INSTRUCTION MANUAL

Monarch® Mag Cell-free RNA (cfRNA) Extraction Kit

NEB #T4080V/S 10/50 preps

Version 1.0 07/26

Table of Contents

Kit Contents and Storage

Component	NEB #	Application/Usage	T4080V 10 preps	T4080S 50 preps	Storage Temperature
Monarch StabiLyse™ DNA/RNA Buffer	T2111	Sample digestion	24 ml	210 ml	15-25°C
Monarch Buffer BX	T2041	Binding buffer, Wash 1 concentrate	20 ml	122 ml	15-25°C
Monarch Buffer WZ	T1115	Wash 2, 3 and 4 concentrate (5X)	12 ml	50 ml	15-25°C
Monarch Mag Beads M2	T4105	Magnetic beads for nucleic acid purification	2.2 ml	9 ml	15–25°C. 4°C after opening
Proteinase K, Molecular Biology Grade	P8200	Protein digestion	0.55 ml	2 ml	15–25°C. -20°C after opening
Monarch DNase I, Lyophilized	T2104	DNA removal	2200 U	11000 U	15–25°C. After rehydration, store in aliquots at -20°C
Monarch DNase I Reaction Buffer	T2105	DNase reaction buffer	4.6 ml	14 ml	15–25°C. 4°C after opening
Nuclease-free Water	B1500	Elution	14 ml	25 ml	15-25°C

Storage Recommendation

- **Monarch Mag Beads M2** should be stored at 4°C after first use.
- **Proteinase K** should be stored at -20°C after opening the kit.
- **Monarch DNase I, Lyophilized** should be stored in aliquots at -20°C after rehydration. Avoid more than 3 freeze-thaws.
- **Monarch DNase I Reaction Buffer** should be stored at 4°C after first use.
- Remaining kit components should be stored at room temperature.
- Always keep reagent bottles tightly closed.
- See individual component labels for specific storage guidance.

Intended Use

The Monarch Mag Cell-free RNA (cfRNA) Extraction Kit is developed for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Safety Information

- Monarch StabiLyse DNA/RNA Buffer and Monarch Buffer BX contain guanidine salts, which can form highly reactive compounds when combined with bleach. Do not add bleach or acidic solutions directly to the buffers or sample preparation waste.
- For more information regarding the composition of buffers, please consult the Safety Data Sheets available on our website www.neb.com/T4080.
- Proper laboratory safety practices should be employed using this kit, including the use of lab coats, gloves, and eye protection.

Quality Control

To help ensure consistent quality and performance, each lot of this kit is tested for predetermined quality control and functional specifications.

Introduction

The Monarch Mag Cell-free RNA (cfRNA) Extraction Kit is a phenol-free, magnetic bead-based extraction solution for efficient and reproducible isolation of circulating cell-free RNA (cfRNA) and exosomal RNA from biofluids. Extracted cfRNA is suitable for application in RNA biomarker discovery and liquid biopsy workflows. The kit includes on-bead DNA removal reagents to obtain cfRNA that is free from contaminating DNA. The silica-coated magnetic beads, combined with the optimized buffer chemistry, ensure optimal binding and recovery of cfRNA that is ready for downstream applications. When integrated with NEB's sequencing and amplification applications, our cfRNA extraction solution enables streamlined workflows from biological sample to signal. Flexible by design, the kit supports both manual and automated workflows and can be readily scaled to accommodate different sample input volumes.

Features of this kit include:

- **High Performance:** Isolate total cell-free RNA including long ncRNA, mRNAs and small RNAs.
- **Reproducibility:** Obtain consistent results for sample types with challenging biological variability.
- **Scalability:** Easily adapt to different sample input volumes.
- **DNA-free output:** Lyophilized DNase supplied with the kit enables DNA removal during extraction.
- **Application ready:** Achieve streamlined sample-to-result workflows by integrating with NEB's sequencing and amplification solutions.

Sustainability and Recycling Information

Monarch DNA and RNA Purification kits are designed for sustainability and performance. Learn more about Monarch sustainability at www.neb.com/monarchsustainability.

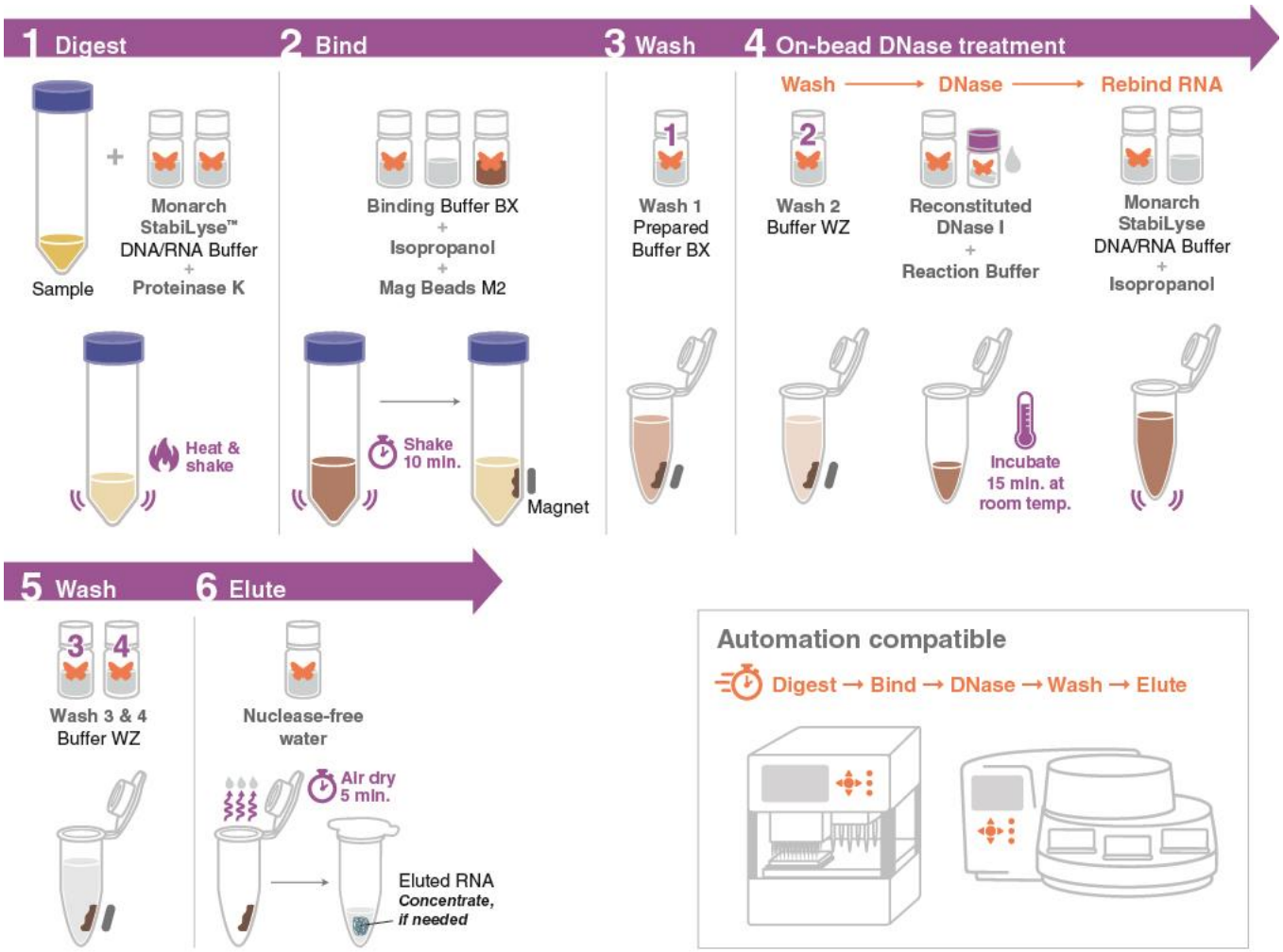
- **Sustainable performance**: Monarch kits use significantly less plastic in spin columns, buffer bottles, and other plastic components than leading alternatives. Magnetic bead formats provide a low-plastic option compared to traditional spin columns.
- **Flexible purchasing options:** Monarch kit components such as magnetic beads, spin columns, and buffers are available individually to suit your workflow needs.
- **Same performance, design and formulations:** Standalone products are the same components and formulations that are included in complete kits.
- **Streamlined packaging:** Monarch kits come in sturdy, right-sized reusable boxes and include quick protocol cards for easy reference.
- **Sustainable and recyclable packaging**: Monarch kits are printed with minimal ink using eco-friendly practices and renewable energy. Kit boxes, inserts and paper materials use recycled and recyclable paper.

Help keep Monarch sustainable by recycling after use. Learn more about how to recycle Monarch boxes and kit components at www.neb.com/monarchrecycling.

Background

The Monarch Mag Cell-free RNA (cfRNA) Extraction Kit offers a flexible solution for reproducible extraction of circulating cell-free RNA for manual or automation workflows. In a typical workflow, plasma obtained from processing whole blood is first digested using the Monarch StabiLyse DNA/RNA Buffer and Proteinase K. This step removes contaminating proteins and ensures efficient recovery of cfRNA. After the plasma digestion is complete, the addition of Monarch Buffer BX, isopropanol and silica-coated magnetic beads creates optimal conditions for RNA to bind to the magnetic beads, while undesirable impurities remain in the supernatant. The supernatant is discarded and the magnetic beads are washed using prepared Monarch Buffer BX and Monarch Buffer WZ. At this point, on-bead DNase treatment, using the supplied lyophilized DNase I, is recommended to remove contaminating DNA. Following DNase treatment, RNA is re-bound to the beads using Monarch StabiLyse DNA/RNA Buffer and isopropanol, and washed using prepared Monarch Buffer WZ. The DNA-free cfRNA is then eluted using nuclease-free water and can be concentrated into smaller volumes using the reagents provided with the kit. The extracted cfRNA is application-ready and seamlessly integrates with NEB's library prep and amplification workflows. Our scalable extraction process enables flexibility in sample volume and promotes efficient utilization of reagents.

Figure 1: Workflow for Monarch Mag Cell-free RNA (cfRNA) Extraction Kit



Properties

Purification Format	Magnetic beads
Compatible Platform	Manual or open automation platforms
Intended Application	Free circulating RNA and exosome RNA extraction from liquid samples
RNA Size Isolated	Total circulating RNA including long RNAs, small RNAs, miRNAs
Sample Type Compatibility	Plasma, urine, cerebrospinal fluid (CSF)
Compatible Downstream Applications	Sequencing and amplification applications including RNA-Seq, Small RNA library prep, RT-qPCR

Important notes before you begin

Cell-free RNA characteristics

Cell-free RNAs (cfRNAs) serve as important biomarkers in liquid biopsy for the detection and monitoring of cancer and other diseases. Cell-free RNAs are fragmented and can exist in diverse fragment sizes ranging from small microRNAs to long non-coding RNAs. These RNAs are free circulating in body fluids or associated with extracellular vesicles (EVs). All forms of cfRNAs are present in extremely low concentrations and variable amounts depending on physiological conditions and disease states. Key pre-analytical conditions in cfRNA testing workflows include cfRNA preservation at harvest, a sensitive extraction process that enables a faithful representation of cfRNA sizes, and removal of contaminating DNA that can lead to false positives in downstream RNA-based workflows.

Cell-free RNA quantification

Circulating RNA in plasma is an intrinsically challenging analyte to detect and characterize due to the low concentration and high sample-to-sample variability. Sensitive methods such as Agilent® Pico Bioanalyzer® or RT-qPCR are recommended to measure extraction outputs. In some cases, users may prefer to proceed without extraction quantification and rely on the sensitivity of library prep and amplification for detection. Downstream integration with NEB's library prep and amplification platforms enables improved detection in end-to-end workflows.

Sample collection

Sample collection and preservation play a crucial role in cell-free RNA yield and recovery. Sample collection tubes and collection processes should be chosen carefully to stabilize cell-free nucleic acids and minimize contamination by cellular nucleic acids. Tube manufacturer's instructions for sample collection and storage should be followed. This kit is compatible with several different collection tubes including standard anticoagulant tubes such as EDTA and sodium citrate, as well as preservative-containing tubes such as those offered by Streck®, Norgen and PAXgene®. Heparin-containing blood collection tubes are not recommended due to downstream interference of heparin in molecular assays.

Cell-free RNA size range

The standard protocol for this kit enables efficient extraction of all sizes of cfRNA, as verified by total RNA-seq. Protocol modifications are provided on the product webpage for modulating the RNA fragment sizes recovered.

Procedure Notes

Sample types

This extraction kit has been evaluated for plasma, urine and cerebrospinal fluid (CSF) samples.

Sample handling and storage

Use appropriate stabilization reagents or collection tubes for sample collection and follow manufacturer's instructions for best practices on sample collection and storage.

Storing and handling of Monarch Mag Beads M2

Store Monarch Mag Beads M2 at 4°C after first use. For optimal performance, Monarch Mag Beads M2 should be equilibrated to room temperature before use. Importantly, the beads should be uniformly mixed by thorough vortexing immediately before dispensing into the sample.

Binding of cfRNA to beads

RNA binds to silica-coated magnetic beads through interactions with the bead surface in the presence of the binding buffer (Monarch Buffer BX) and the appropriate volume of isopropanol. At this stage, the sample-binding mix needs to be mixed for 10 minutes to ensure efficient binding of cfRNA. A thermal mixer, shaker or an end-over-end mixer is recommended.

On-bead DNase treatment

Monarch DNase I, Lyophilized, supplied with the kit, contains the optimal enzyme units needed for complete DNA removal during cfRNA extraction from biofluid samples. This DNase I is an RNase-free formulation. Rehydration of supplied lyophilized DNase must be performed using the instructions in this manual as the final enzyme units required for cfRNA extraction differ from those in other kits and applications. After the DNase reaction mix is added to the sample, a 15 min incubation with intermittent mixing is recommended. During RNA rebinding with Monarch StabiLyse DNA/RNA Buffer and isopropanol, it is important to allow 5 minutes of binding, with shaking, to ensure RNA is rebound to the beads, while the digested DNA remains in the supernatant.

Bead drying

Following the final wash, beads should be dried thoroughly to remove residual ethanol. Optimally dried beads appear shiny and uniformly dark, while over-dried beads appear cracked or matte light brown. Avoid over-drying the beads by following the recommended drying times.

Elution volume considerations

At the elution step, RNA is released from the magnetic beads using nuclease-free water. For optimal release of all bound cfRNA, an elution volume of 100 µl is recommended followed by a magnetic bead-based concentration into smaller volumes. Depending on the workflow design and volume requirement for the downstream assay, some users may prefer to elute in <100 µl. Vigorous mixing on a thermal mixer or vortex is recommended to ensure complete release of RNA into the water. For automated workflows, adjust minimum elution volumes according to the specifications and requirements of the system being used.

Concentrating cfRNA

Because of the intrinsically low concentrations of cell-free RNA, the extracted cfRNA may need to be concentrated into smaller volumes before use in downstream applications. The beads and buffers included in the kit are sufficient for concentrating cfRNA using the concentration protocol provided in the manual. Other options for concentrating cfRNA include column-based kits such as the Monarch Spin RNA Cleanup Kit (10 µg) (NEB #T2030) or vacuum concentrators such as SpeedVac®.

General Guidelines for Monarch Mag Cell-free RNA (cfRNA) Extraction Kit

- Sample collection and preservation play a crucial role in yield and recovery. Choose sample collection tubes that are most suitable for your application and follow the manufacturer's storage protocol.
- Ensure that the Monarch Mag Beads M2 have reached room temperature before use.
- Ensure that the Monarch Mag Beads M2 are thoroughly mixed immediately before transferring to sample tube.
- Prior to use, refer to the reagent preparation instructions to:
    - prepare DNase reaction mix.
    - prepare Wash 1 using Monarch Buffer BX. This should be prepared fresh and used within a day.
    - prepare Monarch Buffer WZ.
- Perform all steps at room temperature unless specifically indicated otherwise.
- Review Total Sample Binding Mix volume provided in Table 1 ahead of starting the protocol to make the appropriate choice of extraction tube, as this varies by the sample input volume.

Equipment and Reagents Required & Supplied by the User

Equipment

- Magnetic separation racks compatible with the tubes used
- For 50 ml conical tubes, we recommend the 6 x 50 ml Magnetic Separation Rack (NEB #T5010)
- Tube racks
- Benchtop thermal mixer or a shaker incubator with appropriate tube adaptor (such as 50 ml conical tube)
- Serological pipette controller
- Benchtop mini-centrifuge

Reagents/supplies

- Isopropanol (≥ 99%)
- Ethanol (≥ 95%)
- RNase-free 1.5 ml microfuge tubes, LoBind or non-stick recommended
- 50 ml conical tubes
- Serological pipettes (10 ml, 25 ml)
- Micropipettes and tips

Preparation of Reagents and Buffers Supplied with the Kit

Rehydrate Monarch DNase I, Lyophilized to 20U/µl

Monarch DNase I is a proprietary lyophilized mix that is stable at room temperature in the supplied dry cake format.

For rehydration and subsequent storage guidance, follow the instructions below:

1. Place the glass vial containing lyophilized enzyme on the RNase-free benchtop.
2. Unscrew the cap and place the cap upside down on the benchtop.
3. Remove the grey rubber stopper and place it securely inside the inverted screw cap.
4. Using a pipette, dispense the appropriate volume of the supplied Nuclease-free Water onto the lyophilized cake.
    a. For NEB #T2104-2 (11000 U) supplied with the 50-prep kit, dispense 550 µl Nuclease-free Water.
    b. For NEB #T2104-1 (2200 U) supplied with the 10-prep kit, dispense 110 µl Nuclease-free Water.
5. Pipette mix 8-10 times, adjusting the tip positioning inside the vial such that all the lyophilized material gets hydrated. Do not vortex.
6. Place the vial back on the bench.
7. The solution will turn clear within 15 seconds. Let it sit for an additional minute.
8. The rehydrated DNase I is ready for use in the RNA isolation protocol.
9. Store the rehydrated DNase I at -20°C in appropriately sized aliquots based on the user's processing needs. Avoid more than 3 freeze-thaw cycles.

Prepare the appropriate volume of DNase reaction mix

Prepare a DNase reaction master mix for the number of extractions using the following table:

	Volume (µl)
Rehydrated Monarch DNase I (20 U/µl)	10
DNase I Reaction Buffer	150
Total DNase reaction mix volume per extraction	**160**

Prepare Wash 1 using Monarch Buffer BX

Wash 1 should be prepared fresh in a user-supplied tube. Prepare sufficient volume for the number of extractions being performed. Use prepared Wash 1 within a day as precipitation may appear after a day. Add in sequence:

Reagent	**Volume per extraction**
Monarch Buffer BX	334 µl
Nuclease-free Water	166 µl
Isopropanol	500 µl
Wash 1 volume per sample	**1000 µl**

Prepare Monarch Buffer WZ

Monarch Buffer WZ is provided as a concentrate. Prior to use, prepare the buffer accordingly using the table below:

	Application	Ratio	NEB #T4080V (10 prep-kit)	NEB #T4080S (50 prep-kit)
Monarch Buffer WZ	Wash 2 Wash 3 Wash 4	Add 4 volumes of ethanol per volume of Buffer WZ	Add 48 ml ethanol to the supplied reagent bottle	Add 200 ml ethanol into the supplied reagent bottle

- Monarch StabiLyse DNA/RNA Buffer is provided at 1x concentration; no additional preparation is needed.
- Monarch Buffer BX is used at 1x concentration at the bead binding steps; no additional preparation is needed.
- Always keep all buffer bottles tightly closed when not in use.
- Store kit components at the recommended storage conditions found on component labels and in the product manual.

Preparation of Monarch Buffers & Reagents Purchased Separately

The reagent and buffer preparation guidance below is specific for components purchased separately for the Monarch Mag Cell-free RNA (cfRNA) Extraction Kit application. Preparation requirements may differ for other kits and applications; refer to the appropriate kit-specific guidance. Standalone buffers and reagents not listed below for this kit application require no additional preparation.

Reagents/Buffers	Application	Ratio	For components purchased separately
Monarch DNase I, Lyophilized, 11000 U (NEB #T2104M)	DNA removal during cfRNA extraction	N/A	Add 550 µl Nuclease-free Water and pipette mix to resuspend. Let it sit for 15 seconds until the solution clears.
Monarch Buffer WZ (NEB #T1115L)	Washes	Add 4 volumes of ethanol per volume of Buffer WZ	Add 104 ml of ethanol.
Monarch Buffer BX (NEB #T2041)	cfRNA binding	N/A	Use undiluted reagent at the binding step; no additional preparation is needed.
	Wash 1	N/A	Wash 1 should be prepared fresh in a user-supplied tube and used within a day. See the guidance above.

Protocol for Manual Extraction of cfRNA Using the Monarch Mag Cell-free RNA (cfRNA) Extraction Kit

The scalability of this product allows for use of different sample input volumes with this kit. **The stepwise protocol below describes the procedure when using 2 ml sample input.** If using a different sample volume as the starting point, refer to the table below to determine reagent volumes accordingly.

Table 1. Reagent volumes required based on extraction input volume. Choose an appropriately sized tube to accommodate the Total Sample Binding Mix.

	For 1 ml sample	For 2 ml sample	For 4 ml sample
Monarch StabiLyse DNA/RNA Buffer	1 ml	2 ml	4 ml
Proteinase K	20 µl	40 µl	80 µl
Monarch Buffer BX	0.5 ml	1 ml	2 ml
Isopropanol	2.5 ml	5 ml	10 ml
Monarch Mag Beads M2*	30 µl	60 µl	120 µl
Total Sample Binding Mix Volume	5.05 ml	10.1 ml	20.2 ml
On-bead DNase reaction	160 µl |	160 µl	160 µl
Wash steps	1 ml	1 ml	1 ml
Elution	100 µl	100 µl	100 µl
Elution after concentration	20 µl	20 µl	20 µl

**equilibrated to room temperature*

Procedure for 2 ml sample input

Perform all steps at room temperature unless otherwise specified.
Note that during elution steps, and in the concentration protocol, vigorous shaking is needed. For example. 1,500 rpm.

Sample processing

1. Process whole blood collected in blood collection tubes according to tube manufacturer's instructions to separate plasma.
2. Transfer 2 ml plasma into a clean, RNase-free tube of sufficient volume to accommodate the total sample binding mix volumes listed above. A 50 ml conical tube is recommended for 2 ml or 4 ml samples.

Sample digestion

3. Add 2 ml **Monarch StabiLyse DNA/RNA Buffer** to the tube containing 2 ml plasma.
4. Add 40 µl **Proteinase K** to the sample tube.
5. Invert the tube 5 times to ensure the contents are mixed well.
6. Incubate the tube on a thermal mixer for 30 minutes at 37°C with shaking at 1,000 rpm.
7. Cool the tube on ice for 5 minutes.

Bead binding

8. Add 1 ml **Monarch Buffer BX** to the digested plasma sample.
9. Add 5 ml **Isopropanol** to the sample.
10. Add 60 µl **well vortexed, Monarch Mag Beads M2** to the sample.
11. Invert the sample tube 5 times to mix.
12. Mix the tube on a mixer/shaker for 10 minutes at room temperature at 1,000 rpm.
13. Place the tube on a compatible magnetic separation rack for 5 minutes.
14. Remove the supernatant and discard.
15. Remove the tube from the magnetic rack.

Wash #1

16. Add 1 ml **Wash 1 (prepared Monarch Buffer BX)** to the sample. Pipette-mix well to ensure beads are resuspended completely.
17. Transfer the bead/Wash1 suspension to a clean 1.5 ml microfuge tube. Do not discard the 50 ml sample tube yet.
18. Place the 1.5 ml microfuge tubes containing the sample on a compatible magnetic separation rack for 2 minutes.

    Optionally, if some residual beads remain in the 50 ml sample tube from Step #16, remove the supernatant from the 1.5 ml tube after 1 minute of its magnetization, add it to the 50 ml tube, rinse to collect any residual beads and dispense the suspension back into the 1.5 ml tube for further 1 minute magnetization.
19. Remove the supernatant from the 1.5 ml sample tube and discard.
20. Remove the tube from the magnetic rack.

On-bead DNase treatment

If not performing on-bead DNase treatment, proceed to Step #34

Wash #2 (Pre-DNase treatment Wash)

21. Add 1 ml prepared **Monarch Buffer WZ** to the tubes.
22. Vortex to uniformly resuspend the beads, then spin briefly on a mini-centrifuge to collect any drops stuck on the sides of the tube.
23. Transfer the tube to a magnetic separation rack for 2 minutes. Remove the supernatant and discard.

DNase reaction

24. Spin the sample tube briefly and place it on the magnetic rack.
25. Remove any residual supernatant using a smaller volume pipette.
26. Remove sample tube from the magnetic separation rack.
27. Add 160 µl **DNase reaction mix** to the sample tube. Pipette-mix to resuspend the beads.
28. Incubate at room temperature for 15 minutes. Vortex intermittently, for example, every 5 minutes.

RNA rebinding

29. Add 320 µl **Monarch StabiLyse DNA/RNA Buffer** to the sample tube.
30. Add 480 µl **Isopropanol** to the sample tube. Do not make a master mix of Monarch StabiLyse DNA/RNA Buffer and Isopropanol.
31. Vortex to uniformly resuspend the beads and mix on a mixer/shaker for 5 minutes at 1000 rpm.
32. Place the tube on the magnetic separation rack for 2 minutes.
33. Remove the supernatant and discard.

Wash #3 and #4

34. Add 1 ml **Monarch Buffer WZ** to the tube. Vortex to resuspend the beads.
35. Place the tube back on the magnetic separation rack for 2 minutes. Remove the supernatant and discard.
36. Repeat Steps 34-36 for an additional wash with **Monarch Buffer WZ**.

Bead drying and elution

37. Spin the tube briefly in a mini-centrifuge and place on the magnetic separation rack.
38. Remove any residual supernatant using a low-volume pipette.
39. Air dry the bead pellet for 5 minutes with the tube on the magnetic rack and tube cap open.
40. Add 100 µl Nuclease-free Water to the bead. Vortex to ensure the beads are resuspended.
41. Mix the tube with vigorous shaking for 5 minutes (for example, 1,500 rpm on a mixer/shaker)
42. Place the tubes on a magnetic rack and let the beads separate for 5 minutes.
43. Carefully transfer the supernatant to a new 1.5 mL centrifuge tube. The supernatant contains DNA-free, purified, cell-free RNA that can be concentrated before use in downstream applications.

Protocol for Concentration of Extracted Cell-Free RNA using Monarch Mag Beads M2

Extracted cfRNA can be concentrated into a smaller volume for enable higher inputs in downstream applications. The magnetic beads and buffers included in the kit are sufficient to concentrate cfRNA using the bead-based protocol provided. If a column-based concentration is desired, the Monarch Spin RNA Cleanup Kit (10 µg) (NEB #T2030) can be used. Other concentration options include a vacuum concentrator such as SpeedVac.

1. To the cfRNA eluate, add 2 volumes of **Monarch Buffer BX**. For example, for 100 µl cfRNA, add 200 µl Monarch Buffer BX.
2. Add an equal volume of Isopropanol. For the above example, add 300 µl Isopropanol.
3. Add 15 µl **Monarch Mag Beads M2**.
4. Mix the tube with vigorous shaking for 5 minutes (for example, 1,500 rpm on a mixer/shaker).
5. Place on the magnetic rack for 3 minutes.
6. Remove the supernatant and discard.
7. Remove the tube from the magnetic rack and add 500 µl prepared **Monarch Buffer WZ.**
8. Vortex the tube for 10 seconds to resuspend the beads.
9. Place the tube on the magnetic rack for 2 minutes or until the supernatant becomes clear.
10. Remove the supernatant and discard.
11. Repeat steps 7-10 for an additional wash with **Monarch Buffer WZ**.
12. Briefly spin the tube on a mini-centrifuge and place it back on the magnetic rack.
13. Remove any residual supernatant using a low-volume pipette.
14. Air dry the beads for 3 minutes with the tube on the magnetic rack and cap open.
15. Remove the tube from the magnet and add 20 µl Nuclease-free Water.
16. Mix the tube with vigorous shaking for 5 minutes (for example, 1,500 rpm on a mixer/shaker).
17. Place the tube on magnet for 5 minutes and transfer the supernatant containing concentrated cfRNA into a new 1.5 ml tube.

For additional protocols, please see the product webpage.

Protocol Guidance for Automated Extraction of cfRNA Using the Monarch Mag Cell-free RNA (cfRNA) Extraction Kit

The Monarch Mag Cell-free RNA (cfRNA) Extraction Kit is compatible with a wide range of open-access automation platforms, including magnetic rod–based systems (e.g., KingFisher® instruments) and liquid handling systems.

Protocols and scripts for automation workflows tested by NEB are available and can be requested via the product webpage at www.neb.com/T4080.

For platforms not specifically tested by NEB, we recommend consulting your automation provider for guidance on adapting the manual protocol to your specific system and application.

Cell-free RNA Quantitation and Downstream Analysis

Due to the low concentration of cell-free RNA circulating in biofluids, UV-vis spectrophotometric methods are not recommended for quantification. Additionally, fluorometric methods such as Qubit™ (Invitrogen®) may not provide accurate quantification since the dye is unsuitable for the conformation/length of cfRNA according to the Qubit manufacturer. Sensitive automated electrophoresis, such as Agilent RNA 6000 Pico Bioanalyzer, is strongly recommended. Quantitative RT-PCR using primers/probes that specifically only target RNA and not DNA also give a more accurate assessment of cfRNA concentrations.

The extracted cell-free RNA is application-ready and can be used in sequencing or amplification-based detection. For sequencing--based analysis, NEBNext library prep solutions are recommended.

Troubleshooting

Problem	Common Cause	Suggestions/Solutions
Lower cfRNA yield than expected	Sample contains low levels of cfRNA	Circulating cfRNA is present in low concentrations in body fluids and current quantification methods are not standardized/reliable. Sample collection processes and biological variability can lead to inherently low loads of cfRNA in the starting sample. Increasing sample input volume can help.
	Incorrect reagent preparation or storage	Ensure all components are stored at the appropriate conditions for optimal performance. Monarch Mag Beads M2 are stored at 4°C but should be allowed to warm to room temperature before use. Check the protocol to confirm that buffers have been properly prepared and that the correct volumes are used. Verify that the appropriate buffer is added at each step.
	Insufficient beads added	Ensure that the Monarch Mag Beads M2 solution is thoroughly mixed before addition to the sample. Because the beads settle quickly, inadequate mixing may result in dispensing fewer beads than required, reducing binding capacity and lowering RNA yield. Use low-retention pipette tips to avoid potential loss of beads on the tip walls.
	Insufficient binding	It is imperative to allow the sample to mix with the bead-binding mix with shaking for the recommended amount of time to create optimal conditions for cell-free RNA to bind to the beads.
	Beads not optimally dried	Beads should be dried for 5 minutes while the tubes remain on the magnetic rack with the tube caps open. Under-dried beads can lead to contaminant carryover and over-dried beads may lead to lower yield. Beads that are over-dried appear matte and light brown, often with visible surface cracks. Optimally dried beads appear shiny and uniformly dark.
	Incomplete elution	Thorough mixing of the beads with the recommended volume of Nuclease-free Water is essential to elute RNA from the beads. Vortex well to ensure that all beads are resuspended, followed by 5 minutes on a mixer/shaker.
	Beads lost during prep	Use a strong magnet to ensure effective bead separation, and carefully remove the supernatant without disturbing the bead pellet, as any beads removed with the supernatant may result in RNA loss.
Magnetic bead carryover	Beads not magnetized sufficiently	During magnetic separation steps, ensure that the supernatant is clear and free of beads before removing it. Leave the tubes on the magnet for a minimum of 2 minutes at all magnetic separation steps.
	Improper pipette handling	When transferring the final elution to a new tube, be careful not to disturb the bead pellet.

For more troubleshooting and FAQs, please visit the product webpage or reach out to our technical support team at info@neb.com

Ordering Information

Monarch® Mag Cell-free RNA (cfRNA) Extraction Kit

PRODUCT	NEB#
Monarch® Mag Cell-free RNA (cfRNA) Extraction Kit	T4080
Kit components available individually	
Monarch® Mag Beads M2	T4105
Monarch StabiLyse™ DNA/RNA Buffer	T2111
Monarch® Buffer BX	T2041
Monarch® DNase I, Lyophilized	T2104
Monarch® Buffer WZ	T1115

NEB Companion Products

PRODUCT	NEB#
NEBNext UltraExpress® RNA Library Prep Kit	E3330
NEBNext® Low-bias Small RNA Library Prep Kit	E3420
NEB 6 x 50 ml Magnetic Separation Rack	T5010

Revision History

REVISION #	DESCRIPTION	DATE

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