

## **EXTRAClean RNA Clean-Up and Concentration Micro-Elute Kit**

### **Product Insert**

**Product #73600**

Norgen's EXTRAClean RNA Clean-Up and Concentration Micro-Elution Kit provides a rapid method for the purification, cleanup and concentration of up to 10 µg of RNA isolated using different methods including phenol/guanidine-based protocols, and from various upstream enzymatic reactions such as DNase treatment, labeling and *in vitro* transcription. The minimum recommended elution volume is 8 µL, which enables the concentration of small amounts of all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other reaction components such as proteins, RNases and nucleotides, without the use of phenol or chloroform. The EXTRAClean columns undergo stringent processing and rigorous quality control measures to minimize contamination traces, ensuring optimal results for sensitive applications such as NGS. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including end-point or quantitative reverse transcription PCR, Northern blotting, RNase protection and primer extension, expression array assays and next generation sequencing.

### **Norgen's Purification Technology**

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first mixing the RNA samples or enzymatic reactions containing RNA with Buffer RL (please see the flow chart on page 4). Ethanol is then added and the mixture is loaded onto an activated spin-column specifically designed for small elution volumes. Norgen's resin binds RNA in a manner that depends on ionic concentrations. Thus, only the RNA will bind to the column, while the contaminating proteins or nucleotides will be removed in the flowthrough. The bound RNA is then washed three times with the provided Wash Solution A in order to remove any remaining impurities. The purified RNA is then eluted using 8 – 15 µL of Elution Solution A. The purified RNA is of the highest integrity, and can be used in a number of downstream applications. Norgen's RNA Clean-Up and Concentration Micro-Elution Kit purifies RNA with minimal amounts of DNA contamination. An optional protocol is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications such as quantitative PCR.

### **Advantages**

- Concentration of small amounts of RNA into 8 µL
- Ideal for concentrating RNA purified from exosomes, plasma, serum, urine, and other bodily fluids and any RNA samples initially purified in large volumes
- Efficient RNA cleanup from enzymatic reactions – labeling, DNase treatment and *in vitro* transcription
- Cleanup of RNA isolated using different methods, including phenol/chloroform extractions
- Fast and easy processing using rapid spin-column format
- Suitable for all sizes of RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions

## Kit Components

Component	Product #73600 (50 preps)
Buffer RL	40 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Column Activation Solution	30 mL
EXTRAClean Micro-Elute RNA Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

## Specifications

Kit Specifications	
Maximum Column Binding Capacity	10 µg
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material:	10 µg of RNA
Minimum Elution Volume	8 µL
Time to Complete 10 Purifications	20 minutes
Average Recovery	≥ 90%

### Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

### Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at [www.norgenbiotek.com](http://www.norgenbiotek.com).

The **Buffer RL** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

### Customer-Supplied Reagents and Equipment

You must have the following in order to use the RNA Clean-up and Concentration Micro Kit:

For RNA Clean-Up and Concentration from Enzymatic Reactions or Previously Isolated RNA

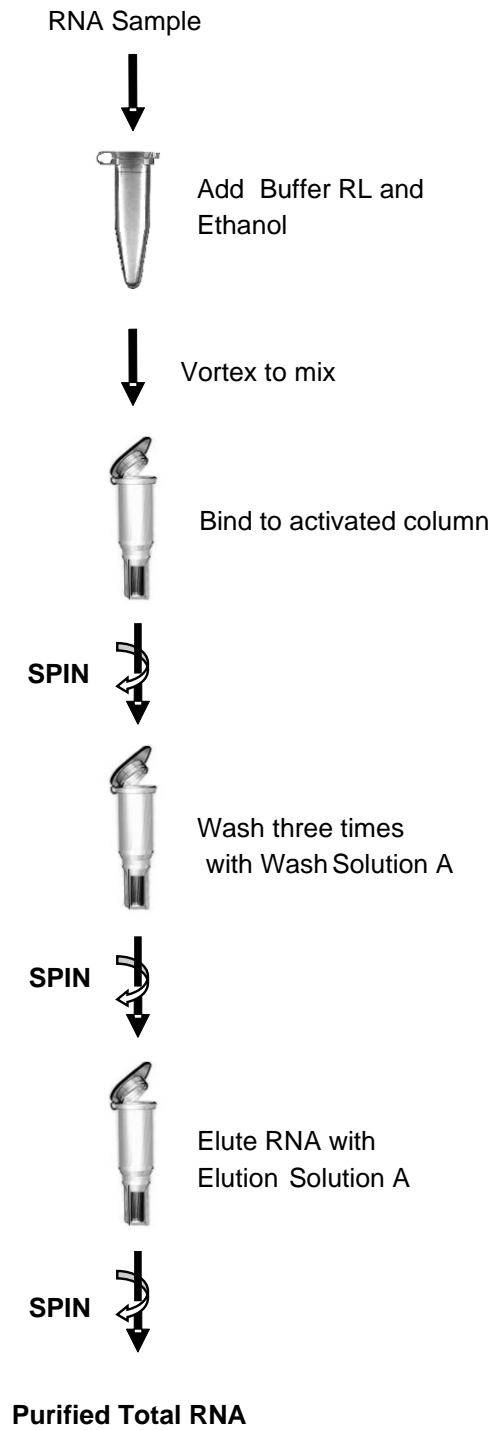
- Benchtop microcentrifuge
- β-mercaptoethanol
- 96 - 100% ethanol
- RNase-free or DEPC-treated water

For RNA Clean-up and Concentration from Aqueous Phase (RNA fraction) of Phenol/Guanidine-Based RNA (Trizol or Tri Reagent) Isolation Methods

- Benchtop microcentrifuge
- 70% ethanol

## Flow Chart

Procedure for Purifying RNA using Norgen's EXTRAClean RNA Clean-up and Concentration Micro Kit



## Procedures

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}$$

where  $RCF$  = required gravitational acceleration (relative centrifugal force in units of g);  $r$  = radius of the rotor in cm; and  $RPM$  = the number of revolutions per minute required to achieve the necessary  $g$ -force.

## Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware are not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

## A. Protocol for RNA Clean-Up and Concentration from Enzymatic Reactions or Previously Isolated RNA

### Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle(s) containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare an appropriate amount of Buffer RL by adding 10  $\mu$ L of  $\beta$ -mercaptoethanol (provided by the user) to each 1 mL of Buffer RL required.  $\beta$ -mercaptoethanol is toxic and should be dispensed in a fume hood.
- It is recommended that no more than 10  $\mu$ g of RNA be used per cleanup.
- The maximum volume of RNA sample that can be processed is 200  $\mu$ L.
- It is important to work quickly during this procedure.
- This kit purifies RNA with minimal amounts of DNA contamination. However, an optional protocol is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications such as quantitative PCR. The procedure in Appendix A is to be carried out prior to performing the kit procedure below.

## 1. Sample Preparation

- a. Adjust the volume of the RNA sample to 100  $\mu\text{L}$  by adding RNase-free or DEPC-treated water. It is recommended that no more than 10  $\mu\text{g}$  of RNA be used for each column.

**Note:** If an input volume between 100 and 200  $\mu\text{L}$  is used, adjust the sample volume to 200  $\mu\text{L}$  (maximum allowable) with RNase-free or DEPC-treated water. In this case, use the volumes indicated in bold in the bracket in Steps 1b and 1c.

- b. Add 250  $\mu\text{L}$  (**or 500  $\mu\text{L}$** ) of Buffer RL to the RNA sample. Mix by vortexing.
- c. Add 200  $\mu\text{L}$  (**or 400  $\mu\text{L}$** ) of 96 – 100% ethanol (provided by the user) to the mixture from Step 1b. Mix by vortexing for 10 seconds.

**Note:** If the sample being processed is highly enriched for small RNA (such as microRNA, siRNA, Piwi-interacting RNA, etc.) increase the amount of 96 – 100% ethanol added to the mixture from Step 1b. In this case, add 350  $\mu\text{L}$  (or 700  $\mu\text{L}$  for 200  $\mu\text{L}$  original input volume) of 96 – 100% ethanol in Step 1c. RNA isolated from bodily fluids (serum, plasma, urine, etc.) and exosomes are considered to belong to this category. If the sample contains a heterogeneous mixture of a range of RNA sizes, including large RNA, follow the procedure as outlined in Step 1c without adjustments.

## 2. Column Activation and Sample Binding to EXTRAClean Micro-Elute Spin Column

- a. Assemble a column with one of the provided collection tubes.
- b. Apply 500  $\mu\text{L}$  of Column Activation Solution onto the column and centrifuge for 1 minute.
- c. Discard the flowthrough. Reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
- d. Apply up to 600  $\mu\text{L}$  of the RNA sample with the ethanol (from Step 1c) onto the activated column and centrifuge for 1 minute.
- e. Discard the flowthrough. Reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
- f. If the volume of the RNA sample is greater than 600  $\mu\text{L}$ , repeat Steps 2d and 2e until all the remaining RNA sample has passed through the column.

## 3. EXTRAClean Micro-Elute Spin Column Wash

- a. Apply 400  $\mu\text{L}$  of Wash Solution A to the column and centrifuge for 1 minute.

**Note:** Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.
- b. Discard the flowthrough and reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
- c. Repeat steps 3a and 3b to wash the column a second time.
- d. Wash column a third time by adding another 400  $\mu\text{L}$  of Wash Solution A and centrifuging for 1 minute.
- e. Discard the flowthrough and reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
- f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

## 4. RNA Elution

- a. Place the EXTRAClean Micro-Elute Spin Column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 8 – 15  $\mu\text{L}$  of Elution Solution A to the column.

**Note:** For maximum concentrations of RNA, the 8 elution  $\mu\text{L}$  volume may be used. For maximum recovery of RNA the 15  $\mu\text{L}$  volume is recommended

- c. Centrifuge for 2 minutes at 200 x g (~2,000 RPM), followed by 1 minute at 14,000 x g (~14,000 RPM). Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

**Note:** For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat Steps 4b and 4c).

## 5. Storage of RNA

- a. The purified RNA sample may be stored at -20°C for a few days. It is recommended that samples be placed at -70°C for long term storage.

## B. Protocol for RNA Clean-up and Concentration from Aqueous Phase (RNA fraction) of Phenol/Guanidine-Based RNA (Trizol or Tri Reagent) Isolation Methods

### Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the Wash Solution A by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle(s) containing the concentrated Wash Solution A. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is recommended that no more than 10 µg of RNA to be used per cleanup.
- It is important to work quickly during this procedure.

### 1. Sample Preparation

- a. Isolate RNA using a phenol/guanidine-based reagent such as Trizol or Tri Reagent, according to manufacturer's instruction. After the separation of the aqueous and organic phases, collect the upper (aqueous) fraction containing the RNA into a new RNase-free microcentrifuge tube (not provided). Note the volume.
- b. Add one volume of 70% ethanol (provided by the user) to the fraction from step 1a. Mix by vortexing for 10 seconds.

**Note:** If the RNA sample being processed is highly enriched for small RNA (such as microRNA, siRNA, Piwi-interacting RNA, etc.) increase the amount of ethanol added to the fraction from Step 1a. In this case, add one volume of 96 – 100% ethanol in Step 1b. RNA isolated from bodily fluids (serum, plasma, urine, etc.) and exosomes are considered to belong to this category. If the sample contains a heterogeneous mixture of a range of RNA sizes, including large RNA, follow the procedure as outlined in Step 1b without adjustments.

### 2. Column Activation and Sample Binding to EXTRAClean Micro-Elute Spin Column

- a. Assemble a column with one of the provided collection tubes.
- b. Apply 500 µL of Column Activation Solution onto the column and centrifuge for 1 minute.
- c. Discard the flowthrough. Reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
- d. Apply up to 600 µL of the RNA sample with the ethanol (from Step 1b) onto the activated column and centrifuge for 1 minute.
- e. Discard the flowthrough. Reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.

- f. If the volume of the RNA sample is greater than 600  $\mu\text{L}$ , repeat Steps 2d and 2e until all the remaining RNA sample has passed through the column.

### 3. EXTRAClean Micro-Elute Spin Column Wash

- a. Apply 400  $\mu\text{L}$  of Wash Solution A to the column and centrifuge for 1 minute.  
**Note:** Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.
- c. Discard the flowthrough and reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
- d. Repeat steps 3a and 3b to wash the column a second time.
- e. Wash column a third time by adding another 400  $\mu\text{L}$  of Wash Solution A and centrifuging for 1 minute.
- f. Discard the flowthrough and reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
- g. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

### 4. RNA Elution

- a. Place the EXTRAClean Micro-Elute Spin Column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 8 – 15  $\mu\text{L}$  of Elution Solution A to the column.  
**Note:** For maximum concentrations of RNA, use the 8  $\mu\text{L}$  elution volume. For maximum recovery of RNA the 15  $\mu\text{L}$  volume is recommended.
- c. Centrifuge for 2 minutes at 200 x  $g$  (~2,000 RPM), followed by 1 minute at 14,000 x  $g$  (~14,000 RPM). Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x  $g$  (~14,000 RPM) for 1 additional minute.  
**Note:** For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat Steps 4b and 4c).

### 5. Storage of RNA

- a. The purified RNA sample may be stored at  $-20^{\circ}\text{C}$  for a few days. It is recommended that samples be placed at  $-70^{\circ}\text{C}$  for long term storage.

## Appendix A: Optional DNA Removal in Solution Followed by RNA Clean-Up and Concentration

Norgen's EXTRAClean RNA Clean-Up and Concentration Micro-Elute Kit purifies RNA with minimal amounts of DNA contamination. An optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications such as quantitative PCR. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step. This procedure is to be performed prior to starting the kit protocol.

1. Adjust the volume of the RNA sample to be treated to 50  $\mu\text{L}$  with RNase-free water.
2. Add 45  $\mu\text{L}$  of the provided Enzyme Incubation Buffer.
3. Add 5  $\mu\text{L}$  of Norgen's DNase I to the RNA sample. **Gently mix by pipetting the sample up and down using a pipette. Do not vortex.**
4. Incubate at 25 to 30 $^{\circ}\text{C}$  for 15 minutes.
5. Proceed directly to Protocol A "**Protocol for RNA Clean-up and Concentration from Enzymatic Reactions or Previously Isolated DNA**".

## Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.
	An alternative elution solution was used	It is recommended that the Elution Solution A supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
Clogged Column	High amounts of RNA in the input	Ensure that no more than 10 µg of RNA is used as the input for each column.
	High amounts of genomic DNA present in sample	The lysate may be passed through a 25-gauge needle attached to a syringe 5-10 times in order to shear the genomic DNA prior to loading onto the column.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.
RNA is Degraded	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to “ <i>Working with RNA</i> ” at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.



Problem	Possible Cause	Solution and Explanation
RNA does not perform well in downstream applications	RNA was not washed three times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
DNA or Genomic DNA contamination	Large amounts of starting material used	Perform RNase-free DNaseI digestion on the RNA sample prior to starting the protocol as outlined in Appendix A. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

Related Products	Product #
RNA Clean-Up and Concentration Micro Kit	23600
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
CleanAll DNA/RNA Clean-Up and Concentration Micro Kit	23800
100b RNA Ladder	15002
1kb RNA Ladder	15003

### Technical Support

Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website ([www.norgenbiotek.com](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

Norgen's purification technology is patented and/or patent pending. See [www.norgenbiotek.com/patents](http://www.norgenbiotek.com/patents)

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