INSTRUCTION MANUAL

Monarch® Spin High-Capacity RNA Cleanup Kit (3 mg)

NEB #T2060S/L

10/50 preps

Version 1.0 05/26

Table of Contents

Kit Contents and Storage

Component	NEB #	Application/Usage	T2060S 10 preps	T2060L 50 preps	Storage Temperature
Monarch Spin Columns S3B	T2067	RNA Binding Matrix	10	50	15-25°C
Monarch Spin Collection Tubes	T2118	Collection of eluted waste material	10	50	15-25°C
Monarch Buffer BX	T2041	RNA Cleanup Binding Buffer	6 ml	40 ml	15-25°C
Monarch Buffer WX (5X concentrate)	T2042	RNA Cleanup Wash Buffer	2.5 ml x 2	20 ml	15-25°C
Nuclease-free water	B1500	Elution	14 ml	122 ml	15-25°C

Storage Recommendation

- All kit components should be stored at room temperature.
- Always keep reagent bottles tightly closed.
- Keep columns sealed in the enclosed bag.
- See individual component labels for specific storage guidance.

Intended Use

The Monarch Spin High-Capacity RNA Cleanup Kit (3 mg) 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 Buffer BX contains guanidine thiocyanate, 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/T2060.
- 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 Spin High-Capacity RNA Cleanup Kit (3 mg) is a rapid and reliable method for the cleanup of up to 3 mg of high-quality RNA in a spin column format, compatible with centrifugation. Removal of proteins, buffer salts and nucleotides from enzymatic reactions such as *in vitro* transcription, DNase I treatment and RNA capping is easily accomplished with minimal effort and time. This kit contains unique columns, designed to prevent buffer retention and ensure no carryover of contaminants, enabling elution of highly pure and concentrated RNA (elute in as little as 250 µl).

Features of this kit include:

- **High Performance:** Recover up to 3 mg of highly pure RNA. Effectively remove enzymes, detergents, nucleotides, and other low molecular weight reaction components.
- **Reliability:** Consistent results for yield and purity.
- **Broad Sample Compatibility:** Purify a wide range of sizes of RNA synthesized by *in vitro* transcription (IVT), RNA synthesized with modified nucleotides, RNA samples following enzymatic reactions such as DNase I treatment and capping, and RNA extracted by other methods.
- **Application Ready:** Purified RNA is suitable for downstream applications, including RT-PCR, RNA library prep for NGS, transfection, and the formation of ribonucleoprotein (RNP) complexes for genome editing studies.

Sustainability and Recycling Information

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

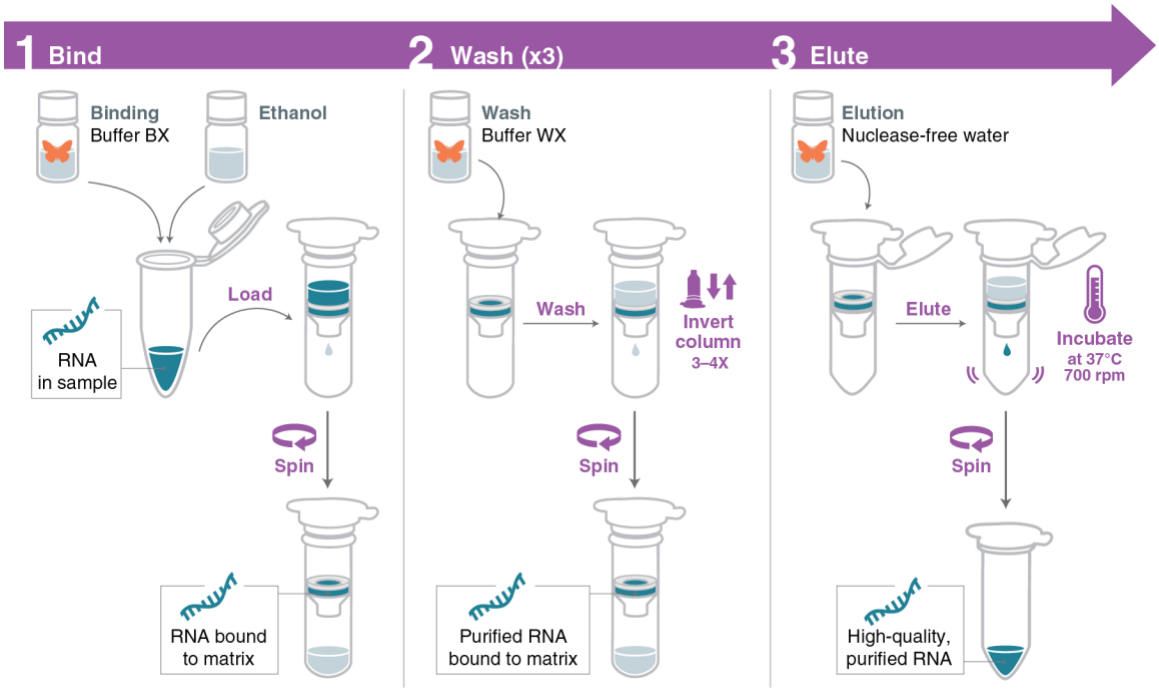
- **Sustainable performance**: Monarch kits use significantly less plastic in spin columns, buffer bottles, and other plastic components than leading alternatives.
- **Flexible purchasing options:** Monarch kit components such as spin columns, magnetic beads and buffers are available individually to 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

Monarch Spin High-Capacity RNA Cleanup Kit (3 mg) utilizes the same buffers (including a single wash buffer for convenience) and similar bind/wash/elute protocol to the other Monarch Spin RNA Cleanup Kits for 10 µg (NEB #T2030), 50 µg (NEB #T2040) and 500 µg (NEB #T2050) capacities. Spin and incubation times allow for purification in 30 minutes with a capacity of up to 3 mg RNA. Monarch Buffer BX and ethanol (not supplied) are used to dilute the samples and ensure binding compatibility onto the proprietary silica matrix. Next, the Monarch Buffer WX ensures enzymes, detergents and other low molecular weight reaction components (e.g., unincorporated nucleotides) are removed. Finally, RNA is eluted with Nuclease-free water. Eluted RNA is highly pure and ready for use in a variety of downstream applications, including RT-PCR, RNA library prep for NGS, transfection, the formation of ribonucleoprotein (RNP) complexes for genome editing studies, and more.

Figure 1: High-Capacity RNA Cleanup Workflow



The Monarch Spin High-Capacity RNA Cleanup Kit (3 mg) uses the bind/wash/elute method and a unique spin column compatible with microcentrifuges.

Properties

Purification Format	Spin Column
RNA Sample Type Compatibility	RNA from various sources including *in vitro* transcription (IVT) samples, IVT samples synthesized with modified nucleotides and RNA extracted by other methods
Typical Recovery	70-100%
RNA Purity	$A_{260/280} > 2.0$ and $A_{260/230} > 1.8$
Binding Capacity	3 mg
RNA Size Range	> 25 nt
Elution Volume	250-1000 µl
Compatible Downstream Applications	RT-PCR, RNA library prep for NGS, RNA labeling, RNAi, microinjection, transfection
Equipment Compatibility	Centrifuge

Applications & Usage

Listed are selected examples of applications and usage. To see an updated list or related protocol guidance, refer to the product webpage.

RNA Cleanup	RNA purified by other methods can be further purified
Enzymatic Reaction Cleanup	Enzymes such as RNA polymerases, DNase I, Proteinase K and phosphatases are removed allowing efficient desalting
***In vitro* Transcription Cleanup**	Enzymes and excess unincorporated dNTPs are removed to yield highly pure synthesized RNA

Important notes before you begin

General Guidelines for Working with RNA

Successful RNA cleanup requires careful sample handling and ensuring that all buffers and labware contacting the RNA are free of contaminating nucleases:

1. RNases are stable and difficult to inactivate. Care must be taken when handling samples during and after purification of RNA.
    a. Plasticware and glassware in direct contact with RNA-containing samples should be RNase-free.
    b. Gloves should be worn at all times when handling samples and kit components.
    c. Frequent glove changes are encouraged.
    d. Bench and equipment surfaces should be clean and can be decontaminated prior to work using commercially available cleaners such as RNaseZap®.
2. Elution with nuclease-free water is standard, but for samples that will be stored for later use, EDTA can be added to 0.1–1.0 mM to limit degradation by magnesium-requiring nucleases. Alternatively, elution with slightly alkaline TE (not supplied) can be used.
3. Avoid unnecessary freeze-thaw cycles of purified RNA. Aliquots should be made, consistent with downstream needs.

General Guidelines for Monarch Spin High-Capacity RNA Cleanup Kit (3 mg)

- This protocol provides an example case with a starting RNA input volume of 300 ul, corresponding to 3 mg of RNA synthesized from an *in vitro* transcription (IVT) reaction (for example, using the HiScribe® T7 High Yield RNA Synthesis Kit (NEB #E2040)). For other sample input concentrations or volumes, scale protocol accordingly, up to a total capacity of 3 mg per column.
- It is recommended to keep the starting RNA input volume < 800 µl (for a maximum of 3 mg) to limit the total number of column loading steps.
- There are two centrifugation speeds in this protocol: 10,000 x *g* for sample loading and 16,000 x *g* for wash and elution, carried out at room temperature.
- This protocol is not compatible with the use of a vacuum manifold. All steps should be completed using centrifugation.
- When cleaning up large amounts of RNA (> 500 µg), precipitation will occur following the addition of the Monarch Buffer BX and ethanol to the sample (steps 1 and 2). Load the entire sample including all the precipitate onto the column.
- Avoid overloading the column when working with high RNA inputs. For RNA inputs >3 mg, divide the sample into aliquots containing a maximum of 3 mg RNA before adding Monarch Buffer BX and ethanol. If >3 mg RNA is processed as a single sample, addition of the Monarch Buffer BX and ethanol results in the formation of a large precipitate., making it difficult to divide the sample evenly across columns and increasing the risk of column overload and RNA loss.
- A pellet containing the RNA of interest may form on the side of the column following the first binding spin (step 3).
- If the RNA input is > 3 mg (maximum capacity per column), and reloading the column > 2 times, a pellet containing the RNA of interest may form in the collection tube. The pellet in the collection tube can be resuspended with nuclease-free water and processed using the RNA cleanup protocol.
- The column has a maximum capacity of 800 µl. If the sample volume (after addition of Monarch Buffer BX and ethanol) exceeds 800 µl, load the sample in 600 µl increments to help ensure high purity in the eluate. Before proceeding to step 5, ensure that all sample has passed through the column during step 4. An additional spin may be used to remove any residual liquid.
- For RNA size > 5 kb, it is recommended to use 500 µl nuclease-free water for the first elution. To maximize recovery, a second elution is necessary for RNA ≥ 5 kb.
- Reloading the column multiple times (> 5) during sample loading steps can make elution more challenging; additional elutions will ensure successful recovery.
- After the first elution, a gel-like pellet containing RNA of interest may remain on the side of the column and membrane layer. Additional elution steps will maximize recovery.

Equipment and Reagents Required & Supplied by the User

Equipment

- Benchtop microcentrifuge
- Thermal mixer

Reagents/supplies

- Ethanol (≥ 95%)
- RNase-free microfuge tubes or appropriate tubes for sample preparation before loading on to column
- 2 ml RNase-free microfuge tubes for elution

Buffer Preparation

- Monarch Buffer WX is provided as a concentrate. Please prepare the buffer using the table below:

Reagents/Buffers	T2060S (10 prep)	T2060L (50 prep)
Monarch Buffer WX Add 4 volumes of ethanol (≥ 95%) to one volume of Buffer WX	Add 10 ml of ethanol to each bottle of Buffer WX	Add 80 ml of ethanol to bottle of Buffer WX

- Monarch Buffer BX is provided at 1X concentration, no additional preparation is needed.

- If a precipitate has formed in the Monarch Buffer BX, warm to room temperature to re-dissolve before use.
- Always keep all buffer bottles tightly closed when not in use.

Protocol

The standard protocol outlined below provides an example case for a starting sample volume of 300 µl with an estimated RNA concentration of 10 µg/µl. For samples with higher or lower RNA concentration, adjust the starting volume accordingly, up to a total input of 3 mg per column. It is recommended to keep the starting RNA input volume < 800 µl (for a maximum of 3 mg) to limit the total number of column loading steps.

When cleaning up large amounts of RNA (> 500 µg), precipitation will occur after adding Monarch Buffer BX and ethanol to the sample (steps 1 and 2). Load the entire sample including all the precipitate onto the column. If starting with a large RNA volume, it is recommended to split the RNA input into 3 mg (maximum) aliquots prior to adding Monarch Buffer BX and ethanol to avoid overloading the columns.

Monarch Buffer WX is provided as a concentrate. Follow buffer preparation guidelines before use.

There are two centrifugation speeds in this protocol: 10,000 x *g* for sample loading and 16,000 x *g* for wash and elution. All centrifugation steps must be carried out at room temperature.

Bind

1. **Add 2 volumes of Monarch Buffer BX to one volume of RNA input (e.g., 600 µl of Monarch Buffer BX to 300 µl of RNA input).** Scale buffer volume according to starting RNA input volume (3 mg maximum capacity per column).
2. **Add one volume (e.g., 900 µl) of ethanol (≥ 95%) to your sample and mix well by pipetting.** Do not vortex. Scale ethanol volume to final volume in step 1.
3. **Insert column into collection tube, load 600 µl of sample onto column and close the cap. Spin at 10,000 x *g* for 1 minute, then discard the flow-through.** If total volume is < 800 µl, entire sample can be loaded on to column and skip step 4.
4. **Re-insert column into collection tube, and repeat step 3 until all of the sample has been loaded.** To save time, spin for 30 seconds, instead of 1 minute.

Wash

Note: Ensure that all of the sample has passed through the column before proceeding to step 5. An additional spin will clear any remaining liquid from the column. Discard the flow-through.

5. **Re-insert column into collection tube. Add 500 µl of Monarch Buffer WX to the column, spin at 16,000 x *g* for 1 minute, then discard the flow-through**. Recommended: After adding Monarch Buffer WX, remove column from collection tube and gently invert column 3 – 4 times, avoid contact with column tip. Re-insert into collection tube and spin.
6. **Repeat step 5 twice for a total of three washes.**

Elute

7. **Transfer column to an RNase-free 2 ml microfuge tube (not provided)**. Use care to ensure that the tip of the column does not come into contact with the flow-through from the last wash step. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over.
8. **Elute RNA by adding 250-500 µl nuclease-free water to the column and incubating in a thermal mixer at 37 °C and 700 rpm for 10 minutes. Spin at 16,000 x *g* for 1 minute.** For RNA size > 5 kb it is recommended to use 500 µl nuclease-free water.
9. **For maximum recovery, additional elution steps are recommended*.** Transfer column to a fresh RNase-free 2 ml microfuge tube. Add 100–500 µl nuclease-free water to the column and incubate in a thermal mixer at 37 °C and 700 rpm for 5-10 minutes. Spin at 16,000 x *g* for 1 minute.

Elution guidance to maximize recovery:

- *Re-loading the column multiple times (> 5) during sample loading steps can make elution more challenging; additional elutions will ensure successful recovery.*
- *For RNA sizes < 5 kb at 3 mg RNA input, a second elution with 100-500 µl nuclease-free water is recommended.*
- *For RNA sizes ≥ 5 kb at 3 mg RNA input, a second elution is necessary.*
- *For RNA sizes ≥ 9 kb at 3 mg RNA input, increasing elution volume to 500 µl and a third elution can maximize recovery*.

10. **Eluted RNA can be used immediately or stored at -70 °C.**

RNA Quantification

- Quantitation of RNA can be performed using direct spectrophotometric measurement (Nanodrop®, Lunatic®), RNA-specific dye-assisted fluorometric measurements (Qubit®, RiboGreen®), or by RT-qPCR. Each method has advantages and disadvantages relating to accuracy, time requirements, equipment requirements and expense.

- Direct spectrophotometric analysis of samples with a micro-volume spectrophotometer (Nanodrop) is easy, rapid, and appropriate for routine measurements where absolute concentrations are not required. These devices do not perform well on dilute samples (below 20 ng/µl). Additionally, the contribution of other macromolecules to the absorbance spectra is not always appreciated with these devices. Use of a spectrophotometer with content profiling (Lunatic) can be helpful.

- Fluorescent dyes that specifically bind to RNA can provide a more accurate way to determine concentration but require additional effort because of the need to generate standard curves with samples of known concentration. Many kits exist for this approach, and the overall workflows have been optimized for efficiency, providing a reasonable balance between accuracy and effort/cost. NEB routinely utilizes these methods during RNA kit development and sample manipulations.

- RT-qPCR remains the gold standard for absolute quantitation of RNA and provides unrivaled limits of detection. Care must be taken to design appropriate primer sets to detect RNA only and appropriate controls must be utilized to ensure amplification is RNA specific and not from residual host DNA. We recommend NEB's Luna® RT-qPCR products.

- Modified nucleotides have a shifted absorbance profile. The type and amount of nucleotides replaced by modified nucleotides influence quantification of RNA by direct spectrophotometric analysis. For quantification calculations refer to manufacturer or literature recommendations for the specific nucleotide in question.

RNA Purity & Integrity

- The purity of eluted RNA samples can be quickly assessed by reviewing OD ratios collected during routine spectrophotometry. Pure RNA typically has an $A_{260/280}$ of 1.9–2.1, and an $A_{260/230}$ of 2.0–2.2. Many factors can influence these values such as the use of a proper reference blank solution, the incorporation of modified nucleotides, the buffer pH, and contaminants such as protein, buffer salts, ethanol, etc.

Troubleshooting

Problem	Common Cause	Suggestions/Solutions
No RNA purified	Ethanol was not added to Monarch Buffer WX	Check protocol to ensure correct buffer reconstitution.
RNA in flow-through and collection tube after sample loading	Overloaded column	Dispense RNA input into 3 mg (maximum) aliquots prior to adding Monarch Buffer BX and ethanol. Any pellet in collection tube can be recovered by resuspending with nuclease-free water and processed using RNA cleanup.
Low RNA yield	Reagents added incorrectly	Check protocol to ensure correct buffer reconstitution, order of addition of buffers and ethanol, and proper handling of column flow-through and eluates.
	Precipitate in binding step was not loaded on the column	Ensure that entire sample along with all precipitate formed in the binding step after adding Monarch Buffer BX and ethanol is loaded onto the column.
	Insufficient mixing of reagents	Ensure the ethanol is thoroughly mixed with RNA sample and Monarch Buffer BX before applying the sample to the RNA Cleanup Column.
	Incomplete elution	Ensure appropriate volume of nuclease-free water is used for elution. For RNA ≥ 9 kb, larger elution volumes combined with heated elution will improve yield. Larger elution volumes, multiple elutions, and longer incubation times can increase yield of RNA, but will dilute the sample and may increase processing times. For typical RNA samples, the recommended elution volumes and incubation times should be sufficient.
	High degree of RNA secondary structure	Binding and elution of smaller RNAs (< 45 nt) can be affected by secondary structure of the RNA molecules. If poor yield of a small RNA is observed, we recommend diluting your sample with 2 volumes of ethanol instead of one volume in step 2 of the protocol.
Purified RNA is Degraded	RNase contamination	In order to avoid RNase contamination during RNA cleanup, make sure to work on a clean lab bench, wear gloves and use disposable RNase-free pipet tips and microfuge tubes (not provided). Keep all kit components tightly sealed when not in use.
	Improper storage of RNA	Purified RNA should be used immediately in downstream applications or stored at -70°C.
Low $A_{260/230}$ Ratios	Residual guanidine salt carry-over	Ensure wash steps are carried out prior to eluting sample. During wash steps, the column can be gently inverted 3–4 times after adding wash buffer. Use care to ensure the tip of the column does not contact the flow-through. If unsure, repeat centrifugation. When reusing collection tubes, blot the rim of the tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer.
	A 2 ml tube was not used for elution	Ensure that a 2 ml microfuge tube is used for the RNA elution step, to avoid any contact between eluate and the column. Use care to ensure the tip of the column does not contact the flow-through.
Low RNA performance in downstream steps	Salt and/or ethanol carry-over	Ethanol and residual salt may inhibit downstream applications. Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-centrifuge for 1 minute to ensure traces of salt and ethanol are not carried over in the eluted RNA.
	DNA contamination	DNA removal may be necessary for certain applications. Incubate RNA sample with DNase I (NEB #M0303), DNase I-XT (NEB #M0570), or Monarch DNase I, Lyophilized (NEB #T2104) and cleanup RNA using the RNA Cleanup Protocol.

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 Spin High-Capacity RNA Cleanup Kit (3 mg)

PRODUCT	NEB#
Monarch Spin High-Capacity RNA Cleanup Kit (3 mg)	T2060
Kit components available individually	
Monarch Spin Columns S3B and Tubes	T2067
Monarch Buffer BX	T2041
Monarch Buffer WX (5X concentrate)	T2042
Nuclease-free water	B1500

NEB Companion Products

PRODUCT	NEB#
DNase I (RNase-free)	M0303
DNase I-XT (RNase-free)	M0570
Monarch DNase I, Lyophilized	T2104
Monarch Spin High-Capacity DNA Cleanup Kit	T1135
HiScribe® T7 High Yield RNA Synthesis Kit	E2040
HiScribe T7 Quick High Yield RNA Synthesis Kit	E2050
HiScribe® T7 mRNA Kit with CleanCap® Reagent AG	E2080
NEBNext UltraExpress® RNA Library Prep Kit	E3330
NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina®	E7760
Luna® Universal One-Step RT-qPCR Kit	E3005
Luna® Universal Probe One-Step RT-qPCR Kit	E3006
LunaScript® RT SuperMix Kit	E3010
Proteinase K, Molecular Biology Grade	P8107
N1-Methyl-Pseudouridine-5′-Triphosphate (N1-Methyl-Pseudo-UTP)	N0431
Faustovirus Capping Enzyme	M2081
mRNA Cap 2′-O-Methyltransferase	M0366

NEB Related Products

PRODUCT	NEB#
Monarch Spin RNA Cleanup Kit (10 µg)	T2030
Monarch Spin RNA Cleanup Kit (50 µg)	T2040
Monarch Spin RNA Cleanup Kit (500 µg)	T2050
Monarch Spin RNA Isolation Kit (Mini)	T2110
Monarch Spin Plasmid Miniprep Kit	T1110
Monarch Spin PCR & DNA Cleanup Kit	T1130

Revision History

REVISION #	DESCRIPTION	DATE