

**EXTRAClean CleanAll DNA/RNA Clean-up and Concentration Micro Kit**  
**Product # 74600** **Product Insert**

Norgen's EXTRAClean CleanAll DNA/RNA Clean-up and Concentration Micro Kit provides a rapid method for the purification, cleanup and concentration of DNA or RNA from different isolation methods or upstream applications. This kit purifies all sizes of DNA, from small PCR products, native or linearized plasmids, to genomic DNA. The kit can be used as an alternative to organic extraction and ethanol precipitation to clean up various enzymatic reactions. It effectively removes PCR by-products including primers, dimers, enzymes, and unincorporated nucleotides. The purified DNA is fully compatible with restriction enzyme digestion, ligation, labeling, PCR and sequencing.

This kit also purifies RNA from phenol/guanidine-based protocols or from various upstream enzymatic reactions such as DNase treatment, labeling and *in vitro* transcription. The kit purifies 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 and nucleotides, without the use of phenol or chloroform. 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, and expression array assays. Norgen's EXTRAClean CleanAll DNA/RNA Clean-up and Concentration Micro Kit also provides a protocol for the rapid removal of endotoxins from previously purified DNA down to 0.1 EU/ $\mu$ g DNA or less. Endotoxins, also known as lipopolysaccharides, are cell-membrane components of Gram-negative bacteria such as *E. coli*. Endotoxins are released during the lysis step of plasmid purification and significantly reduce transfection efficiencies in endotoxin sensitive cell lines. Therefore, the removal of endotoxins from plasmid preparations is often necessary prior to the use of the DNA in downstream applications.

**Norgen's Purification Technology**

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. 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 DNA or RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first mixing the nucleic acid samples or enzymatic reactions containing DNA or RNA with Binding Buffer H (please see the flow chart on page 5). For RNA purification, ethanol is then added, and the mixture is loaded onto a spin-column. Norgen's resin binds nucleic acids in a manner that depends on ionic concentrations. Thus, only the nucleic acids will bind to the column, while the contaminating proteins or nucleotides will be removed in the flowthrough. The bound nucleic acid is then washed two times with the provided Wash Solution K in order to remove any remaining impurities. The purified DNA or RNA is eluted with Elution Buffer L. The purified DNA or RNA is of the highest integrity and can be used in a number of downstream applications.

**Kit Components**

Component	Product # 74600 (50 samples)
Binding Buffer H	60 mL
Wash Solution K	27 mL
Elution Buffer L	15 mL
EXTRAClean Micro Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50 (2 x 25)
Product Insert	1

### Advantages

- Versatile performance for cleaning up all nucleic acids
- Fast and easy processing using rapid spin-column format
- Suitable for all sizes of RNA, from large rRNA down to microRNA (miRNA)
- Suitable for all sizes of DNA, from large gDNA down to small PCR fragments
- Optional endotoxin removal
- No phenol or chloroform extractions
- Cleanup nucleic acid from a variety of isolation methods and enzymatic reactions

### Kit Specifications

Kit Specifications	
Column Binding Capacity	50 µg for RNA, 10 µg for DNA
Maximum Column Loading Volume	600 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Size of DNA Purified	≥ 100 bp*
Maximum Amount of Starting Material:	50 µg of RNA, 10 µg of DNA
Time to Complete 10 Purifications	20 minutes
Minimum Elution Volume	20 µL
Average Recovery	≥ 90% for RNA ≥ 90% for DNA from 100 bp to 10 kbp ≥ 75% for DNA ≥ 10 kbp

\* Applicable to smaller fragments or oligonucleotides with alternative protocol.

### Kit Applications:

Applications Requiring RNA Cleanup	Contaminants Removed
DNase treatment	DNase, salts
<i>In vitro</i> transcription	DNA template, nucleotides, enzymes, salts
RNA Probe Labeling (Radioactive, Fluorescent or Digoxigenin)	RNA polymerase, nucleotides, salts, label or coupling reagent
Organic-based RNA Isolation (such as Trizol-based lysate)	Phenol, chloroform

Applications Requiring DNA Cleanup	Contaminants Removed
Restriction Enzyme Digestion	Restriction enzymes, salts
DNA Modifications (Klenow, T4 Polynucleotide Kinase, Alkaline Phosphatase)	DNA modification enzymes, nucleotides, salts
PCR Reactions	DNA polymerases, nucleotides, salts, primers, mineral oils
DNA Probe Labeling (Radioactive, Fluorescent or Digoxigenin)	DNA modification enzymes or polymerase, nucleotides, label or coupling reagent, salts, primers
Ligation	Ligase, nucleotides, salts
Transfection, Microinjection	Endotoxin
DNA Staining (Including CsCl-gradients)	Intercalating dyes such as ethidium bromide and SYBR Green, salts

### Storage Conditions and Product Stability

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

### Quality Control

In accordance with Norgen's Quality Management System, each lot of Norgen's EXTRAClean CleanAll DNA/RNA Clean-up and Concentration Micro Kit is tested against predetermined specifications to ensure consistent product quality.

### Product Use Limitations

Norgen's EXTRAClean CleanAll DNA/RNA Clean-up and Concentration Micro Kit is designed for research purposes only. They are not intended for human or diagnostic use.

### Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

### Safety Information

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).

### Customer-Supplied Reagents and Equipment

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

#### *For All Protocols*

- Benchtop microcentrifuge
- Microcentrifuge tubes
- 96 - 100% ethanol

#### *For Endotoxin Removal*

- Isopropanol

For RNA Clean-up and Concentration

- $\beta$ -mercaptoethanol

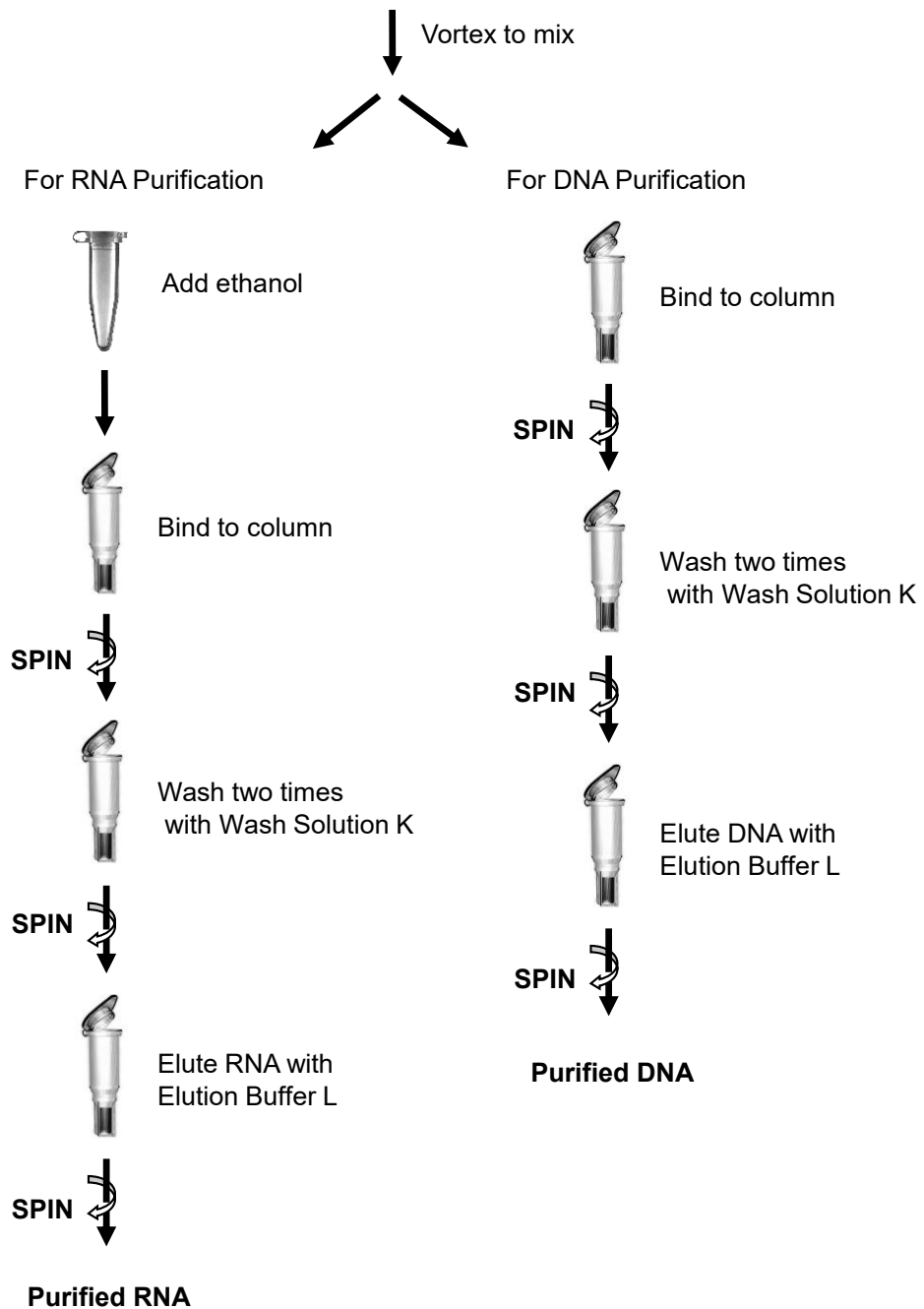
For RNA Clean-up and Concentration from Phenol/Guanidine-based RNA (Trizol or Tri Reagent) Isolation Methods

- 70% ethanol

### Flow Chart

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

Obtain Nucleic Acid Sample (RNA or DNA) and add Binding Buffer H



## 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.

Since Norgen's EXTRAClean CleanAll DNA/RNA Clean-up and Concentration Micro Kit uses common solutions for DNA and RNA clean-up, it is important for user to maintain an RNase-free environment if both procedures are to be carried out, either simultaneously or separately.

### 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 workstations
- 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 DNA Cleanup

### Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution K** by adding 54 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution K**. This will give a final volume of 81 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 DNA be used per cleanup.
- The maximum volume of DNA sample that can be processed is 200 µL.

### 1. Sample Preparation

- a. Adjust the volume of the DNA sample to 100 µL by adding molecular biology-grade water. It is recommended that no more than 10 µg of DNA be used for each column.  
**Note:** If an input volume between 100 and 200 µL is used, adjust the sample volume to 200 µL (maximum allowable) with molecular biology-grade water. In this case, use the volumes indicated in **bold** in the bracket in Steps **1b**
- b. Add 500 µL (or **1 mL**) of **Binding Buffer H** to the DNA sample. Mix by vortexing

### 2. Binding to Column

- a. Assemble an EXTRAClean micro spin column with one of the provided collection tubes.

- b. Apply up to 600  $\mu\text{L}$  of the DNA sample (from **Step 1b**) onto the EXTRAClean micro spin column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).
- c. Discard the flowthrough. Reassemble the EXTRAClean micro spin column with its collection tube.
- d. If the volume of the DNA sample is greater than 600  $\mu\text{L}$ , repeat **Step 2b** and **2c** until all the remaining DNA sample has passed through the EXTRAClean micro spin column.

### 3. Column Wash

- a. Apply 500  $\mu\text{L}$  of **Wash Solution K** to the EXTRAClean micro spin column assembly and centrifuge the unit for 2 minutes at 14,000 x g.
- b. Discard the flowthrough and reassemble the unit.
- c. Spin the EXTRAClean micro spin column for an additional 1 minute at 14,000 x g, in order to completely dry the resin. Discard the collection tube.
- d. Ensure that the columns are dry. Spin for an additional 1 minute at 14,000 x g if necessary.
- e. Discard the collection tube with the flowthrough.

### 4. Elution of Clean DNA

- a. Assemble the EXTRAClean micro spin column with one of the provided 1.7 mL **Elution tubes**.
- b. Elute DNA with the provided **Elution Buffer L** according to the following table:

	10,000 bp or larger	1000 bp to 10,000 bp	Smaller than 1000 bp
<b>Recommended Elution Volume</b>	100 $\mu\text{L}$	50 $\mu\text{L}$	50 $\mu\text{L}$
<b>Minimum Elution Volume</b>	50 $\mu\text{L}$	20 $\mu\text{L}$	20 $\mu\text{L}$
<b>Elution Speed</b>	2 minutes at 200 x g followed by 1 minute at 14,000 x g	2 minutes at 200 x g followed by 1 minute at 14,000 x g	1 minute at 14,000 x g

- c. (Optional): An additional elution can be performed by repeating steps **3a** and **3b**. This elution should be collected into a separate tube to avoid diluting the DNA solution in the first elution.

## B. Protocol for DNA Cleanup with Endotoxin Removal

### Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution K** by adding 54 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution K**. This will give a final volume of 81 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  $\mu\text{g}$  of DNA be used per cleanup.
- The maximum volume of DNA sample that can be processed is 200  $\mu\text{L}$ .

### 1. Sample Preparation

- a. Transfer up to 100  $\mu\text{L}$  of DNA into a microcentrifuge tube. Add 500  $\mu\text{L}$  of **Binding Buffer H** to the DNA and mix well by inversion or vortexing.
- b. Assemble an EXTRAClean micro spin column with a provided collection tube. Add the DNA solution to the top of the column.
- c. Let the column assembly stand for 5 minutes at room temperature.

**Note:** If dripping occurs into the collection tube during the 5-minute incubation period, just proceed with protocol as written.

- d. After 5 minutes, add 60  $\mu\text{L}$  of isopropanol to the liquid on the column. Close lid and vortex column assembly **gently** to mix.

## 2. Binding to Column

- a. Spin the EXTRAClean micro spin column at 14,000 x g (~14,000 RPM) for 1 minute in a microcentrifuge.
- b. Discard the flowthrough and reassemble the EXTRAClean micro spin column with its collection tube.

## 3. Washing Bound DNA

- a. Apply 500  $\mu\text{L}$  of **Wash Solution K** to the EXTRAClean micro spin column assembly and centrifuge the unit for 2 minutes at 14,000 x g.
- b. Discard the flowthrough and reassemble the unit.
- c. Spin the EXTRAClean micro spin column for an additional 1 minute at 14,000 x g, in order to completely dry the resin. Discard the collection tube.

## 4. Elution of Clean DNA

- a. Assemble the column with one of the provided 1.7 mL **Elution tubes**.
- b. Elute DNA with the provided **Elution Buffer L** according to the following table:

	10,000 bp or larger	1000 bp to 10,000 bp	Smaller than 1000 bp
<b>Recommended Elution Volume</b>	100 $\mu\text{L}$	50 $\mu\text{L}$	50 $\mu\text{L}$
<b>Minimum Elution Volume</b>	50 $\mu\text{L}$	20 $\mu\text{L}$	20 $\mu\text{L}$
<b>Elution Speed</b>	2 minutes at 200 x g followed by 1 minute at 14,000 x g	2 minutes at 200 x g followed by 1 minute at 14,000 x g	1 minute at 14,000 x g

- c. (Optional): An additional elution can be performed by repeating steps **4a** and **4b**. This elution should be collected into a separate tube to avoid diluting the DNA solution in the first elution.

## C. Protocol for RNA Clean-up and Concentration from Enzymatic Reactions or Other Non-Phenol/Guanidine-Based RNA Isolation Methods

### Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution K** by adding 54 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution K**. This will give a final volume of 81 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 Binding Buffer H by adding 10  $\mu\text{L}$  of  $\beta$ -mercaptoethanol (provided by the user) to each 1 mL of Binding Buffer H required.  $\beta$ -mercaptoethanol is toxic and should be dispensed in a fume hood.
- It is recommended that no more than 50  $\mu\text{g}$  of RNA be used per cleanup.
- The maximum volume of RNA sample that can be processed is 200  $\mu\text{L}$ .
- 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.

## 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 50  $\mu\text{g}$  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 **Binding Buffer H** 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.

## 2. Binding to Column

- a. Assemble an EXTRAClean micro spin column with one of the provided collection tubes.
- b. Apply up to 600  $\mu\text{L}$  of the RNA sample with the ethanol (from **Step 1c**) onto the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).
- c. Discard the flowthrough. Reassemble the EXTRAClean micro spin column with its collection tube.
- d. If the volume of the RNA sample is greater than 600  $\mu\text{L}$ , repeat Step **2b** and **2c** until all the remaining RNA sample has passed through the column.

## 3. Column Wash

- a. Apply 500  $\mu\text{L}$  of **Wash Solution K** to the column and centrifuge for 1 minute at 14,000 x g.  
**Note:** Ensure the entire wash solution 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 spin column with its collection tube.
- c. Wash the EXTRAClean micro spin column a second time by adding another 500  $\mu\text{L}$  of **Wash Solution K** and centrifuge for 2 minutes at 14,000 x g.
- d. Ensure that the column is dry. Spin for an additional minute at 14,000 x g, if necessary.
- e. Discard the collection tube with the flowthrough.

## 4. RNA Elution

- a. Place the EXTRAClean micro spin column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 50  $\mu\text{L}$  of **Elution Buffer L** to the column.  
**Note:** For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 20  $\mu\text{L}$  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

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.

## D. Protocol for RNA Clean-up and Concentration from Phenol/Guanidine-based RNA (Trizol or Tri Reagent) Isolation Methods

### Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.

- Prepare a working concentration of the **Wash Solution K** by adding 54 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution K**. This will give a final volume of 81 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 50 µg of RNA be used per cleanup.
- It is important to work quickly during this procedure.

### 1. Sample Preparation

- 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) fractions containing the RNA into a new RNase-free microcentrifuge tube (not provided). Note the volume.
- Add one volume of 70% ethanol (provided by the user) to the fraction from step **1a**. Mix by vortexing for 10 seconds.

### 2. Binding to Column

- Assemble an EXTRAClean micro spin column with one of the provided tubes.
- Apply up to 600 µL of the RNA mixed with the ethanol (from **Step 1b**) onto the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).
- Discard the flowthrough. Reassemble the EXTRAClean micro spin column with its collection tube.
- If the volume of RNA mix is greater than 600 µL, repeat Steps **2b** and **2c** until all the remaining RNA mix has passed through the column.

### 3. Column Wash

- Apply 500 µL of **Wash Solution K** to the EXTRAClean micro spin column and centrifuge for 1 minute at 14,000 x g.  
**Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.
- Discard the flowthrough and reassemble the EXTRAClean micro spin column with its collection tube.
- Repeat steps **3a** and **3b** to wash the EXTRAClean micro spin column a second time.
- Wash column a third time by adding another 500 µL of **Wash Solution K** and centrifuge for 2 minutes at 14,000 x g.
- Ensure that the column is dry. Spin for an additional minute at 14,000 x g, if necessary.
- Discard the collection tube with the flowthrough.

### 4. RNA Elution

- Place the EXTRAClean micro spin column into a fresh 1.7 mL Elution tube provided with the kit.
- Add 50 µL of **Elution Buffer L** to the column.  
**Note:** For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 20 µL is recommended.
- 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

- 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.

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

Norgen's EXTRAClean CleanAll DNA/RNA Clean-up and Concentration Micro 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 an RNase-free DNase I be used.

1. Adjust the volume of the RNA sample to be treated to 80  $\mu$ L with RNase-free water.
2. Prepare a working stock of 0.5 Kunitz unit/ $\mu$ L RNase-free DNase I solution according to the manufacturer's instructions. Alternatively, dissolve or dilute stock DNase I in a reaction buffer (40 mM Tris pH 7.0, 10 mM MgCl<sub>2</sub> and 3 mM CaCl<sub>2</sub>, made RNase-free) to give a final concentration of 0.5 Kunitz unit/ $\mu$ L.
3. Add 20  $\mu$ L of 0.5 Kunitz unit/ $\mu$ L DNase I to the RNA sample.
4. Incubate at 25 to 30°C for 15 minutes.
5. Proceed directly to Protocol A "**Protocol for RNA Clean-up and Concentration from Enzymatic Reactions or Other Sample Preparation Methods**".

## Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA recovery	Binding of DNA to the column was inefficient	Binding of the DNA is dependent on both pH and salt concentration. Ensure that an appropriate amount of <b>Binding Buffer H</b> was used for the volume of the DNA Input
	The appropriate amount of ethanol was not added to the <b>Wash Concentrate</b>	The <b>Wash Solution K</b> has been specifically designed to contain the appropriate number of components. Ensure that the <b>Wash Solution K</b> was prepared using the correct amount of ethanol.
	<b>Binding Buffer H</b> was not completely removed in the wash step.	Traces of salt left on the column from the binding step may interfere with the elution of the DNA. Ensure that the column is washed with the <b>Wash Solution K</b> .
	Proper Elution Buffer was not used	The provided <b>Elution Buffer L</b> has been optimized for high elution recoveries. If water or TE buffer is used instead, ensure the pH is around 8.
	<b>Elution Buffer L</b> was not placed directly onto the resin	It is important that the <b>Elution Buffer L</b> be placed directly onto the resin, as this helps to increase recovery by ensuring an even passing of the buffer through the resin. Do not pipette the <b>Elution Buffer L</b> onto the side of the column.

DNA does not perform well in downstream applications	Insufficient washing of resin with bound DNA	Traces of salt from the binding step may remain in the sample if the column is not properly washed with the <b>Wash Solution K</b> . Ensure that the column is spun for 2 minutes during the washing step. Salt may interfere with downstream applications, and thus must be washed from the column.
Endotoxin levels in the eluted DNA are slightly higher than 0.1 EU/ $\mu$ g DNA	Proper <b>Elution Buffer</b> was not used	The provided <b>Elution Buffer L</b> has been optimized for endotoxin-free recoveries. If endotoxin-free water is used for the elution, ensure that the pH is between 7 and 8.
	A different <b>Elution Buffer</b> was used	The provided <b>Elution Buffer L</b> has been optimized for endotoxin-free recoveries. The endotoxin-free properties of the eluted DNA will be compromised if another elution buffer is used. If a different <b>Elution buffer</b> other than the one provided is used, the buffer should also be checked for endotoxin levels.
	The endotoxin levels of the input were extremely high	If the initial input DNA had extremely high endotoxin levels, the levels may not be completely reduced to 0.1 EU/ $\mu$ g of DNA or less. In this case, the eluted DNA could be applied to a second column and the procedure repeated in order to further reduce the endotoxin levels.
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 <b>Elution Buffer L</b> 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 <b>Wash Solution K</b>	Ensure that 54 mL of 96-100% ethanol is added to the supplied <b>Wash Solution K</b> prior to use.
Clogged Column	High amounts of RNA as input	Ensure that no more than 50 $\mu$ g of RNA is used as input
	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.
RNA does not perform well in downstream applications	RNA was not washed twice with the provided <b>Wash Solution K</b>	Traces of salt from the binding step may remain in the sample if the column is not washed twice times with <b>Wash Solution K</b> . 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 DNase I digestion on the RNA sample before clean-up according to the protocol provided in <b>Appendix A</b> .

Related Products	Product #
Total RNA Purification Kit	17200
Cytoplasmic & Nuclear RNA Purification Kit	21000
Leukocyte RNA Purification Kit	21200
microRNA Purification Kit	21300
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)

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6  
Phone: (905) 227-8848  
Fax: (905) 227-1061  
Toll Free in North America: 1-866-667-4362