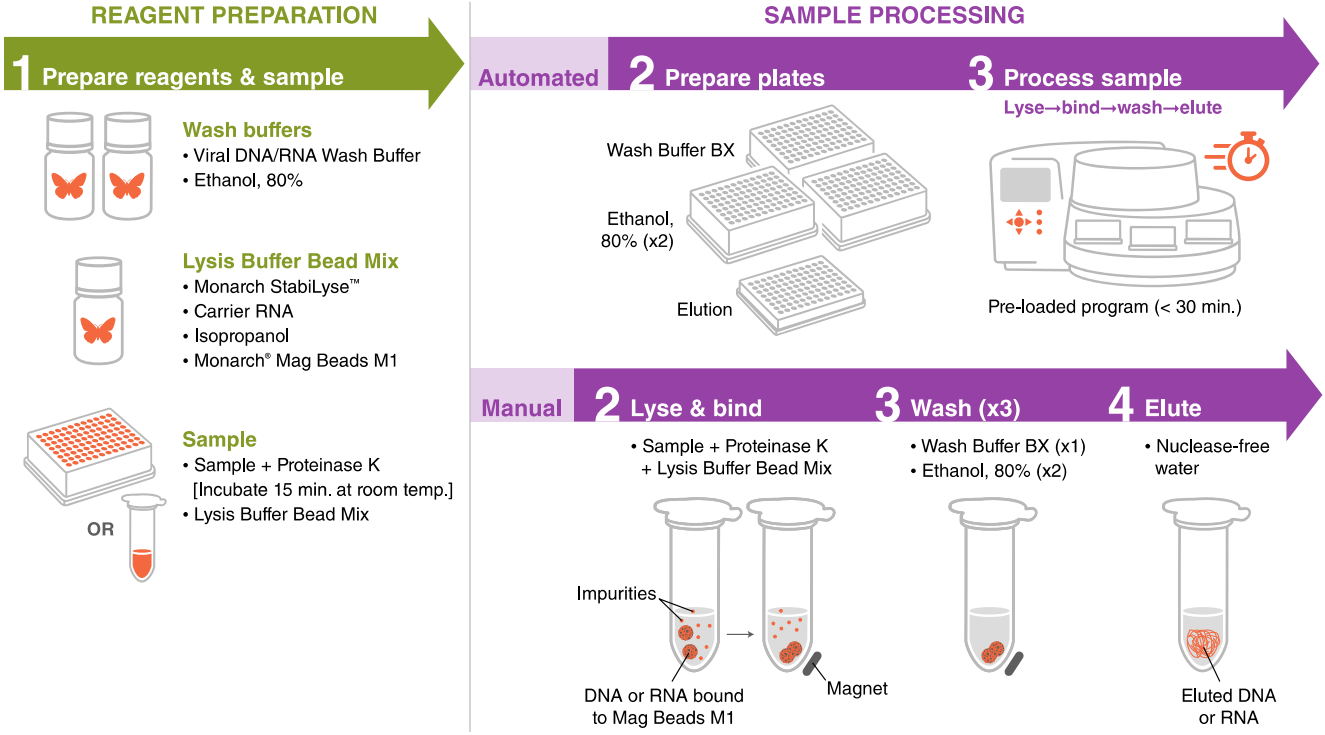
Recommended use and safety

- For purification of total viral DNA or RNA from saliva and respiratory swab samples in VTM.
- Monarch StabiLyse DNA/RNA Buffer and Monarch Buffer BX contain chaotropic agents. For information regarding the composition of buffers, please consult the Safety Data Sheets available at www.neb.com. Proper laboratory safety practices should be employed, including the use of lab coats, gloves, and eye protection.
- If using potentially infectious materials, follow institutional protocols for handling and disposing of biohazardous materials.
- This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Workflow

Samples are processed either by a manual workflow or an automated workflow on instrument platforms compatible with processing magnetic beads, such as the KingFisher, Agilent Bravo, and others.

Figure 1. Monarch Mag Viral DNA/RNA Extraction Kit Workflow



Important Considerations for Starting Materials

Saliva and Respiratory Swab Samples in Viral Transport Media (VTM)

- This protocol has been optimized for use with raw saliva or respiratory swab samples 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.
- The protocol may be scalable to larger sample input volumes (i.e., > 200 µl) with proportional scaling of sample preparation reagents, but the workflow may require further optimization.
- Other sample types may be compatible with this kit. Refer to the product webpage for the most up-to-date information on compatible sample types, protocols, and performance data.

Transport Media

CDC VTM (SOP# DSR-052-05), Hardy Diagnostics VTM (Cat. R99) and BD Universal Viral Transport (SKU 220220) formulations are compatible with the Monarch Mag Viral DNA/RNA Extraction Kit. Compatibility of other transport medium formulations may require evaluation by the user.

Monarch Carrier RNA

The addition of carrier RNA (poly(A)) to samples during processing enhances the recovery of low amounts of viral RNA and DNA. It also reduces the likelihood of viral RNA degradation due to RNases. Users may omit the use of carrier RNA; however, viral DNA and RNA recovery may be reduced. Carrier RNA should be omitted if the downstream application utilizes a poly(A) RNA enrichment step.

Use of an Internal Control

An exogenous internal control (e.g., virus particle or virus-like particle containing nucleic acid; not supplied) may be utilized to monitor sample extraction. The exogenous control should be added to the sample before extraction, and nucleic acid from this material will be copurified. If using an exogenous control material that may be vulnerable to degradation, add the control DNA or RNA to the Lysis Buffer Bead Mix along with Monarch Carrier RNA (i.e., before the addition of isopropanol and magnetic beads to the bead mix).

Important Notes about Viral DNA and RNA Purification

Copurification of Viral Nucleic Acid and Carrier RNA

- Viral nucleic acid and carrier RNA are purified in parallel. Depending on the input sample, viral nucleic acid yield may be relatively low and is often too low to be determined spectrophotometrically or fluorometrically. RT-qPCR/qPCR is recommended to determine viral RNA and DNA yields.
- Carrier RNA may account for much of the purified nucleic acid and its recovery can be quantified spectrophotometrically.
- To remove unwanted DNA or RNA from a purified sample, further treatment with the appropriate nuclease is required (not supplied).

Procedure Notes

This optimized viral nucleic acid extraction procedure employs a sample lysis step followed by a simple bind-wash-elute process. Manual and automated workflows allow samples to be processed in microfuge tubes or 96-well plates. Perform all steps at room temperature unless directed otherwise.

Sample Lysis and Binding of Nucleic Acid to Beads

Samples are lysed using Proteinase K and a user-prepared lysis buffer/bead mixture containing lysis buffer, carrier RNA, isopropanol (user supplied), and magnetic beads. The buffer conditions, and thorough mixing, promote binding of extracted nucleic acid onto silica-coated beads.

Magnetic Bead Collection Times

The superparamagnetic properties of the silica-coated beads result in a fast magnetic response, contributing to ease of handling during use. Recommended bead collection times are listed for the relevant protocol steps; however, bead collection times may vary depending on the sample type and magnet used.

Wash Steps

Following the binding of viral nucleic acid onto silica-coated beads, beads are subjected to washes with mixing to remove residual contaminants, including proteins and salts. Two user-prepared wash buffers are utilized in three wash steps. Users prepare Viral DNA/RNA Wash Buffer for the first wash step, and 80% ethanol for the second and third wash steps. Recommended wash volumes vary and are listed in each protocol.

A thermal mixer or vortex mixer can be used to mix bead washes in microcentrifuge tubes. For bead washes in deep well plates, a thermal mixer or plate shaker can be used. Using KingFisher Flex automation, the magnetic head and tip comb are utilized for mixing.

Bead Drying

Following a third wash step, the magnetic beads are air dried. Recommended bead drying times are listed in the protocol. The drying time can be extended if any visible droplets of liquid remain, or if the beads appear wet; however, overdrying of beads is not recommended as yield may be reduced.

Heated Elution

Nucleic acid is eluted from dried beads in a heated elution step. Nuclease-free water is added to the beads and samples are incubated at 65°C with mixing. Beads are then collected on a magnet, and the eluate is carefully transferred to a new tube or plate, without disturbing the beads. Purified nucleic acid can be stored on ice for short-term storage or at –80°C for long-term storage.

Considerations for Maximizing Viral RNA Recovery

Viral DNA and RNA are copurified using this optimized viral nucleic acid extraction protocol. To maximize viral RNA recovery, keep the following principles in mind:

1. Successful RNA extraction and purification are greatly influenced by the type of sample and the user's ability to maintain RNA integrity during sample collection, storage, and processing.
2. RNases are stable and difficult to inactivate, therefore, care must be taken when handling samples during and after preparation of RNA. Plasticware and glassware in direct contact with RNA-containing samples should be RNase-free. Gloves should be worn at all times when handling samples and kit components. Frequent glove changes are encouraged. Bench and equipment surfaces should be clean and can be decontaminated before work using commercially available cleaners such as RNaseZAP™.
3. Elution with nuclease-free water is standard, but for samples that will be stored for use later, EDTA can be added to 0.1–1.0 mM to limit degradation due to magnesium-requiring nucleases. Alternatively, elution with slightly alkaline TE can be employed.
4. Avoid repeated freeze-thaw cycles of purified RNA. Aliquots should be made consistent with downstream needs.

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.

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

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.

Protocol for Manual Isolation of Viral DNA/RNA in Microfuge Tubes (1.5 or 2.0 ml)

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% absolute 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.

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. 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.
   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

	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:	
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 II. Sample Lysis

1. Add 5 µl Proteinase K to each 1.5 ml microfuge tube.
2. Add 200 µl sample (e.g., saliva or nasal swab in VTM) to each tube, and pipette thoroughly to mix.
3. Incubate tubes at room temperature for 15 minutes.
4. Gently vortex Lysis Buffer Bead Mix before adding 421 µl to each sample tube. Pipette gently but thoroughly to mix.

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

Bind nucleic acid to beads:

1. Mix samples in a thermal mixer for 5 minutes at 2000 rpm.
2. Spin tubes briefly in a benchtop mini centrifuge to collect liquid at the bottom of the tube.
3. Place tubes on magnet for 3 minutes.
4. With tubes on the magnet, carefully remove and discard the supernatant.

Wash beads:

5. Remove tubes from the magnet and add 500 µl Viral DNA/RNA Wash Buffer.
6. Mix samples in a thermal mixer for 1 minute at 2000 rpm (or vortex mix, 5 seconds).
7. Spin tubes briefly in a benchtop mini centrifuge.
8. Place tubes on the magnet for 3 minutes.
9. With tubes on the magnet, carefully remove and discard the supernatant.
10. Repeat wash steps 5–9, washing beads with 500 µl 80% ethanol.
11. Repeat wash steps 5–9 for a second wash with 500 µl 80% ethanol.
12. Spin tubes briefly in a benchtop mini centrifuge.
13. Place tubes on the magnet for 3 minutes.
14. With tubes on the magnet, carefully remove and discard any residual supernatant.

Dry beads:

15. Air-dry beads for 5 minutes. If visible liquid droplets remain in the tube or on the bead pellet, extend the drying time up to 10 minutes. (During this bead drying step, set the thermal mixer to 65°C).

Elute nucleic acid:

16. Remove tubes from the magnet and add 33–100 µl nuclease-free water.
17. Mix samples in a thermal mixer at 65°C for 5 minutes at 2000 rpm.
18. Place tubes on the magnet for 5 minutes.
19. Transfer the supernatant (i.e., eluted nucleic acid) to a nuclease-free microfuge tube.
20. Place eluate on ice for immediate use or at –80°C for long-term storage.

Protocol Guidance for Automated Isolation of Viral DNA/RNA in Deep Well Plates

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.

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. Instrument Preparation

1. 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.

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.
   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

	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

	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. Sample Lysis

1. Add 5 µl Proteinase K to plate wells (1.0 ml, 96-well deep well plate).
2. Add 200 µl sample (e.g., saliva or nasal swab in VTM), and pipette thoroughly to mix.
3. Seal the plate with an 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.

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

Bind nucleic acid to beads:

1. Mix sample plate at ~1000 rpm for 5 minutes.
2. Collect beads on magnet for 3 minutes. Remove supernatant.

Wash beads:

3. Add 160 µl Viral DNA/RNA Wash Buffer to beads.
4. Mix at ~700 rpm for 2 minutes.
5. Collect beads on magnet for 3 minutes.
6. Remove supernatant.
7. Repeat wash steps 3–6 with 160 µl 80% ethanol.
8. Repeat wash steps 3–6 for a second wash with 160 µl 80% ethanol.

Dry beads:

9. Dry beads for 30 seconds – 1 minute.

Elute nucleic acid:

10. Add 33–100 µl nuclease-free water to beads.
11. Incubate plate at 65°C with mixing for 5 minutes.
12. Collect beads on magnet for 6 minutes.
13. Transfer eluate to 96-well plate.
14. Place eluted nucleic acid on ice for immediate use or at –80°C for storage.

Appendix A: Guidance for KingFisher Flex Automation

The Monarch Mag Viral DNA/RNA Extraction Kit workflow (i.e., bind, wash, and elute steps) can be automated using the KingFisher Flex magnetic particle processor and a compatible MagMAX® program for nucleic acid purification. Refer to the product webpage for the most up-to-date information on additional guidance and protocols for other automation/liquid handling instruments.

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.

Appendix B: Protocol for KingFisher Flex Automated Isolation of Viral DNA/RNA

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.
   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

	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:	
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.

Troubleshooting

Low yield

- **Incorrect preparation and/or storage of buffers and reagents:**
  - Store Monarch Mag Beads M1 at room temperature (do not freeze). Freezing of beads may affect performance. Vortex beads immediately before use to form a homogeneous suspension.
  - Store reconstituted Carrier RNA in single-use aliquots at –20°C. Avoid multiple freeze-thaws.
  - Ensure the Lysis Buffer Bead Mix is prepared immediately before use and that the mixture is homogeneous before adding to the sample.
  - Ensure the user-prepared Viral DNA/RNA Wash Buffer is prepared the same day as needed; a precipitate may form upon storage.
  - Ensure buffers and samples are thoroughly mixed as directed.
  - Check protocol to ensure correct buffer preparation and order of addition for reagents.
- **Incomplete Proteinase K digestion:** Ensure Proteinase K is used as directed for optimal sample lysis and inactivation of nucleases.
- **Carrier RNA omitted:** Ensure carrier RNA (poly(A)) is used to enhance recovery of low amounts of viral RNA and DNA and to reduce the likelihood of viral RNA degradation due to RNases.
- **Beads over-dried:** After the final wash step, ensure the wash is completely removed and beads are dried as directed in the protocol. Over drying of beads may result in reduced yield.
- **Incomplete elution:** Mix beads thoroughly with nuclease-free water and heat at 65°C with mixing for complete elution.
- **Microvolume spectrophotometer used to measure concentration:** Viral RNA and DNA yields are often too low to be determined spectrophotometrically or fluorometrically. Additionally, carrier RNA may account for much of the purified nucleic acid. RT-qPCR/qPCR is recommended to determine viral RNA and DNA yields.
- **Sample is degraded:** Process samples as soon as possible following collection. If samples are collected in transport media, follow manufacturer's storage recommendations.
- **Incompatible viral transport media used for sample collection and storage:** The compositions of commercially available transport media vary, with some formulations demonstrating incompatibility with nucleic acid purification workflows. The use of certain VTM formulations may result in lower-than-expected recovery of viral nucleic acid.

Purified nucleic acid is degraded

- Starting material not handled/stored properly. Nucleic acid degradation may occur if the sample is not collected and stored properly. For samples collected in viral transport media, follow manufacturer's recommendations for sample collection and storage.
- RNase contamination. To avoid RNase contamination, wear gloves and use disposable RNase-free tips and collection tubes (not provided) during the procedure. Keep all kit components tightly sealed when not in use.
- Improper storage of eluted nucleic acid. Store purified nucleic acid on ice for immediate use or at –80°C for long-term storage.

Low OD Ratios

- Low $A_{260/230}$ values indicate residual guanidine salts or ethanol has been carried over during elution. Ensure wash steps are carried out as directed before eluting sample. Ensure beads are dried as directed after final 80% ethanol wash step.
- Low $A_{260/280}$ values indicate residual protein in the purified sample. Ensure the proper amount of Proteinase K was used for the recommended time.

Low Performance of RNA in Downstream Steps

- Salt and/or ethanol carryover. Ethanol and salt remaining after the washes may inhibit downstream applications. Ensure washes are performed as directed and all traces of 80% ethanol are removed after the final wash. An additional 80% ethanol wash step may be used if salt carryover is suspected. Ensure beads are dry after final wash before performing elution step.
- Presence of qPCR/RT-qPCR inhibitors. For samples that contain high amounts of reaction inhibitors, these inhibitors may be copurified and inhibit qPCR or RT-qPCR. The effect of inhibitors may be reduced or eliminated by reducing the amount of purified nucleic acid used in the qPCR or RT-qPCR reaction.
- Presence of carrier RNA in purified viral nucleic acid. Users may omit the use of carrier RNA; however, viral DNA and RNA recovery may be reduced. Carrier RNA should be omitted if the downstream application utilizes poly(A) RNA enrichment.

Copurification of unwanted viral DNA, viral RNA, or carrier RNA

- Viral DNA and RNA are purified in parallel. Perform a DNase I or RNase A treatment step to remove unwanted DNA or RNA from sample, followed by a reaction cleanup.
- Carrier RNA is copurified. Carrier RNA may account for much of the purified nucleic acid. Users may omit the use of carrier RNA; however, viral DNA and RNA recovery may be reduced.

Magnetic Beads in eluate

- Bead carryover may be observed in the eluate. A small amount of bead carryover may occur and typically does not affect downstream applications. To remove residual beads, place the tube containing the eluate onto a magnet, collect beads, and transfer the supernatant to a new RNase-free tube.

How to Recycle Monarch Kit Components*

Learn more on how to recycle Monarch kit components at www.neb.com/monarchrecycling.

Ordering Information

NEB #	PRODUCT	SIZE
T4010S/L/X	Monarch Mag Viral DNA/RNA Extraction Kit	100/600/1,800 (3 x 600) preps

COMPANION PRODUCTS

NEB #	PRODUCT
T2111L	Monarch StabiLyse DNA/RNA Buffer
P8107S	Proteinase K, Molecular Biology Grade

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	1/24

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.