INSTRUCTION MANUAL

Monarch® RNA Cleanup Kits

NEB #T2030S/L, #T2040S/L, #T2050S/L

10/100 preps

Version 4.0_04/24

Table of Contents

Kit Components

This kit should be stored at room temperature. Always keep buffer bottles tightly closed and keep columns sealed in the enclosed zip-lock bag. For 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, including the use of lab coats, gloves, and eye protection.

	Application/Usage	T2030S/ T2040S/T2050S 10 preps	T2030L/ T2040L/T2050L 100 preps	STORAGE TEMPERATURE
Monarch RNA Cleanup Columns	RNA Binding Matrix	10 columns	100 columns	Room Temp.
Monarch Collection Tubes II	Collection of eluted and waste material	10 tubes	100 tubes	Room Temp.
Monarch Buffer BX	RNA Cleanup Binding Buffer	4 ml	40 ml	Room Temp.
Monarch Buffer WX (5X Concentrate)	RNA Cleanup Wash Buffer Concentrate	2.5 ml	2 x 20 ml	Room Temp.
Nuclease-free Water	Elution	2.5 ml	25 ml	Room Temp.

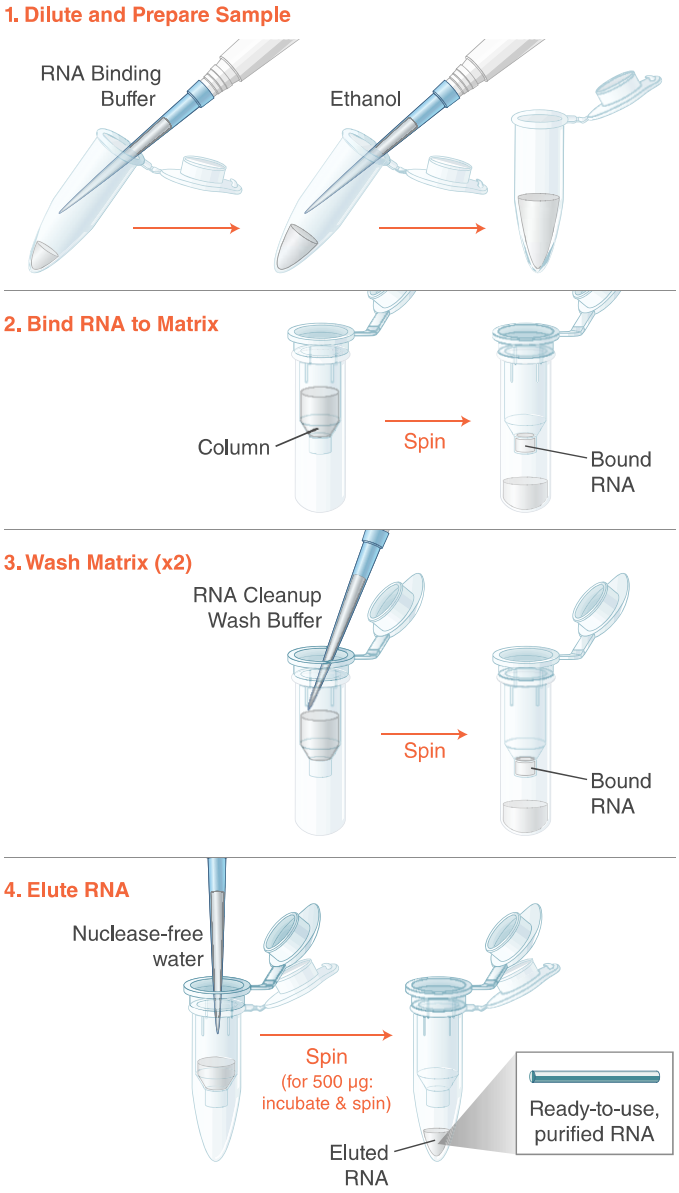
Introduction

The Monarch RNA Cleanup Kit is a rapid and reliable method for the cleanup and concentration of high-quality RNA. Removal of proteins, buffer salts and nucleotides from enzymatic reactions such as *in vitro* transcription, RNA capping and DNase I-treatment is easily accomplished with minimal effort and time. The Monarch RNA Cleanup Kit is available in 3 different binding capacities: 10 µg (NEB #T2030), 50 µg (NEB #T2040) and 500 µg (NEB #T2050). Each kit contains unique columns, all designed to prevent buffer retention and ensure no carryover of contaminants, enabling low-volume elution of highly-pure RNA (T2030: ≥ 6 µl, T2040: ≥ 20 µl and T2050: ≥ 50 µl). Following the standard protocol, RNA ≥ 25 nt is purified with this kit; however, a modified protocol is available to enable the binding of RNA as small as 15 nt (including miRNAs).

The various RNA Cleanup Kits utilize the same buffers (including a single wash buffer for convenience) and similar bind/wash/elute protocols. Minimal spin and incubation times allow for purification in less than 5 minutes for the 10 µg and 50 µg capacities, and in 10–15 minutes for the 500 µg capacity. Monarch Buffer BX and ethanol (not supplied) are used to dilute the samples and ensure they are compatible for binding onto the proprietary silica matrix. Next, the Monarch Buffer WX ensures enzymes, detergents and other low-molecular weight reaction components (e.g., nucleotides) are removed. Finally, RNA is eluted with nuclease-free water. Eluted RNA is highly-pure and is 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.

Supplemental protocols are also available for cleanup of RNA from the aqueous phase following TRIzol or similar extraction, fractionation of RNA to selectively enrich the sub-200 nt pool, and for purification of RNA from agarose gels.

Figure 1: Workflow for RNA Cleanup Kits



Specifications

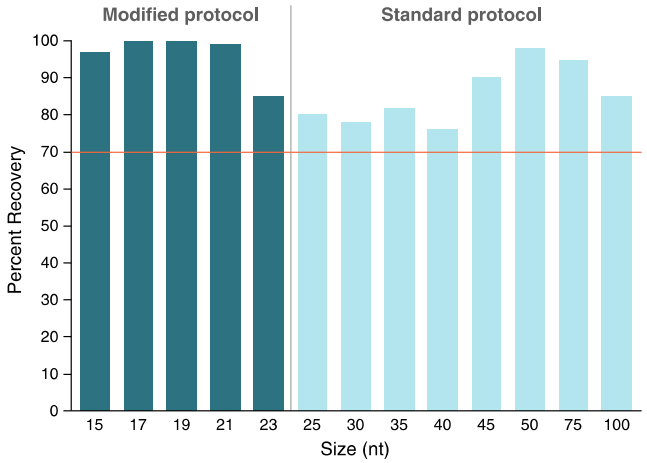
Monarch RNA Cleanup Kit	NEB #T2030 (10 µg)	NEB #T2040 (50 µg)	NEB #T2050 (500 µg)
Binding Capacity:	10 µg	50 µg	500 µg
RNA Size Range:	≥ 25 nt (≥ 15 nt with modified protocol)		
Typical Recovery:	70–100%		
Elution Volume:	6–20 µl	20–50 µl	50–100 µl
Purity:	$A_{260/280}$ > 1.8 and $A_{260/230}$ > 1.8		
Protocol Time:	5 minutes of spin and incubation time		10–15 minutes of spin and incubation time
Common Downstream Applications:	RT-PCR, RNA library prep for NGS, small RNA library prep for NGS, RNA labeling	RT-PCR, RNA library prep for NGS, formation of RNP complexes for genome editing, microinjection, RNA labeling, transfection	RT-PCR, RNA library prep for NGS, RNA labeling, RNAi, microinjection, transfection

Applications

APPLICATIONS	
RNA Cleanup and Concentration (including from the TRIzol aqueous phase)	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 NTPs are removed to yield highly pure synthesized RNA
RNA Gel Extraction	Purification of RNA from agarose gels
RNA Fractionation	Fractionation of RNA into small and large RNA pools

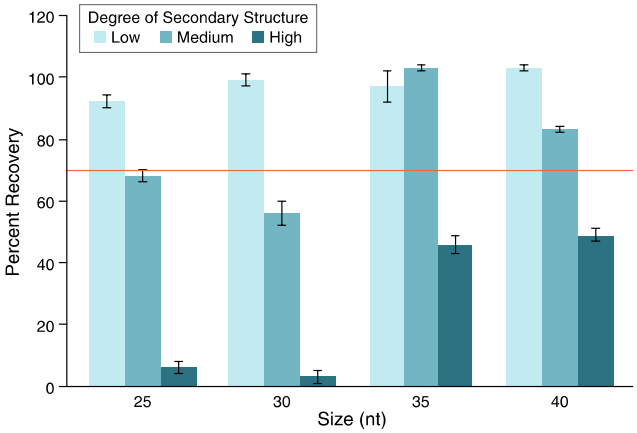
Performance Data

Figure 2: Recovery of Small RNAs using the Monarch RNA Cleanup Kit



Synthesized RNA oligonucleotides (1 µg in 50 µl H_2O) of varying lengths (15–100 nt) were purified using the Monarch RNA Cleanup Kit (NEB #T2040) and eluted in 50 µl nuclease-free water. The percent recovery of RNA was calculated from the resulting A_{260}, measured using a Trinean DropSense 16. The Monarch RNA Cleanup Kit standard protocol results in > 70% recovery and cleanup of RNA ≥ 25 nt, while use of the Monarch RNA Cleanup Kit with the modified protocol (addition of 2 volumes of ethanol in Step 2) results in > 70% recovery and cleanup of even smaller RNAs (as low as 15 nt). Please note that recovery of small RNAs (< 45 nt) can be affected by sequence, interactions with other nucleic acids and/or secondary structure (see Figure 3).

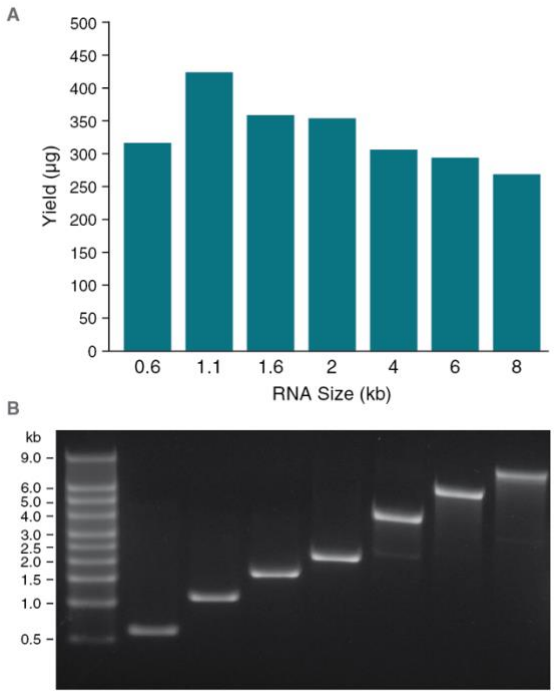
Figure 3: Secondary structure affects recovery of small RNAs (< 45 nt)



Synthesized RNA oligonucleotides (1 µg in 50 µl H_2O) of varying lengths (25–40 nt) and varying predicted secondary structure were purified using the Monarch RNA Cleanup Kit (NEB #T2040) and eluted in 50 µl nuclease-free water. The percent recovery of the RNA was calculated from the resulting A_{260} as measured using a Trinean DropSense 16. Small RNAs (25–40 nt) with low predicted secondary structure were recovered efficiently using the Monarch RNA Cleanup Kit standard protocol. However, increased levels of secondary structure decreases the recovery of small RNAs (25–40 nt in length). Recovery can typically be increased to > 70% (orange line) using the Monarch RNA Cleanup Kit with the modified protocol (addition of 2 volumes of ethanol in Step 2).*

** Secondary structure was predicted using RNAstructure Web Server: Reuter, J. S., & Mathews, D. H. (2010). RNAstructure: software for RNA secondary structure prediction and analysis. BMC Bioinformatics. 11,129*

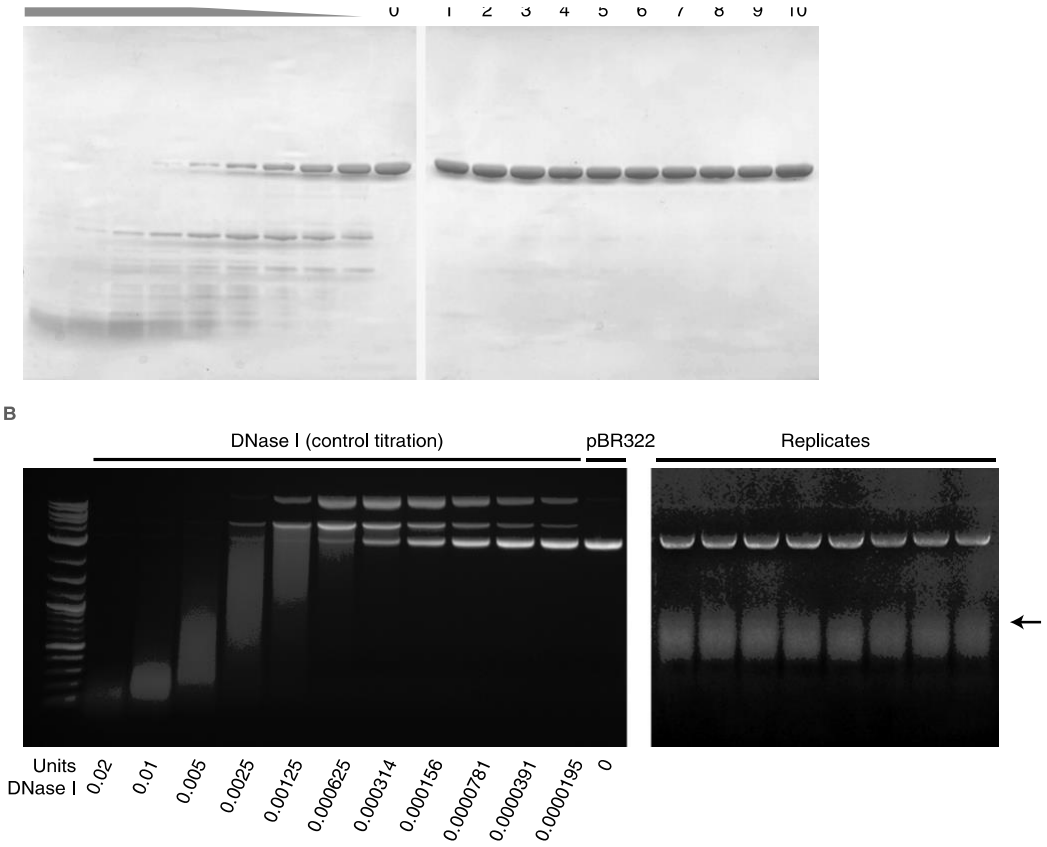
Figure 4: The Monarch RNA Cleanup Kit (500 µg) is suitable for cleaning up large quantities (> 250 µg) of RNA from *in vitro* transcription reactions



A. *RNA transcripts of varying sizes (0.6-8 kb) were synthesized using the HiScribe® T7 Quick High Yield RNA Synthesis Kit (NEB #E2050) using 1.5–1.8 µg of DNA template for 2 hours at 37°C. 40 µl of each in vitro transcription (IVT) reaction was cleaned up using the Monarch RNA Cleanup Kit (NEB #T2050). RNA yields were calculated from the resulting A_{260}, measured using a Nanodrop® spectrophotometer and ranged from 268–425 µg of RNA per IVT reaction.*

B. *RNA integrity (200 ng/lane) was assessed on a 1% agarose-TBE gel stained with SYBR® Gold.*

Figure 5: The Monarch RNA Cleanup Kit efficiently removes proteins (e.g., Proteinase K and DNase I) from enzymatic reactions



A. 1 µg of rRNA (16S- and 23S-ribosomal RNA from E. coli MRE, Sigma) was treated with 0.8 units of Proteinase K (NEB #P8109), cleaned up using the NEB Monarch RNA Cleanup Kit (50 µg, NEB #T2040) and eluted in 25 µl nuclease-free water. The eluted RNA was tested for residual Proteinase K by incubating the eluent (25 µl) with 10 µg MBP5 (NEB #E8046) in 1X NEBuffer 4 at 37°C for 5 minutes. Lanes 1–10 represent 10 "replicate" samples showing MBP5 remains undigested at 42.5 kDa. A Proteinase K control titration (32–0.125 units, Proteinase K) is shown for comparison.

B. 5 µg of rRNA (16S- and 23S-ribosomal RNA from E. coli MRE, Sigma) was treated with DNase I (2 units), cleaned up using the NEB Monarch RNA Cleanup Kit (50 µg, NEB #T2040) and eluted in 25 µl nuclease-free water. Column eluent from eight "replicate" RNA cleanup columns was tested for residual DNase I activity by incubating the eluent (15 µl) with 1 µg pBR322 (NEB #N3033) in 1X DNase I Reaction Buffer at 37°C for 10 minutes. No digestion of the DNA substrate was observed. A DNase I control titration (0.02–0.000195 units DNase I, #M0303) is shown for comparison.

General Guidelines for Working with RNA

Successful RNA Cleanup requires using care when handling samples and ensuring the buffers and labware in contact with the RNA is free of contaminating nucleases.

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

1. RNases are stable and difficult to inactivate, and care must be taken when handling samples during and after purification 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 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 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.
3. Avoid unnecessary freeze-thaw cycles of purified RNA. Aliquots should be made, consistent with downstream needs.

Buffer Preparation

Add ethanol to Monarch Buffer WX prior to use (4 volumes of ≥ 95% ethanol per volume of Monarch Buffer WX).

- For the 10-prep kit, add 10 ml of ethanol to 2.5 ml of Monarch Buffer WX
- For the 100-prep kit, add 80 ml of ethanol to 20 ml of Monarch Buffer WX

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.

Monarch RNA Cleanup Kit Protocol

The standard protocol outlined below will purify RNA ≥ 25 nt. A simple modification in Step 2 can allow for the purification of RNA as small as 15 nt.

Centrifugation should be carried out at 16,000 x g in a standard laboratory microcentrifuge at room temperature.

1. Add 100 µl Buffer BX to the 50 µl sample. A starting sample volume of 50 µl is recommended. For smaller samples, nuclease-free water can be used to adjust the volume. For samples larger than 50 µl, scale buffer volumes accordingly. Samples with a starting volume > 150 µl will require reloading of the column during Step 3.
2. Add 150 µl (1 volume) of ethanol (≥ 95%) to your sample and mix by pipetting or flicking the tube. Do not vortex. This will enable the binding of RNA ≥ 25 nt. If you wish to bind RNA as small as 15 nt, add 2 volumes (300 µl) of ethanol to your sample instead of 1 volume (150 µl). The addition of 2 volumes of ethanol shifts the cutoff size of RNA binding from 25 nt down to 15 nt.
3. Insert column into collection tube, load sample onto column and close the cap. Spin for 1 minute, then discard flow-through. For diluted samples > 900 µl, load a portion of the sample, spin, and then repeat as necessary.

   To save time, spin for 30 seconds, instead of 1 minute.

4. Re-insert column into collection tube. Add 500 µl Buffer WX and spin for 1 minute. Discard the flow-through.

   To save time, spin for 30 seconds, instead of 1 minute.

5. Repeat wash (Step 4).
6. Transfer column to an RNase-free 1.5 ml microfuge tube (not provided). Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to next step.
7. Elute in nuclease-free water according to the table below. The eluted RNA can be used immediately or stored at -70°C. Care should be used to ensure the elution buffer is delivered onto the matrix and not the wall of the column to maximize elution efficiency.

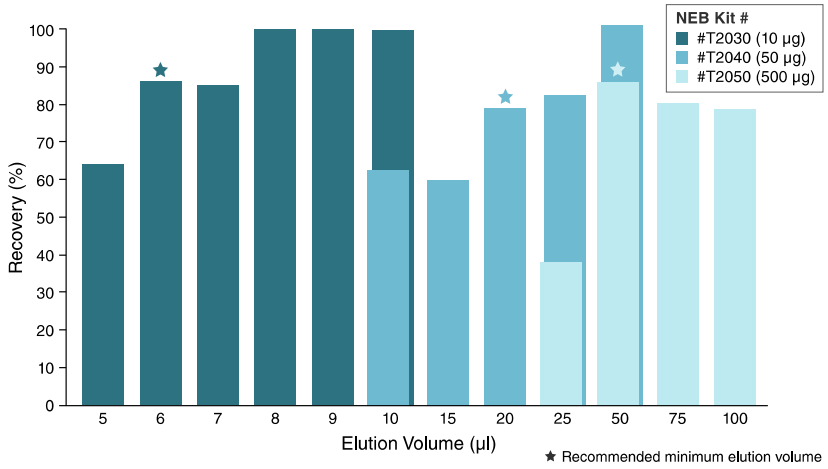
KIT	ELUTION VOLUME	INCUBATION TIME	SPIN TIME
T2030	6–20 µl	N/A	1 minute
T2040	20–100 µl	N/A	1 minute
T2050*	50–100 µl	5 minutes (room temp.)	1 minute

Note: cleaning up large amounts of RNA (> 100 µg, NEB #T2050), some precipitation may occur following the addition of the Monarch Buffer BX and ethanol to the sample (Steps 1 and 2). A pellet containing the RNA of interest may form on the side of the column following the first binding spin (Step 3). To maximize recovery of this RNA, a second elution is recommended.

* Yield may slightly increase if a larger volume is used, but the RNA will be less concentrated.

To save time, spin for 30 seconds, instead of 1 minute.

Figure 6: Recovery of RNA from Monarch RNA Cleanup Kits with Varying Elution Volumes



10, 50 or 500 µg of rRNA (16S and 23S Ribosomal Standard from E. coli, Sigma) was purified using a Monarch RNA Cleanup Kit (10 µg, NEB #T2030) (50 µg, NEB #T2040) (500 µg, NEB #T2050). Nuclease-free water was used to elute the RNA. The percent recovery of the RNA was calculated from the resulting A_{260} as measured using a Trinean DropSense 16. ~80% of RNA can be efficiently recovered in 6 µl from the Monarch RNA Cleanup Kit (10 µg, NEB #T2030), 20 µl from the Monarch RNA Cleanup Kit (50 µg, NEB #T2040), and 50 µl from the Monarch RNA Cleanup Kit (500 µg, NEB #T2050).

Supplemental Protocols

Purification of RNA from the Aqueous Phase Following TRIzol/Chloroform Extraction

RNA isolation reagents containing guanidine thiocyanate and phenol (e.g., TRIzol, RNAzol®, QIAzol®, etc) combined with chloroform extraction, are often used for sample lysis and RNA purification. The aqueous phase from any guanidinium thiocyanate-phenol-chloroform extraction can be cleaned up using the Monarch RNA Cleanup Kit, thereby eliminating the need for tedious RNA precipitation steps.

Before You Begin:

- Add 4 volumes of ethanol (≥ 95%) to the Monarch Buffer WX before use, as directed on the bottle.
- All centrifugation steps should be carried out at 16,000 x *g*. (~13K RPM in a typical microcentrifuge). This ensures all traces of buffer are eluted at each step.

1. Following guanidinium-thiocyanate-phenol-chloroform extraction, carefully transfer the upper aqueous phase into an RNase-free tube (not provided).
2. Add 1 volume of ethanol (≥ 95%). Mix well by pipetting up and down or flicking the tube. Do not vortex.
3. Insert an RNA cleanup column into a collection tube, load sample onto the column and close the cap. Spin for 1 minute, then discard flow-through. For diluted samples ≥ 900 µl, load a portion of the sample, spin, and then repeat as necessary.

   To save time, spin for 30 seconds, instead of 1 minute.
4. Re-insert the column into the collection tube. Add 500 µl Buffer WX and spin for 1 minute. Discard the flow-through.

   To save time, spin for 30 seconds, instead of 1 minute.
5. Repeat wash (Step 4).
6. Transfer the column to an RNase-free 1.5 ml microfuge tube (not provided). Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to next step.
7. Elute in nuclease-free water according to the table below. The eluted RNA can be used immediately or stored at -70ºC. Care should be used to ensure the elution buffer is delivered onto the center of the matrix and not the wall of the column to maximize elution efficiency.

KIT	ELUTION VOLUME	INCUBATION TIME	SPIN TIME
T2030	6–20 µl	N/A	1 minute
T2040	20–100 µl	N/A	1 minute
T2050*	50–100 µl	5 minutes (room temp.)	1 minute

Note: When cleaning up large amounts of RNA (> 100 µg, NEB #T2050), some precipitation may occur following the addition of the Monarch Buffer BX and ethanol to the sample (Steps 1 and 2). A pellet containing the RNA of interest may form on the side of the column following the first binding spin (Step 3). To maximize recovery of this RNA, a second elution is recommended.

* Yield may slightly increase if a larger volume is used, but the RNA will be less concentrated.

To save time, spin for 30 seconds, instead of 1 minute.

Separation of Large and Small RNA into Fractions

Following this protocol enriches RNA below 200 nt into the “small RNA” fraction, while RNA above 200 nt is enriched in the “large RNA” fraction. No fractionation protocol allows precise separation of similarly sized RNA based on length alone. We find that RNA between 100–200 nt will preferentially partition to the different fractions based on various factors, including predicted secondary structure and interactions with other RNAs within the sample. When this protocol is used, highly structured molecules between 100–200 nt typically fall into the small RNA fraction, while unstructured RNA between 100–200 nt fall into the large RNA fraction.

Before You Begin:

- Add 4 volumes of ethanol (≥ 95%) to the Monarch Buffer WX before use, as directed on the bottle.
- All centrifugation steps should be carried out at 16,000 x *g*. (~13K RPM in a typical microcentrifuge). This ensures all traces of buffer are eluted at each step.

1. A starting sample volume of 50 µl is recommended. For smaller samples, nuclease-free water can be used to adjust the volume. For samples larger than 50 µl, adjust volumes accordingly.
2. Add 50 µl (1 volume) Buffer BX to the 50 µl sample.
3. Add 50 µl (1/2 volume) ethanol (≥ 95%). Mix well by pipetting up and down or flicking the tube. Do not vortex.
4. Insert an RNA Cleanup Column into a collection tube and load sample onto column (Column #1, labeled "large RNAs") and close the cap. Spin for 1 minute. **Save the flow through!** For samples with a starting volume larger than 300 µl, load a portion of the sample, spin, and then repeat as necessary.

Small RNAs are in the flow through. To isolate small RNAs, continue with Step 5. Large RNAs are bound to column #1. To isolate large RNAs, continue with Step 7.

5. Add 150 µl (1 volume) ethanol (≥ 95%) to the flow through and mix.
6. Transfer the mixture to a new column (Column #2, labeled "Small RNAs") and centrifuge for 1 minute. Discard the flow through.
7. Insert columns into collection tubes. Add 500 µl Buffer WX to each and spin for 1 minute. Discard the flow-through.
8. Repeat wash (Step 7) for both columns.
9. Transfer columns to an RNase-free 1.5 ml microfuge tube (not provided). Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to next step.
10. Elute in nuclease-free water according to the table below. The eluted RNA can be used immediately or stored at -70°C. Care should be used to ensure the elution buffer is delivered onto the matrix and not the wall of the column to maximize elution efficiency.

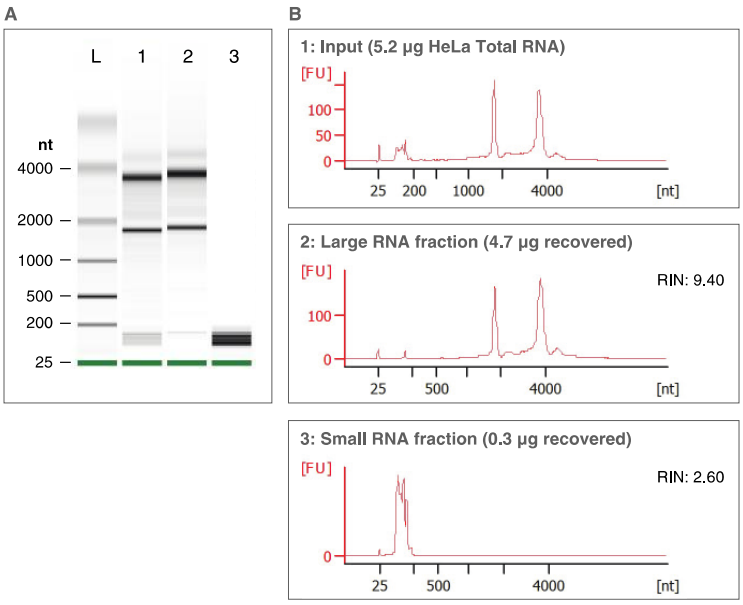
KIT	ELUTION VOLUME	INCUBATION TIME	SPIN TIME
T2030	6–20 µl	N/A	1 minute
T2040	20–100 µl	N/A	1 minute
T2050*	50–100 µl	5 minutes (room temp.)	1 minute

Note: cleaning up large amounts of RNA (> 100 µg, NEB #T2050), some precipitation may occur following the addition of the Monarch Buffer BX and ethanol to the sample (Steps 1 and 2). A pellet containing the RNA of interest may form on the side of the column following the first binding spin (Step 3). To maximize recovery of this RNA, a second elution is recommended.

* Yield may slightly increase if a larger volume is used, but the RNA will be less concentrated.

To save time, spin for 30 seconds, instead of 1 minute.

Figure 7: The Monarch RNA Cleanup Kit can be used to separate RNA into large and small RNA fractions



HeLa Total RNA (5.2 µg, A1 and B1) was separated into large RNA (A2 and B2) and small RNA (A3 and B3) fractions following the Purification of Small and Large RNAs into Separate Fractions Protocol and running on an Agilent® Bioanalyzer® RNA Pico chip. Over 96% (5 µg) of the RNA input was recovered in the separate RNA fractions. Samples 1 and 2 were diluted 1:20 prior to analysis.

Extraction of RNA from Agarose Gels

The Monarch RNA Cleanup Kits can be used to extract RNA from agarose gels, although this is not their primary application. RNA recovery from agarose gel extraction ranges from extraction of RNA from agarose range from 40-70%, which is less than expected recovery for RNA Cleanup from enzymatic reactions.

Before You Begin:

- Add 4 volumes of ethanol (≥ 95%) to the Monarch Buffer WX before use, as directed on the bottle
- All centrifugation steps should be carried out at 16,000 x *g*. (~13K RPM in a typical microcentrifuge). This ensures all traces of buffer are eluted at each step.

1. Excise the RNA fragment to be purified from the agarose gel using a razor blade, scalpel or other clean cutting tool. Use care to trim excess agarose from the perimeter of the band to minimize the amount of binding buffer needed, and reduce the time necessary to extract the RNA.
2. Transfer the excised gel slice to a 1.5 ml microcentrifuge tube and weigh it. Using UV light to visualize the slice is common, but exposure time should be kept as short as possible to minimize damage to the RNA. Use long-wave UV when possible, as shorter wavelengths induce greater damage.
3. Add 4 volumes of Monarch Buffer BX to the tube with the slice. For example, add 400 µl to a 100 mg gel slice. Incubate the sample between 37–55°C (typically 50°C), gently mixing periodically until the gel slice is completely dissolved (generally 5–10 minutes). Failure to dissolve all the agarose will decrease the recovery yield due to incomplete extraction of the RNA and potential clogging of the column by particles of agarose.
4. Add 1 volume of ethanol (≥ 95%) to the sample and mix well by pipetting up and down or flicking the tube. Do not vortex; vortexing may cause degradation of the RNA.
5. Incubate the sample between 37–55°C (typically 50°C) for an additional 5 minutes to ensure the agarose remains completely dissolved and to avoid potential clogging of the column.
6. Insert an RNA cleanup column into a collection tube, load the sample onto the column and close the cap. Spin for 1 minute, then discard flow-through. For diluted samples with a volume larger than 900 µl, load a portion of the sample, spin, and then repeat as necessary.

To save time, spin for 30 seconds, instead of 1 minute.

7. Re-insert the column into the collection tube. Add 500 µl Buffer WX and spin for 1 minute. Discard the flow-through.
8. Repeat wash (Step 7).
9. Transfer the column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column has not come into contact with the flow-through. If in doubt, re-spin for 1 minute before placing into clean microfuge tube.
10. Elute in nuclease-free water according to the table below. Incubate the column and elution for 5 minutes at 65°C prior to spinning. The eluted RNA can be used immediately or stored at -70°C.

KIT	ELUTION VOLUME	INCUBATION TIME	SPIN TIME
T2030	6–20 µl	5 minutes	1 minute
T2040	20–100 µl	5 minutes	1 minute
T2050*	50–100 µl	5 minutes	1 minute

Note: cleaning up large amounts of RNA (> 100 µg, NEB #T2050), some precipitation may occur following the addition of the Monarch Buffer BX and ethanol to the sample (Steps 1 and 2). A pellet containing the RNA of interest may form on the side of the column following the first binding spin (Step 3). To maximize recovery of this RNA, a second elution is recommended.

* Yield may slightly increase if a larger volume is used, but the RNA will be less concentrated.

To save time, spin for 30 seconds, instead of 1 minute.

RNA Quantification

- Quantitation of RNA can be performed using direct spectrophotometric measurement (Nanodrop, Trinean), 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 (Trinean DropSense) 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.

RNA Purity & Integrity

- 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 buffer pH, and contaminants such as protein, buffer salts, ethanol, etc.

Troubleshooting

Low RNA Yield

- Reagents added incorrectly. Check protocol to ensure correct buffer reconstitution, order of addition for buffers and ethanol, and proper handling of column flow-through and eluents.
- Insufficient mixing of reagents. Ensure the ethanol is thoroughly mixed with mixture of RNA sample and Buffer BX before applying sample to the purification column.
- Incomplete elution during prep. Ensure the nuclease-free water used for elution is delivered directly to the center of the column so that the matrix is completely covered and elution is efficient. Larger elution volumes and longer incubation times can increase yield of RNA off the column at the cost of dilution of the sample and increased processing times. For typical RNA samples, the recommended elution volumes and incubation times should be sufficient. Additionally, multiple rounds of elution can be employed to increase the amount of RNA eluted, at the expense of dilution of the sample. The first elution can be used to elute a second time to maximize recovery and minimize sample dilution.
- Binding and elution of smaller RNAs (< 45 nt) can be affected by secondary structure of the RNA molecules (Figure 3). If poor yield of a small RNA is observed, we recommend diluting your sample with 2 volumes of ethanol instead of 1 volume in Step 2.
- If using the Extraction of RNA from Agarose Gel Protocol (page 10), make sure to incubate the sample between 37-55°C after addition of both the Buffer BX and the ethanol. Additionally, incubate the column with nuclease-free water at 65°C for 5 minutes prior to spinning to elute the RNA.

Purified RNA is Degraded

- RNase contamination. In order to avoid RNase contamination during RNA cleanup, make sure to wear gloves and use disposable RNase-free tips and collection tubes (not provided) during the procedure. Keep all kit components tightly sealed when not in use.
- Improper storage of RNA. Purified RNA should be used immediately in downstream applications or stored at -70°C.

Low OD Ratios

- Low $A_{260/230}$ values indicate residual guanidine salts have been carried over during elution. Ensure wash steps are carried out prior to eluting sample. 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 Kimwipe prior to reattachment to the column to remove any residual wash buffer.

Low Performance of RNA in Downstream Steps

- Salt and/or ethanol carry-over. Ethanol and salt remaining after the washes 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-spin 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) and cleanup RNA using the Monarch RNA Cleanup Protocol.

Ordering Information

PRODUCT	NEB #	SIZE
Monarch RNA Cleanup Kit (10 µg)	T2030S/L	10/100 preps
Monarch RNA Cleanup Kit (50 µg)	T2040S/L	10/100 preps
Monarch RNA Cleanup Kit (500 µg)	T2050S/L	10/100 preps
Columns sold separately		
Monarch RNA Cleanup Columns (10 µg)	T2037L	100 columns + collection tubes
Monarch RNA Cleanup Columns (50 µg)	T2047L	100 columns + collection tubes
Monarch RNA Cleanup Columns (500 µg)	T2057L	100 columns + collection tubes
Monarch Collection Tubes II	T2018L	100 collection tubes
Buffers sold separately		
Monarch Buffer BX	T2041L	80 ml
Monarch Buffer WX	T2042L	40 ml
Nuclease-free Water	B1500S/L	25/100 ml

COMPANION PRODUCTS

PRODUCT	NEB #	SIZE
DNase I (RNase-free)	M0303S/L	1,000/5,000 units
Proteinase K, Molecular Biology Grade	P8107S	2 ml
EnGen® sgRNA Synthesis Kit, *S. pyogenes*	E3322S	20 rxns
HiScribe T7 High Yield RNA Synthesis Kit	E2040S	50 rxns
HiScribe T7 Quick High Yield RNA Synthesis Kit	E2050S	50 rxns
NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina®	E7760S/L	24/96 rxns
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 rxns
NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 rxns
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 rxns
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)	E7330S/L	24/96 rxns
Luna Universal One-Step RT-qPCR Kit	E3005S/L	200/500 rxns
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L	200/500 rxns

LunaScript® RT SuperMix Kit	E3010S/L	25/100 rxns
RELATED PRODUCTS		
Monarch Total RNA Miniprep Kit	T2010S	50 preps
Monarch Plasmid Miniprep Kit	T1010S/L	50/250 preps
Monarch DNA Gel Extraction Kit	T1020S/L	50/250 preps
Monarch PCR & DNA Cleanup Kit (5 µg)	T1030S/L	50/250 preps
Thermolabile Proteinase K	P8111S	30 units

Revision History

REVISION #	DESCRIPTION	DATE
1.0	New Document	6/18
2.0		8/18
3.0	New format applied	4/20
4.0	Updated names of buffers, formatting, header and footer	4/24

How to Recycle Monarch Kit Components*

Component	Recycling Notes**
Kit Box (paper)	For the greatest environmental benefit, please reuse this box. It is fully recyclable in paper recycling. The small magnets do not prohibit recycling.
Columns and Collection Tubes (hard plastic)	Columns and collection tubes are made from polypropylene 5 and are recyclable. After use, please refer to your institutional policies for proper disposal, especially when working with biohazardous materials.
Plastic Bottles (hard plastic)	Bottles are made from high-density polyethylene 2, and caps are polypropylene 5. Please rinse before recycling.
Plastic Bags (plastic film)	Bags are made from low-density polyethylene 4 and can be recycled with other plastic bags and films.
Protocol Card (paper)	Recycle with mixed paper, or keep in your lab notebook for reference. The finish on this card does not prohibit recycling.

* Information as of November 2015. Please visit NEBMonarchPackaging.com for updates.
** Please defer to your institutional policies for proper disposal of this kit and its components.
Consult with your local and institutional authorities to learn how to maximize your landfill diversion and materials recovery.

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

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