INSTRUCTION MANUAL

Monarch® Spin RNA Isolation Kit (Mini)

NEB #T2110G/V/S/L

10/50/200 preps

Version 2.0 11/25

Table of Contents

Kit Contents

Component	NEB #	Application/usage	#T2110G/V (10 preps)	#T2110S (50 preps)	#T2110L (200 preps)	Storage temperature
Monarch StabiLyse DNA/RNA Buffer	T2111	Sample lysis and stabilization	12.5 ml	63 ml	210 ml	15–25°C
Monarch Buffer BX	T2041	RNA priming reagent concentrate (3X)	2 ml	10 ml	40 ml	15–25°C
Monarch Buffer WZ	T1115	RNA wash buffer concentrate (5X)	5 ml	26 ml	100 ml	15–25°C
Nuclease-free Water	B1500	RNA elution, BX reconstitution and rehydration of Monarch DNase I, Lyophilized	2.5 ml	25 ml	122 ml	15–25°C
Monarch DNase I Reaction Buffer	T2105	Reaction Buffer for DNase I	0.9 ml	4.6 ml	14 ml	15–25°C. After first use, store at 4°C
Monarch DNase I, Lyophilized	T2104	Removal of residual genomic DNA	880 U	2200 U	8800 U	15–25°C. After rehydration, store in aliquots at -20°C
Proteinase K, Molecular Biology Grade	P8200	Protein digestion	0.11 ml	1 ml	3 ml	-20°C after opening
Monarch Spin Columns S2C	T3017	Spin column for gDNA removal	10 columns	50 columns	200 columns	15–25°C
Monarch Spin Columns S2A	T2047	Spin column for RNA capture	10 columns	50 columns	200 columns	15–25°C
Monarch Spin Collection Tubes	T2118	Collection tube	20 tubes	100 tubes	400 tubes	15–25°C

Storage Recommendations

- **After opening,** store Proteinase K at -20°C.
- **After first use,** store Monarch DNase I Reaction Buffer at 4°C to help minimize risk of contamination.
- **After rehydration**, aliquot and store Monarch DNase I at -20°C. Avoid more than 3 freeze-thaws.
- Remaining 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 RNA Isolation Kit (Mini) 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 solution directly to the buffers or the sample preparation waste.
- For more information regarding the composition of buffers, please consult the Safety Data Sheets available on our website (www.neb.com).
- Proper laboratory safety practices should be employed when 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 RNA Isolation Kit (Mini) is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from a wide variety of biological samples, including cultured cells, mammalian tissues, microbes, plants, insects and blood. Cleanup of enzymatic reactions and purification of RNA from TRIzol®-extracted samples is also possible using this kit. Purified RNA has high-quality metrics with $A_{260/280}$ and $A_{260/230}$ ratios typically > 2.0, high RNA integrity scores, and minimal residual gDNA. Captured RNA ranges in size from full-length rRNAs down to intact miRNAs. Purified RNA is suitable for downstream applications such as RT-qPCR, cDNA synthesis, RNA-seq, and RNA hybridization-based technologies. Designed with sustainability in mind, Monarch kits use significantly less plastic than other kits on the market.

Features of this kit include:

- **Versatility:** A single kit enables RNA isolation from a wide range of sample types including cells, fibrous and lipid-rich tissues, bacteria, plants, insects and more.
- **High Performance:** Achieve high yield, purity and integrity of total RNA with minimal residual gDNA.
- **High Concentration:** Elute in as low as 10 µl, allowing for highly concentrated RNA suitable for low input applications.
- **Unique Design:** Unique column design enables elution in low volumes and minimizes buffer retention and contaminant carryover.
- **Application Compatibility:** RT-qPCR, RNA-Seq, Small RNA libraries, hybridization-based workflows.

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:** Significantly less plastic is used in spin columns, bottles and other plastic parts, than similar kits from leading suppliers, with no compromise on high yield, purity, and performance.
- **Thinner-walled columns:** Reduction in total plastic without affecting performance.
- **Flexible purchasing options:** Columns and buffers are also available separately. Purchase only what you need and avoid wasted materials.
- **Same performance, design, and formulations:** Standalone products are the same components and formulations as those included in complete kits.
- **Streamlined packaging:** Monarch kits come in sustainable, sturdy, reusable boxes at just the right size with concise protocol cards that replace printed manuals.
- **Sustainable and recyclable packaging**: Packaging is printed with less ink using eco-friendly practices and powered by sustainable sources such as wind, where possible. Packaging is sourced for recyclability, and recycled paper is used where possible to make the kit boxes, inserts, and paper materials.

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

Background of Monarch Spin RNA Isolation Kit (Mini)

The Monarch Spin RNA Isolation Kit (Mini) combines an optimized buffer chemistry with improved silica membrane technology to produce high-quality RNA suitable for downstream applications. The included Monarch StabiLyse DNA/RNA Buffer provides a dual function of sample stabilization and lysis, creating optimal conditions to lyse cells and preserve the integrity of released RNA during sample homogenization. Additionally, our protocol is compatible with samples preserved in other RNA stabilization reagents such as RNALater® and DNA/RNA Shield. After sample homogenization and lysis, the lysate flows through the Monarch Spin Column S2C containing our proprietary silica matrix that effectively binds and removes genomic DNA while the RNA is collected in the flowthrough. The addition of ethanol to the flowthrough creates optimal conditions for RNA to bind to the Monarch Spin Column S2A. An on-column DNase step can be performed using the supplied Monarch DNase I, Lyophilized, to further remove residual DNA. The application of Monarch Buffer BX helps ensure DNase removal and achieves optimal priming of the silica matrix to keep RNA bound in the subsequent wash steps. The wash steps with Monarch Buffer WZ are critical to ensure removal of contaminants, salts and residual proteins. Finally, elution with nuclease-free water results in concentrated, purified RNA suitable for various molecular applications.

Figure 1: Monarch Spin RNA Isolation Kit Workflow

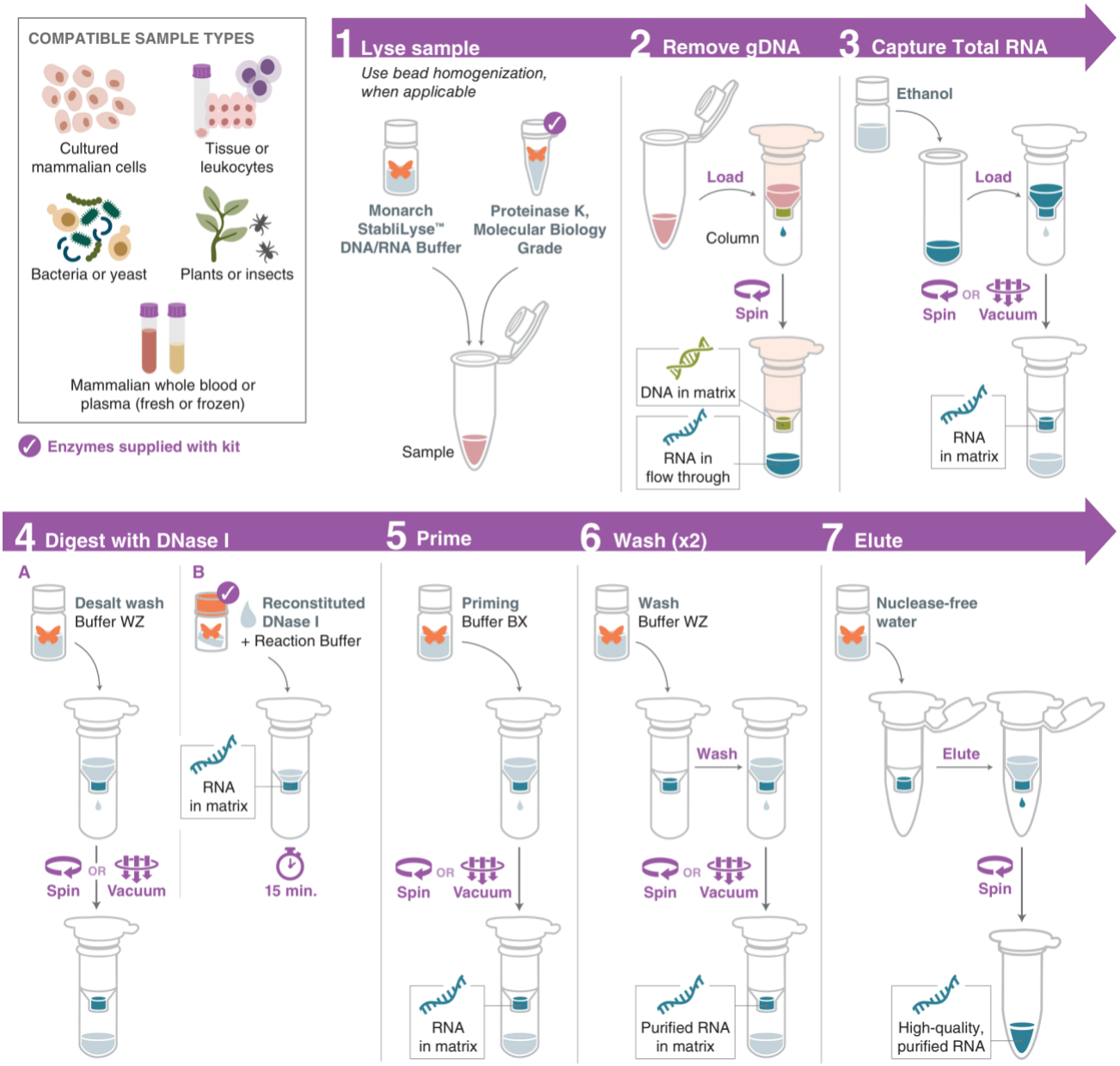
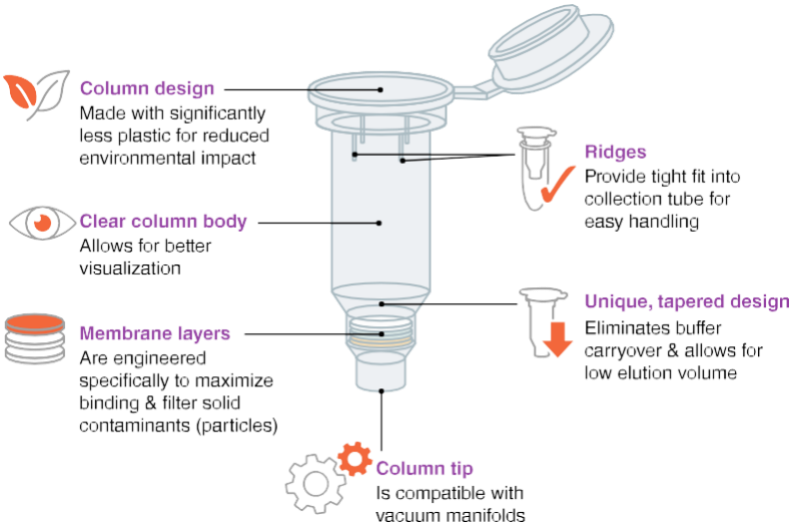


Figure 2. Monarch Column Design



NEB Monarch's unique column design and membrane assembly allow efficient genomic DNA removal and high-quality RNA purification with low elution volume without any loss of yield. The column is designed and made with significantly less plastic for a reduced environmental impact.

Properties

To view functional performance data, please visit the product webpage.

Purification Format	Spin column for nucleic acid purification
Compatible Methods	Centrifugation or vacuum manifold
Intended Usage	Total RNA isolation
Compatible Sample Types	Cells, bacteria, yeast, plants, insects, blood, plasma, mammalian tissues including soft, fibrous and lipid-rich
Yield	Varies depending on sample type; see webpage for details
Column Binding Capacity	Up to 100 µg total RNA
RNA Size	≥ 20 nt, total RNA including small RNA, miRNA, mRNA, lncRNA, rRNA
Elution Volume	≥ 10 µl
RNA Purity	Typically $A_{260/280} \geq 2.0$ and $A_{260/230} \geq 1.8$
Compatible Downstream Applications	cDNA synthesis, RT-qPCR, RNA-Seq, small RNA library prep, Northern blotting, hybridization-based workflows

Important Notes Before Starting

The yield and quality of RNA are highly influenced by several critical factors, including sample collection and storage techniques, growth stage, and input amounts. It is crucial to consider these factors and use best practices to maximize RNA yield and quality.

Choosing Input Amounts

Using the right amount of starting material ensures sufficient RNA yield without exceeding the binding capacity of the column. To ensure the column is not overloaded, do not exceed the maximum input recommended. Large sample input amounts reduce lysis efficiency, introduce excessive amounts of cellular components other than RNA (DNA, polysaccharides, proteins, lipids, etc), and compromise RNA binding to the silica-based capture column. For recommendations on appropriate input amounts, please see Choosing Sample Input Types for RNA Purification and Average Yields Expected.

Working with RNA

Successful RNA extraction and purification are greatly influenced by the type and physiological condition of the sample, as well as the user's ability to maintain RNA integrity during preservation, lysis, and purification. In general, samples should be flash frozen or rapidly processed after harvest to ensure RNA remains intact and accurately reflects the gene expression profile at the time of harvest. Sample lysis and homogenization should include RNase inactivation measures, and lysis methods should ensure complete cell disruption to enable maximal RNA recovery. RNA capture should be efficient for large and small RNAs and should include vigorous wash conditions to remove contaminants.

To maximize RNA yield, integrity and purity, please keep the following principles in mind:

1. RNases are highly stable and difficult to inactivate, and 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®.
2. Quickly processing fresh samples can avoid concerns associated with sample storage.
3. Samples to be stored should be flash frozen and stored at -80°C. Storage at -20°C can be used for short periods of time (1 week), but RNA integrity will be better maintained at lower temperatures. We recommend storage in an RNA preservation reagent such as Monarch StabiLyse DNA/RNA Buffer, or other similar reagents.
4. Mechanical disruption and homogenization of tissue samples using a bead mill, homogenizer or a similar device efficiently releases RNA and maximizes recovery. Bead size and homogenization times choices are critical in obtaining complete lysis without reducing RNA integrity and should be chosen with care depending on the user's sample type.
5. After lysis and homogenization are completed, perform all steps at room temperature.
6. Elution with nuclease-free water is standard and sufficient. 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.
7. Avoid unnecessary freeze-thaw cycles of purified RNA. Aliquots should be made consistent with downstream needs.

Considerations for Sample Lysis and Homogenization

Sample lysis and homogenization are critical parts of every RNA extraction. Maximal recovery will only be achieved by releasing all available cellular RNA. Lysis of cell walls and plasma membranes, as well as reduction of the viscosity of the lysate, ensures quantitative binding to the capture matrix. Each sample type has different requirements for lysis, so the method used should consider this. **Incomplete lysis and homogenization will reduce the yield of recovered RNA.**

Cultured mammalian cells: Cells grown in suspension, in a monolayer, or as adherent cells should be pelleted before using this kit to ensure the cell culture medium is removed. Alternatively, adherent cells can be lysed directly in plate wells. The plasma membrane of these samples is easily lysed by the detergents in the Monarch StabiLyse DNA/RNA Buffer and no additional disruption is necessary.

Solid tissues, cells from blood (PBMCs, buffy coat): Solid tissues and white blood cells (leukocytes) can be effectively lysed after resuspension in Monarch StabiLyse DNA/RNA Buffer and incubated with Proteinase K. Using a bead homogenizer is strongly recommended as it ensures maximal tissue disruption that leads to maximal RNA release.

Microorganisms, plants, insects: Microbial cultures should be pelleted and all culture medium removed before sample disruption and homogenization. These samples benefit from mechanical disruption and homogenization to effectively release RNA due to carbohydrate-containing cell walls. Alternatively, samples may be lysed enzymatically by the addition of lytic enzymes.

Whole blood or plasma: Mammalian whole blood and plasma contain little RNA and abundance protein (hemoglobin). RNA in blood samples is prone to degradation. Collecting blood samples in appropriate collection tubes with preservation reagents is highly recommended. Effective lysis can be achieved with Monarch StabiLyse DNA/RNA Buffer. Proteinase K treatment is necessary to lower hemoglobin levels before loading the column.

General Guidelines for Monarch Spin RNA Isolation Kit (Mini)

- Centrifugation should be carried out at 16,000 x g (~13,000 RPM) in a standard laboratory microcentrifuge at room temperature with proper balancing.
- Do not exceed the maximum recommended input for a sample as this may cause column overloading and reduce yield.
- Do not load the columns with more than 700 µl per spin.
- Optional steps are not necessary but recommended.
- General recommendations for bead homogenization and enzymatic lysis are outlined for different samples. Customers are encouraged to make lysis choices based on their specific conditions and sample types.
- If using a vacuum manifold, read and follow the manufacturer's instructions before starting.
- Always keep columns tightly sealed in the provided bag.
- Always keep all buffer bottles tightly closed when not in use.
- Addition of Monarch StabiLyse DNA/RNA Buffer and all subsequent steps should be performed at room temperature. If samples are accidentally placed on ice and precipitate forms, allow the samples to return to room temperature to resolubilize before loading onto the column.
- If a precipitate has formed in Monarch Buffer BX, warm the buffer to room temperature to re-dissolve before use.

Equipment and Reagents Supplied by the User

- Benchtop microcentrifuge
- Water bath, heat block or thermomixer
- Bead homogenizer
- Vacuum manifold and adaptors (for the vacuum manifold protocol)
- Vacuum pump (for the vacuum manifold protocol)

Reagents/supplies

- RNase-free 1.5 ml microfuge tubes
- Ethanol (≥ 95%), Molecular Biology Grade
- Isopropanol (≥ 99%) for whole blood samples
- Glycanases for microbes, for example, NEBExpress® T4 Lysozyme (NEB #P8115)
- Xylene or similar for deparaffinization of FFPE tissue.

Reagent Preparation

Rehydration of Monarch DNase I, Lyophilized

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 appropriate volume of the supplied Nuclease-free Water onto the lyophilized cake.
    a. For T2110G/V (10-prep kit), dispense 220 µl Nuclease-free Water.
    b. For T2110S (50-prep kit), dispense 550 µl Nuclease-free Water.
    c. For T2110L (200-prep kit), dispense 550 µl Nuclease-free Water in each vial of lyophilized DNase that you plan to use.

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 stay 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. *Avoid more than 3 freeze-thaw cycles. We strongly recommend storing the rehydrated DNase in appropriately sized aliquots based on the user's processing needs.*

Preparation of Monarch Buffer BX

Monarch Buffer BX serves as the RNA priming reagent and is provided as a concentrate. Before using in the workflow, follow the steps below for reconstitution using nuclease-free water and ethanol in the sequence listed:

1. Add the appropriate volume of the supplied Nuclease-free Water to the reagent bottle.
    a. For T2110G/V (10-prep kit), add 2 ml Nuclease-free Water.
    b. For T2110S (50-prep kit), add 10 ml Nuclease-free Water.
    c. For T2110L (200-prep kit), add 40 ml Nuclease-free Water.
2. Add the appropriate volume of ≥ 95% ethanol (user-supplied.)
    a. For T2110G/V (10-prep kit), add 2 ml ethanol.
    b. For T2110S (50-prep kit), add 10 ml ethanol.
    c. For T2110L (200-prep kit), add 40 ml ethanol.

* If using standalone Monarch Buffer BX (NEB #T2041L) in place of the bottle supplied with the kit, prepare the buffer using the following steps:

1. Transfer 10 ml Buffer BX into a user-supplied, nuclease-free tube (for example, a 50 ml conical tube.)
2. Add 10 ml Nuclease-free Water.
3. Add 10 ml ethanol.

Larger volumes of Buffer BX can be prepared by keeping the proportions of buffer, water and ethanol the same as above.

Preparation of Monarch Buffer WZ

Monarch Buffer WZ serves as the RNA wash buffer and is provided as a concentrate. Before using:

1. Add the appropriate volume of ≥ 95% ethanol (user-supplied).
    a. For T2110G/V (10-prep kit), add 20 ml ethanol.
    b. For T2110S (50-prep kit), add 104 ml ethanol.
    c. For T2110L (200-prep kit), add 200 ml ethanol per bottle.

Protocols

RNA Isolation protocol consists of two stages:

Part I. Sample Lysis and Homogenization–variable workflows depending on the input sample type.
Part II. RNA Binding and Elution– generally common workflow among all sample types.

Part I. Sample Lysis and Homogenization

After sample collection, please follow the sample lysis protocol specific to your starting material.

Cultured Mammalian Cells

The buffers provided in this kit are best optimized for pelleted cells.

For very low cell inputs, such as 100 cells, when pelleting is not feasible, add Monarch StabiLyse DNA/RNA Buffer directly to the cell suspension making the starting volume 350 µl or 700 µl.

1. **Pellet cells by centrifugation at conditions suitable for the cell type used (usually at 500 x *g*, up to 5 minutes at 4°C). Discard the supernatant**. Fresh cell pellets can be processed immediately or frozen on dry ice and stored at -80°C.

2. **Resuspend the pellet in Monarch StabiLyse DNA/RNA Buffer (according to table below) by pipetting gently to avoid foaming**. Pulse vortex gently to ensure homogeneous mixing. Do not place samples on ice. For frozen pellets, thaw briefly (approx. 30 seconds) before resuspension.

Number of cells	Volume of Monarch StabiLyse DNA/RNA Buffer
up to 1 x 10^6	350 µl
1 x 10^6 to 5 x 10^6	700 µl

3. **Proceed to Step 1 of Part II: RNA Binding and Elution.**

In-well Processing of Adherent Cells

Adherent cells can be lysed directly in the wells of a multi-well plate by following the suggested protocol below.

For Immediate Processing:

1. **Remove the media from the well and rinse with PBS.**
2. **Add Monarch StabiLyse DNA/RNA Buffer directly to the well according to the table below:**

Multi-well Plate Size	Volume of Monarch StabiLyse DNA/RNA Buffer
6 well	≥ 700 µl
12 well	350-700 µl
24 well	350 µl
48 well	350 µl

3. **Mix thoroughly and proceed to Step 1 of Part II: RNA Binding and Elution.**

For Storage:

1. **Remove the media from the wells and rinse with PBS.**
2. **Add Monarch StabiLyse DNA/RNA Buffer directly to the well according to the table below:**

Multi-well Plate Size	Volume of Monarch StabiLyse DNA/RNA Buffer
6 well	≥ 700 µl
12 well	350-700 µl
24 well	350 µl
48 well	350 µl

3. **Mix thoroughly and freeze the sample plate at –20°C or –80°C until ready for processing.**
4. **When ready to process, thaw the plate at room temperature and proceed to Step 1 of Part II: RNA Binding and Elution.**

Tissue or Leukocytes

A wide variety of tissues can be processed with the Monarch Spin RNA Isolation Kit (Mini), including soft tissues, fibrous tissues and lipid-rich tissues. Bead homogenization is recommended. **Users should choose appropriately sized beads depending on the sample type. Bead homogenization is not required for leukocytes.**

Monarch StabiLyse DNA/RNA Buffer is ideal for use as a sample homogenization buffer, and for Proteinase K digestion, when used according to the guidance provided.

For sample preserved in RNALater, please see page 14 for instructions.

1. **Determine the volume of Monarch StabiLyse DNA/RNA Buffer needed for your sample according to the table below:**

Sample input Amount	Volume of Monarch StabiLyse DNA/RNA Buffer
Tissue (up to 10 mg)	200 µl
Tissue (10-50 mg)	400 µl
Leukocytes (up to 1 x 10^6)	200 µl
Leukocytes (1 x 10^6 to 5 x 10^6)	400 µl

2. **Add the appropriate volume of Monarch StabiLyse DNA/RNA Buffer to the sample.**
3. **Add an equal volume of Nuclease-free Water to the sample.** For example, to a 10 mg tissue sample in 200 µl Monarch StabiLyse DNA/RNA Buffer, add 200 µl Nuclease-free Water.
4. **Perform sample homogenization using bead mill or similar device, according to the instrument's protocol.**
   For leukocyte samples, or if not performing mechanical homogenization, proceed to Step 8.
5. **Place the sample tubes on ice for 2 minutes.** This step ensures the samples recover from the heat generated during mechanical homogenization.

6. **Centrifuge at 16,000 x g for 2 minutes.** This step helps to pellet debris and minimize the foam generated during mechanical homogenization.
7. **Transfer the supernatant to a new 1.5 ml RNase-free microfuge tube.** It is common for the volume recovered at this step to be slightly less than input. For example, for samples homogenized in 400 µl Monarch StabiLyse/sample mixture, 350 µl supernatant should be expected.
8. **For every 350-400 µl of Monarch StabiLyse DNA/RNA Buffer/Sample mixture, add 15 µl Proteinase K*. Vortex for 5 seconds, and incubate at 55°C according to the chart below:**

Sample type	Incubation time at 55°C**
Homogenized Tissues	5 minutes
Solid Tissues (if not performing bead homogenization)	5-30 minutes
Leukocytes	30 minutes

* Doubling the amount of Proteinase K may increase RNA yield for some tissues.

** Longer incubation times may result in decreased RNA integrity. Optimal time should be determined empirically as yield and integrity vary with incubation time.

9. **Vortex sample for 5 seconds and spin at 16,000 x *g* for 2 minutes to pellet debris.** Digested proteins will form a pellet at this stage.
10. **Transfer supernatant to an RNase-free microfuge tube.**
11. **Add an equal volume of Monarch StabiLyse DNA/RNA Buffer and vortex for 5 seconds.**
12. **Proceed to** **Step 1 of Part II: RNA Binding and Elution.**

Bacteria or Yeast

Most microbial samples are considered tough to lyse and mechanical lysis/homogenization or enzymatic lysis is recommended. **Users should choose appropriately sized beads for mechanical homogenization.**

Some Gram-negative bacteria (e.g., *E. coli*) do not require mechanical or enzymatic lysis and can be lysed directly by resuspension in Monarch StabiLyse DNA/RNA Buffer; however, yields may be lower.

Mechanical Lysis of Bacteria or Yeast

1. **Determine the volume of Monarch StabiLyse DNA/RNA Buffer needed for bead homogenization according to the table below**:

Sample input amount		Volume of Monarch StabiLyse DNA/RNA Buffer
BACTERIA	YEAST	
< 5 x 10^7	< 5 x 10^6	200 µl
5 x 10^7 – 1 x 10^9	5 x 10^6–5 x 10^7	400 µl

2. **Add the appropriate volume of Monarch StabiLyse DNA/RNA Buffer to the sample.**
3. **Add an equal volume of Nuclease-free Water to the sample.** For example, to a pellet of 1 x 10^9 bacterial cells in 400 µl Monarch StabiLyse DNA/RNA Buffer, add 400 µl Nuclease-free Water.
4. **Perform sample homogenization using bead-mill or similar device, according to instruments protocol.**
5. **Place the sample tubes on ice for 2 minutes.** This step ensures the samples recover from the heat generated during mechanical homogenization.
6. **Centrifuge at 16,000 x g for 2 minutes.** This step helps to pellet debris and minimize the foam generated during mechanical homogenization.
7. **Carefully transfer the supernatant to a new RNase-free microfuge tube without disturbing the beads.** It is common for the volume recovered at this step to be slightly less than input. For example, for samples homogenized in 400 µl Monarch StabiLyse/sample mixture, 350 µl supernatant should be expected.
8. ***Optional: Centrifuge the sample tubes at 16,000 x g for 2 minutes to pellet any residual beads that may have been aspirated.*** Homogenization beads for microbes are small and may not remain packed after centrifugation. Care should be taken to not aspirate any beads. If beads are aspirated, this additional centrifugation can help reset the contents of the tube for optimal supernatant removal. Transfer supernatant to an RNase-free microfuge tube.

9. **Add an equal volume of Monarch StabiLyse DNA/RNA Buffer and vortex for 5 seconds.**
10. **Proceed to Step 1 of Part II: RNA Binding and Elution.**

Enzymatic Lysis of Bacteria or Yeast:

1. **Add appropriate reaction buffer and glycanase (user-supplied) in a 100 µl enzymatic reaction, and incubate at appropriate temperature according to the chart below:**

Sample type	Enzyme	Reaction buffer	Incubation time	Digestion temperature
Bacteria (Gram negative)	Chicken Egg Lysozyme (1 mg/ml)	TE Buffer (10mM Tris, 1mM EDTA)	5 minutes	25°C
	NEBExpress® T4 Lysozyme (5 µg/ml)	Low EDTA TE Buffer (10mM Tris, 0.5mM EDTA)		
Bacteria (Gram positive)	Lysozyme (3 mg/ml)	TE Buffer (10mM Tris, 1mM EDTA)	10 minutes	25°C
	NEBExpress T4 Lysozyme (20 µg/ml)	Low EDTA TE Buffer (10mM Tris, 0.5mM EDTA)		
Yeast	Zymolyase® (25 U)	PBS or Sorbitol Buffer	30-40 minutes	30°C

2. **Add 250 µl Monarch DNA/RNA StabiLyse Buffer to make up the sample volume to 350 µl. Vortex vigorously for ~10 seconds.**
3. **Centrifuge for 2 minutes at 16,000 x *g* to pellet cellular debris.**
4. **Transfer supernatant to an RNase-free microfuge tube.**
5. **Proceed to Step 1 of Part II: RNA Binding and Elution.**

Plants or Insects

Cell walls of plants and exoskeleton of insects are biological materials that are hard to lyse. Mechanical lysis using bead mill or similar device is recommended for maximal recovery from these tissues. **Users should choose appropriately sized beads for mechanical homogenization.**

For plant tissue, pulverize the tissue by grinding under liquid nitrogen before using the powder for mechanical lysis.
For insects, preservation in an ethanol-dry ice bath is recommended.

Sample type	Input amount	Volume of Monarch StabiLyse DNA/RNA Buffer
Plant tissue (frozen pulverized)	100 mg	700 µl
Plant tissue (fresh)	10–50 mg	700 µl
Insects (ethanol or ethanol-dry ice preserved)	10–25 mg	700 µl

1. **Add 700 µl Monarch StabiLyse DNA/RNA Buffer to the sample in a tube compatible with bead homogenization.**
2. **Perform sample homogenization using bead-mill or similar device, according to instruments protocol.**
3. **Place the sample tubes on ice for 2 minutes.** This step ensures the samples recover from the heat generated during mechanical homogenization.
4. **Centrifuge at 16,000 x g for 2 minutes.** This step helps to pellet debris and minimize the foam generated during mechanical homogenization.
5. **Carefully transfer the supernatant to a new RNase-free microfuge tube without disturbing the beads.** It is common for the volume recovered at this step to be slightly less than input. For example, for samples homogenized in 400 µl Monarch StabiLyse/sample mixture, 350 µl supernatant should be expected.
6. ***Optional: If any beads get aspirated in the previous step, centrifuge the sample tubes at 16,000 x g for 2 minutes to pellet any residual beads.*** Transfer supernatant to an RNase-free microfuge tube.
7. **Proceed to Step 1 of Part II: RNA Binding and Elution.**

Mammalian Whole Blood or Plasma (fresh or frozen)

Blood and plasma are low RNA-yielding samples, and the RNA output is highly dependent on collection tubes, sample preservation strategy and the RNA load in circulation at the time of donation. Appropriate blood collection tubes with preservation reagents should be utilized.

1. **Add two volumes (400 µl) of Monarch StabiLyse DNA/RNA Buffer to an aliquot of 200 µl whole blood and vortex for 10 seconds. Do not place samples on ice.** For frozen samples, quickly thaw in the presence of two volumes of Monarch StabiLyse DNA/RNA Buffer while vortexing or shaking. Blood cells are lysed during this step, releasing the RNA.
2. **For every 600 µl of Monarch StabiLyse DNA/RNA Buffer/blood mixture, add 15 µl of Proteinase K. Vortex for 10 seconds and incubate at room temperature for 30 minutes with intermittent vortexing.**
3. **Centrifuge at 16,000 x g for 2 minutes to pellet the debris.** This ensures removal of residual blood proteins that may interfere with the RNA binding steps.
4. **Carefully transfer the supernatant into a new RNase-free tube without disturbing the pellet.**
5. **Add an equal volume of isopropanol (user-supplied) and vortex for 5 seconds.** Addition of isopropanol is necessary to create favorable binding conditions before application onto the Monarch Spin Column S2A.
6. **Proceed to Step 4 of Part II: RNA Binding and Elution to load the sample/isopropanol mixture on the Monarch Spin Column S2A.**

Supplementary Protocols

Fractionation of Small or Large RNAs

This protocol can be used on cultured cells to allow for selective depletion or enrichment of the sub-200 nucleotide RNA pool. This modification may also be used on previously purified total RNA to enable fractionation. The procedure is compatible with up to 1 million animal cells, or previously isolated RNA (with a sample volume adjusted to 50 µl).

1. Add 350 µl Monarch StabiLyse DNA/RNA Buffer to the cell pellet. If using previously isolated RNA (50 µl), add 300 µl Monarch StabiLyse DNA/RNA Buffer.

2. Mix well by pipetting to ensure the sample is mixed well with the buffer.

Optional gDNA removal using a column separation can be performed at this stage by transferring the sample mixture (350 µl) from Step 1 to a Monarch Spin Column S2C (orange) fitted in a collection tube. Centrifuge for 1 minute at 16000 x g and use the resulting flow-through as the sample mixture in the next step (Step 3).

3. To the sample mixture, add 350 µl 70% Ethanol. Mix well.

4. Transfer the mixture to a Monarch Spin Column S2A (clear) fitted in a collection tube.

5. Centrifuge for 1 minute at 16000 x g. Save the column and the flow-through. RNAs > 200 nt bind to the column, whereas RNAs < 200 nt partition into the flow-through. For obtaining the desired RNA fractions, proceed with the protocol steps below.

To purify RNAs > 200 nt that are bound to the column, proceed to **Step 6** (if performing on-column DNase I treatment) or **Step 9** of **Part II: RNA Binding and Elution.**

To enrich for RNAs < 200 nt:

a. Add an equal volume of ethanol (≥ 95%) to the flow-through from Step 5. Mix well.

b. Transfer to a new Monarch Spin Column S2A (clear) in a collection tube.

c. Centrifuge for 1 minute at 16,000 x g. Discard the flowthrough.

d. Proceed to either **Step 6** (if performing on-column DNase I treatment) or **Step 9** of **Part II: RNA Binding and Elution.**

Reaction Cleanup (IVT, Labeling, DNase Treatment etc.):

The Monarch Spin RNA Isolation Kit (Mini) can also be used to clean up RNA after enzymatic reactions including IVT, labeling reactions, or DNase I treatment. For customers interested in RNA cleanup as the primary application, NEB also supplies Monarch RNA Cleanup Kits for faster, more streamlined cleanup of RNA (NEB #T2030, #T2040, and #T2050).

1. For every 50 µl of reaction volume, add 100 µl Monarch StabiLyse DNA/RNA Buffer and mix thoroughly.
2. Add an equal volume of ethanol (≥ 95%) (user-supplied). For example, if sample is now 150 µl, add 150 µl ethanol.
3. Mix by pipetting or gently vortexing.
4. Proceed to **Step 4 of Part II: RNA Binding and Elution**.

RNAlater Samples:

Samples stored in RNAlater can be processed according to sample type after the RNAlater is removed by pipetting as the manufacturer recommends. If RNAlater is not removed before processing (for example, liquid samples), please follow the protocol below.

1. Add four volumes of Monarch StabiLyse DNA/RNA Buffer to the sample and mix thoroughly.
2. Proceed to **Step 1 of Part II: RNA Binding and Elution**.

TRIzol Extracted Samples:

1. Transfer the aqueous phase of a TRIzol-extracted sample to an RNase-free tube.
2. Add an equal volume of ethanol (≥ 95%) (not included) and mix thoroughly.
3. Proceed to **Step 4 of Part II: RNA Binding and Elution**.

FFPE Tissue Deparaffinization:

Paraffin is removed from FFPE samples by treating with xylene or a commercially available FFPE deparaffinization solution (see manufacturer's recommendations for details).

Rapid deparaffinization with xylene (tissue sections)

1. Remove (trim) as much excess paraffin from the sample as possible and transfer to a 1.5 ml tube (not included).
2. Add 1 ml xylene (not included) and vortex vigorously for 30 seconds.
3. Centrifuge for 1 minute at 16,000 x *g* and remove xylene.
4. Wash sample with 1 ml ethanol (≥ 95%) (not included) and vortex vigorously for 30 seconds.
5. Centrifuge for 1 minute at 16,000 x *g* and remove ethanol. Repeat Steps 4 and 5.
6. Dry samples by vacuum centrifugation (e.g. Speed-Vac) or by incubating uncapped tubes at ≤ 37°C for up to 40 minutes.
7. Proceed to Tissue Digestion.

Tissue Digestion

1. Prepare a mixture of the following:

Monarch StabiLyse DNA/RNA Buffer	100 µl
Nuclease-free Water	100 µl
Proteinase K	10 µl

2. Add the mixture to the deparaffinized tissue sample (≤ 25 mg).
3. Incubate at 55°C for 15 minutes.
4. Transfer the tube to a preheated block at 65°C and incubate for 15 minutes. Note: If only one heat block is available, place sample at room temperature after the 55°C incubation until the heating block has reached 65°C.
5. Add 500 µl Monarch StabiLyse DNA/RNA Buffer and mix thoroughly. Centrifuge for 1 min at 16,000 x *g* to pellet debris. Transfer supernatant to an RNase-free microfuge tube (not included). Proceed to **Step 1 of Part II: RNA Binding and Elution.**

Part II. RNA Binding and Elution using Centrifugation

Monarch Buffer BX and Monarch Buffer WZ need to be prepared per instructions under Reagent Preparation section.
All centrifugation steps should be performed at 16,000 x g.
For sample volumes > 700 µl (column reservoir capacity), columns may be reloaded.

1. **Transfer up to 700 µl of the sample from Part I to Monarch Spin Column S2C (orange) fitted in a Monarch Spin Collection Tube.** For sample identification, **label collection tubes**, as columns will be discarded in the next step.
2. **Centrifuge for 1 minute.** ***Discard the column. Save the flow-through.*** Genomic DNA is captured on the Monarch Spin Column S2C matrix, while RNA is collected in the flow-through.
3. **To the flow-through, add an equal volume of ethanol (≥ 95%) (user-supplied) and mix thoroughly by pipetting.** **Do not vortex**. The addition of ethanol creates favorable conditions for RNA binding. A cloudy residue may appear when ethanol is added that disappears upon mixing and has no impact on performance.
4. **Transfer 700 µl of the sample/ethanol mixture to a Monarch Spin Column S2A (clear) fitted in a Monarch Spin Collection Tube.**
5. **Centrifuge for 1 minute. Discard the flow-through.** Reload the column with any remaining sample/ethanol mixture, centrifuge and discard the flow-through after each spin.
6. **Add 700 µl Monarch Buffer WZ and centrifuge for 1 minute. Discard the flow-through.** This step ensures removal of excess salts, creates conditions optimal for DNase treatment and improves the purity of resulting RNA.
7. **Optional but recommended DNase treatment**: If further gDNA removal is essential for downstream applications, proceed to on-column DNase I treatment, Step 7A– 7C. If not, proceed to Step 8.

   7A. **In an RNase-free microfuge tube (not included), combine 10 µl Monarch DNase I, Lyophilized with 70 µl Monarch DNase I Reaction Buffer.**

   7B. **Pipet 80 µl DNase reaction mixture directly to the top of the matrix.**

   7C. **Incubate for 15 minutes at room temperature.** Proceed to Step 8.
8. **Add 500 µl Monarch Buffer BX and centrifuge for 1 minute. Discard the flow-through. This buffer serves as a priming step.**
9. **Add 500 µl Monarch Buffer WZ and centrifuge for 1 minute. Discard the flow-through.**
10. **Add another 500 µl Monarch Buffer WZ and centrifuge for 2 minutes.** This step ensures removal of residual ethanol.
11. **Transfer the column to a 1.5 ml RNase-free microfuge tube (user-supplied).** Use care to ensure the tip of the column does not contact the flow-through. If in doubt, centrifuge again for 1 minute to ensure no ethanol is carried over.
12. **Add 20-100 µl Nuclease-free Water directly to the center of column matrix and spin for 30 seconds.** For maximal recovery, elute with at least 20 µl. Elution with 10 µl is possible, with up to 5-10% loss in recovery.
13. **Place eluted RNA on ice if being used for downstream steps, at -20°C for short-term storage (less than 1 week), or at -80°C for long-term storage.** Addition of EDTA to 0.1–1.0 mM may reduce the activity of any contaminating RNases.

Alternative Steps for RNA Binding Using Vacuum Manifold

The kit workflow allows for usage of a vacuum manifold once the RNA is captured on the Monarch Spin Column S2A. Appropriate Sample Lysis and Homogenization, as well as the application of Monarch Spin Column S2C needs to be performed off-board, upstream of the vacuum manifold steps.

1. **Perform Part I. Sample Lysis and Homogenization according to the sample type.**
2. **Transfer up to 700 µl of the sample from Part I to Monarch Spin Column S2C (orange) fitted in a Monarch Spin Collection Tube.** For sample identification, **label collection tubes**, as columns will be discarded in the next step.
3. **Centrifuge for 1 minute.** ***Discard the column. Save the flow-through.*** Genomic DNA is captured on the matrix of the Monarch Spin Column S2C, while RNA is collected in the flow-through.
4. **To the flow-through, add an equal volume of ethanol (≥ 95%) (user-supplied) and mix thoroughly by pipetting.** **Do not vortex**. The addition of ethanol creates favorable conditions for RNA binding. A cloudy residue may appear when ethanol is added that disappears upon mixing and has no impact on performance.
5. **Insert the Monarch Spin Column S2A (clear) into the vacuum adaptor (recommended) or manifold directly.**
6. **Load 700 µl of the sample/ethanol mixture onto the Monarch Spin Column S2A and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.** Reload the column with any remaining sample/ethanol mixture and repeat the vacuum step as needed.
7. **Add 700 µl Monarch Buffer WZ and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.** This step ensures removal of excess salts, creates conditions optimal for DNase treatment and improves the purity of resulting RNA.

8. ***Optional but recommended DNase treatment**:* If further gDNA removal is essential for downstream applications, proceed to on-column DNase I treatment, Step 8A– 8C (recommended). If not, proceed to Step 9.

   8A. **In an RNase-free microfuge tube (not included), combine 10 µl Monarch DNase I, Lyophilized with 70 µl Monarch DNase I Reaction Buffer.**

   8B**. Pipet 80 µl DNase reaction mixture directly to the top of the matrix.**

   8C. **Incubate for 15 minutes at room temperature.** Proceed to Step 9.

9. **Add 500 µl Monarch Buffer BX and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.**
10. **Add 500 µl Monarch Buffer WZ and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.**
11. **Add another 500 µl Monarch Buffer WZ and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.**
12. **Transfer the column to a 1.5 ml RNase-free microfuge tube (user-supplied).** Use care to ensure the tip of the column does not contact the flow-through. If in doubt, re-spin for 1 minute to ensure no ethanol is carried over.
13. **Add 20-100 µl Nuclease-free Water directly to the center of column matrix and spin for 30 seconds.** For maximal recovery, elute with at least 20 µl. Elution with 10 µl is possible, with up to 10-20% loss in recovery.
14. **Place eluted RNA on ice if being used for downstream steps, at -20°C for short-term storage (less than 1 week), or at -80°C for long-term storage.** Addition of EDTA to 0.1–1.0 mM may reduce the activity of any contaminating RNases.

Appendix

Protocol for In-tube DNase Treatment on RNA eluates

Sensitive applications such as RT-qPCR require that RNA input samples contain little to no genomic DNA. To further reduce residual genomic DNA, you can treat your eluted sample with DNase I in a separate reaction and clean up the sample using a second Monarch Spin Column S2A from this kit or a separate cleanup kit.

In-tube DNase treatment can be performed with rehydrated Monarch DNase I, Lyophilized supplied with the kit or as a standalone (NEB #T2104) available for purchase separately.

1. For each sample to be treated, prepare a DNase I reaction mix in an RNase-free tube (user-supplied) according to the table below. Mix well by gentle pipetting.

Mixture component	Volume
RNA Sample (≤ 10 µg) Volume Adjusted with Water or TE Buffer	40 µl
Monarch DNase (rehydrated)	5 µl
Monarch DNase I Reaction Buffer	5 µl
Total Volume	**50 µl**

2. Incubate at room temperature (20–30°C) for 15 minutes.
3. Proceed to Reaction Cleanup, page 14.

Assessing RNA

RNA Quantification

RNA can be quantified using direct spectrophotometric measurements (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), nor do they allow de-convolution of the contribution of genomic DNA to the absorbance at 260 nm. 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, Unchained Labs®) 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.

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

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 using a proper reference blank solution, the buffer pH, and contaminants such as protein, buffer salts, ethanol, etc.

RNA integrity can be assessed using an Agilent Bioanalyzer, Agilent TapeStation or a similar device to resolve the sample and determine the ratio of 28S to 18S rRNA, and the amount of lower molecular weight species. This approach produces an RNA Integrity Number (RIN for Bioanalyzer, RINe for TapeStation) that encapsulates these generally agreed upon metrics. RIN values greater than 7 are often referred to as "good," though the specific downstream application of the purified RNA determines the true needs of RNA quality. Researchers are encouraged to establish their own criteria.

Note that the RNA Integrity metrics from Agilent's automated electrophoresis systems are applicable for samples with typical upper and lower ribosomal fragments. For samples with atypical ribosomal profiles such as green tissues of plants with plastidal RNA, and insects with breaks in upper ribosomal fragment, the instrument algorithm is not the best predictor of RNA quality and users may observe a discrepancy between instrument-generated RIN and the actual visual profile of the gel trace. Additionally, there is a growing understanding within the scientific community regarding comparisons between RIN (from Bioanalyzer) and RINe (from TapeStation). Users are encouraged to learn more about the RNA integrity metrics through available resources such as manufacturer's technical notes and peer-reviewed literature that may help set expectations for their sample type.

Troubleshooting

Problem	Common Cause	Suggestions/Solutions
Column clogging	Sample input higher than recommended	Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded.
	Insufficient Lysis	Increase time of digestion or homogenization. Centrifuge sample to pellet debris and use only supernatant for next steps. Use larger volume of buffer for lysis and homogenization.
Low RNA yield	Insufficient Lysis	Increase time of digestion or homogenization. Centrifuge sample to pellet debris and use only supernatant for next steps. Use larger volume of buffer for lysis and homogenization.
	Sample is degraded	Use RNA preservation reagents to maintain RNA integrity during storage. Use best practices when working with RNA; harvest samples quickly, flash-freeze samples or preserve in stabilization reagent as soon as possible. To minimize risk of contaminating RNases, clean surfaces and tools with RNase-removal reagents, and always handle samples with appropriate PPE.
	Sample input higher than recommended	Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. Overloading leads to inefficient purification, insufficient elution and reduced yield.
Low RNA quality	Sample is degraded	Use RNA preservation reagents to maintain RNA integrity during storage. Use best practices when working with RNA; harvest samples quickly, flash-freeze samples or preserve in stabilization reagent as soon as possible. To minimize risk of contaminating RNases, clean surfaces and tools with RNase-removal reagents, and always handle samples with appropriate PPE.
	Salt/Ethanol carryover	Low $A_{260/230}$ values indicate residual guanidine salts have been carried over during elution. Ensure wash steps are carried out before eluting sample. Do not skip any washes with Buffer BX and Buffer WZ. Use care to ensure the tip of the column does not contact the flow-through. If unsure, please repeat centrifugation. When reusing collection tubes, blot rim of tube on a soft paper towel before reattachment to the column to remove any residual wash buffer. Add additional wash step and/or extend spin time for final wash.
	Residual protein carryover	Low $A_{260/280}$ values indicate residual protein in the purified sample. Ensure the Proteinase K step was utilized for the recommended time. Ensure samples have no debris before addition of ethanol and loading onto RNA purification column. Do not skip any washes with Buffer BX and Buffer WZ.
DNA contamination	DNA carryover	Perform optional on-column DNase I treatment to remove unwanted gDNA from lysed sample. Perform in-tube/off-column DNase I treatment to remove gDNA
	Sample input higher than recommended	Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded.

For more troubleshooting and FAQs, please visit product webpage.

Ordering Information

View the entire Monarch DNA & RNA Purification portfolio at NEBMonarch.com.

Monarch® Spin RNA Isolation Kit (Mini)

PRODUCT	NEB #
Monarch Spin RNA Isolation Kit (Mini)	T2110
Columns and collection tubes also offered separately	
Monarch Spin Columns S2C	T3017
Monarch Spin Columns S2A	T2047
Monarch Spin Collection Tubes	T2118

NEB Companion Products

PRODUCT	NEB #
Monarch DNase I, Lyophilized	T2104
NEBExpress T4 Lysozyme	P8115
Monarch Mag Viral DNA/RNA Extraction Kit	T4010
Monarch RNA Cleanup Kits	T2030, T2040, T2050
Monarch StabiLyse DNA/RNA Buffer	T2111
Proteinase K, Molecular Biology Grade	P8107

Revision History

Revision #	Description	DATE
1.1	Corrected step number on page 12	04/25
2.0	Added new sizes to kit: G, V, L	11/25