

## RNA/DNA Purification Micro Kit

Product # 50300

## Product Insert

Norgen's RNA/DNA Purification Micro Kit provides a rapid method for the isolation and purification of total RNA and genomic DNA sequentially from a single sample of cultured animal cells, tissue samples (particularly hard-to-extract fibrous tissues) or blood. The total RNA and genomic DNA are both column purified in less than 30 minutes. The purified RNA and DNA fractions can be eluted in as little as 20  $\mu$ L. This kit is ideal for researchers who are interested in studying the genome and transcriptome of a single sample, such as for studies of microRNA profiling, gene expression including gene silencing experiments or mRNA knockdowns, studies involving biomarker discovery, and for characterization of cultured cell lines. Norgen's RNA/DNA Purification Micro Kit is especially useful for researchers who are isolating macromolecules from precious, difficult to obtain or small samples such as biopsy materials or single foci from cell cultures, as it eliminates the need to fractionate the sample. Furthermore, analysis will be more reliable since the RNA and DNA are derived from the same sample, thereby eliminating inconsistent results. The purified macromolecules are of the highest purity and can be used in a number of different downstream applications

### Norgen's Purification Technology

#### *RNA and DNA Purification*

Purification is based on spin column chromatography. The process involves first lysing the cells or tissue of interest with the provided Buffer SKP. For hard-to-extract fibrous tissues (such as heart and muscle), an additional Proteinase K digestion step is provided for maximum recovery of the RNA and DNA. The DNA in the lysate is then captured and purified on a gDNA Purification Micro Column. Ethanol is then added to the flowthrough of the DNA purification step, and the solution is loaded onto an RNA Purification Micro Column. Norgen's resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the RNA including microRNAs will bind to the column while the proteins are removed in the flowthrough. Next, the bound RNA is washed with the provided Wash Solution A to remove impurities, and the purified RNA is eluted with the Elution Solution A. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). Both RNA and DNA fractions can be eluted in as little as 20  $\mu$ L, resulting in a higher concentration of nucleic acids. The purified RNA is of the highest integrity and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays. The genomic DNA is of the highest quality, and can be used in PCR reactions, sequencing, Southern blotting, methylation studies and SNP analysis.

#### Advantages

- The RNA and DNA are both column purified
- Both the RNA and DNA can be eluted in as little as 20  $\mu$ L
- RNA and DNA are isolated from a single sample with no splitting of the lysate, thus reducing inconsistent results and variability
- Sequential isolation of RNA and DNA from a single sample. Ideal for precious, difficult to obtain or small samples such as biopsy material or single foci from cell cultures.
- Isolate total RNA and genomic DNA from a single sample in less than 30 minutes
- Additional Proteinase K provided for hard-to-extract fibrous tissues.
- All sizes of RNA are isolated, from large mRNA down to microRNA
- The purified RNA and DNA are of the highest quality and can be used in a number of downstream applications

## Specifications

Kit Specifications	
Maximum Column Binding Capacity	35 µg for RNA 10 µg for DNA
Maximum Column Loading Volume	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Animal Cells Animal Tissues Blood	5 x 10 <sup>5</sup> cells 5 mg (for most tissues) 50 µL
Time to Complete 10 Purifications	30 minutes
Average Yields* Liver (5 mg) Liver (5 mg)	10 – 12 µg RNA 1 – 2 µg DNA

\* average yields will vary depending upon a number of factors including species, growth conditions used and developmental stage.

## Kit Components

Component	Used For	Product #50300 (50 samples)
Buffer SKP	RNA Lysis	40 mL
Wash Solution A	RNA Wash      gDNA Wash	2 x 38 mL 1 x 18 mL
Elution Solution A	RNA Elution	6 mL
Elution Buffer F	gDNA Elution	6 mL
RNase-Free Water	Fibrous Tissue Processing	40 mL
Proteinase K	Fibrous Tissue Processing	2 x 12 mg
gDNA Purification Micro Columns	gDNA Purification	50
RNA Purification Micro Columns	RNA Purification	50
Collection Tubes		100
Elution Tubes (1.7 mL)		100
Product Insert		1

## Storage Conditions and Product Stability

Store Proteinase K at -20°C upon arrival. All other solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 1 year 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).

**Buffer SKP** 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

Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.

## Customer-Supplied Reagents and Equipment

You must have the following in order to use the RNA/DNA Purification Micro Kit:

### *For All Protocols*

- Benchtop microcentrifuge
- $\beta$ -mercaptoethanol (Optional)
- 96 - 100 % ethanol
- Molecular biology grade water (Milli-Q® water)

### *For Animal Cell Protocol*

- PBS (RNase-free)

### *For Animal Tissue Protocol*

- Liquid nitrogen
- Mortar and pestle

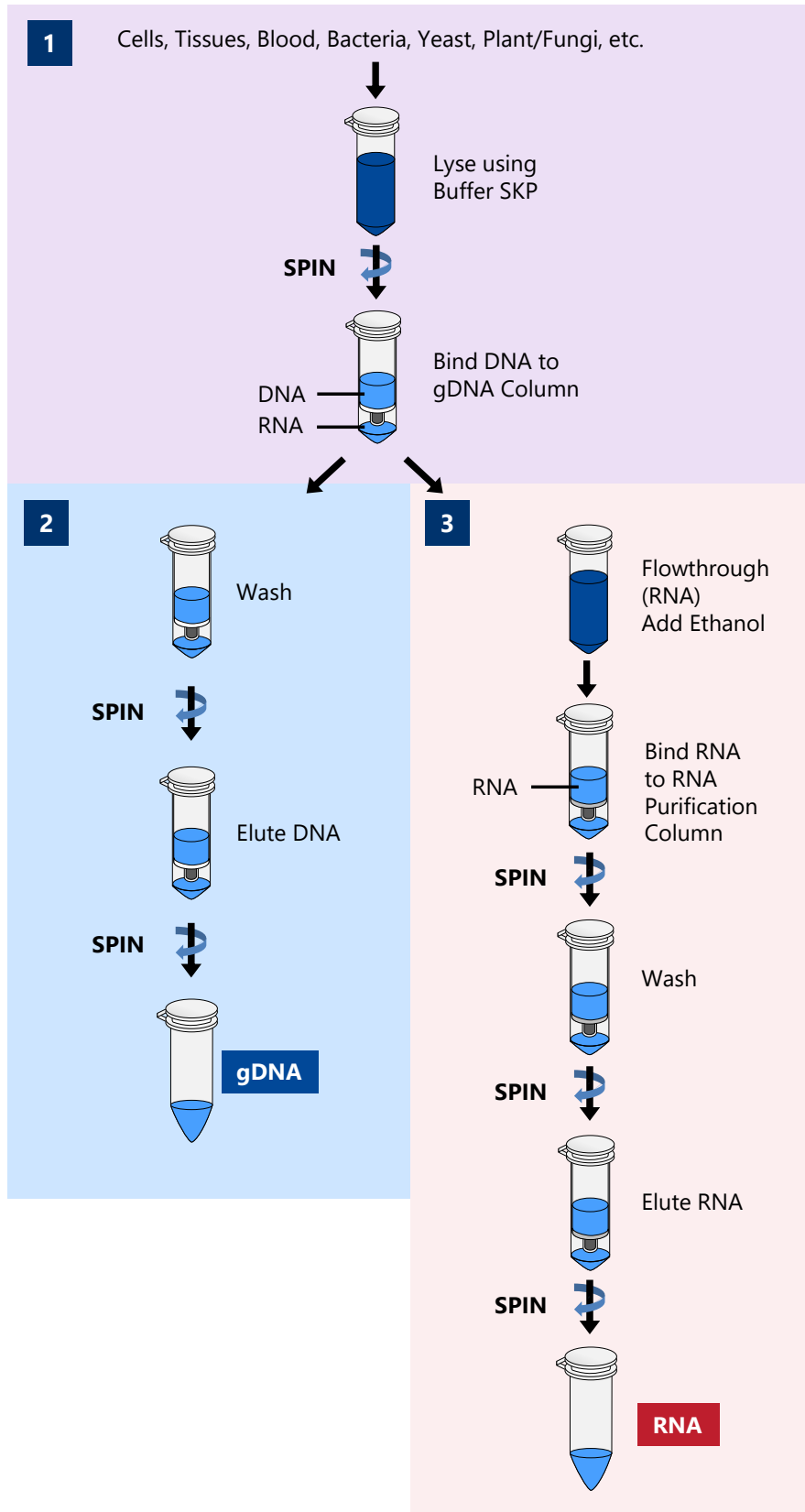
## Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is 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

# Flow Chart

Procedure for Purifying Total RNA and DNA using Norgen's RNA/DNA Micro Purification 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.

### IMPORTANT NOTE:

This procedure is written in three steps. Section 1 contains the lysate preparation protocols from different types of starting materials. Please ensure that the proper protocol is followed for your sample. Section 2 contains the protocol to isolate genomic DNA from the sample. Section 3 contains the protocol to isolate total RNA from the sample. The same protocols for Section 2 to Section 3 will apply to all the different starting materials.

#### Notes Prior to Use for all RNA/DNA Purification Procedures

- The steps for preparing the lysate are different depending on the starting material (**Step 1**). However, the subsequent steps are the same in all cases (**Steps 2 – 9**).
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed.
- 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 each of the bottles containing 38 mL of concentrated **Wash Solution A**. This will give a final volume of 128 mL.
  - 42 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing 18 mL concentrated **Wash Solution A**. This will give a final volume of 60 mL.The labels on the bottles have a box that may be checked to indicate that the ethanol has been added. The **Wash Solution A** is used for both RNA and DNA Purification
- **Optional:** The use of β-mercaptoethanol in lysis is highly recommended for most tissues, particularly those known to have high RNase content (ex: pancreas). It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 μL of β-mercaptoethanol (provided by the user) to each 1 mL of **Buffer SKP** required. β-mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the **Buffer SKP** can be used as provided.
- It is important to work quickly when purifying RNA.
- This kit is provided with 2 separate columns. When columns are removed from the labelled bags they are supplied in they can easily be identified as follows:
  - gDNA Purification Micro Columns - column has predominately white contents
  - RNA Purification Micro Columns – column has predominately black contents

## Section 1. Preparation of Lysate From Various Cell Types

### 1A. Lysate Preparation from Cultured Animal Cells

#### Notes Prior to Use

- For optimal results, it is recommended that  $5 \times 10^5$  cells or fewer be used for the input
- A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain  $10^6$  cells.
- Cell pellets can be stored at  $-70^\circ\text{C}$  for later use or used directly in the procedure. Determine the number of cells present before freezing.
- Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- Frozen cell pellets should not be thawed prior to beginning the protocol. Add the **Buffer SKP** directly to the frozen cell pellet (**Step 1A(ii) d**).

#### 1A (i). Cell Lysate Preparation from Cells Growing in a Monolayer

- a. Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
- b. Add 300  $\mu\text{L}$  of **Buffer SKP** directly to culture plate.
- c. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes.
- d. Transfer lysate to a microcentrifuge tube.

**Optional:** The lysate could be heated at  $55^\circ\text{C}$  for 10 minutes to enhance lysis

- e. **Proceed to Step 2.**

#### 1A (ii). Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

- a. Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than  $200 \times g$  ( $\sim 2,000$  RPM) for 10 minutes to pellet cells.
- b. Carefully decant the supernatant

**Note:** For inputs of over  $10^5$  cells, 5-10  $\mu\text{L}$  of media may be left behind with the pellet in order to ensure that the pellet is not dislodged. For inputs of fewer than  $10^5$  cells, 30-50  $\mu\text{L}$  of media may be left behind in order to ensure that the pellet, which could be invisible, is not dislodged.

- c. Add 300  $\mu\text{L}$  of **Buffer SKP** to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step.

**Optional:** The lysate could be heated at  $55^\circ\text{C}$  for 10 minutes to enhance lysis

- d. **Proceed to Step 2.**

## 1B. Lysate Preparation from Animal Tissues (Soft, Non-Fibrous)

### Notes Prior to Use

- RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Thus it is important that the procedure is carried out as quickly as possible, particularly the Cell Lysate Preparation step.
- Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
- The maximum recommended input of tissue for optimal separation of RNA and DNA is 5 mg. If your tissue of interest is not included in the table below we recommend starting with an input of no more than 3 mg.

**Table 1. Recommended Maximum Input Amounts of Different Tissues**

Tissue	Maximum Input Amount
Brain	5 mg
Kidney	5 mg
Liver	5 mg
Lung	5 mg
Spleen	5 mg
Heart or Muscle	Please use Section 1C

### 1B. Cell Lysate Preparation from Animal Tissues (Soft, Non-Fibrous)

- Excise the tissue sample from the animal.
- Determine the amount of tissue by weighing. Please refer to Table 1 for the recommended maximum input amounts of different tissues. For tissues not included in the table, we recommend starting with an input of no more than 3 mg.
- Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.
- Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
- Add 300  $\mu$ L of **Buffer SKP** to the tissue sample and continue to grind until the sample has been homogenized. Homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.

**Optional:** Alternatively, the lysate could be heated at 55°C for 10 minutes to enhance lysis

- Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
- Spin lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube (not provided). Note the volume of the supernatant/lysate.
- Proceed to Step 2.**

## 1C. Lysate Preparation from Animal Tissues (Fibrous)

### Notes Prior to Use

- RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Thus it is important that the procedure is carried out as quickly as possible, particularly the Cell Lysate Preparation step.
- Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
- Reconstitute the provided Proteinase K with 600  $\mu$ L molecular biology grade water to a final concentration of 20 mg/mL as follows. Make small aliquots and store un-used enzyme at -20°C.
- The maximum recommended input of heart or muscle for optimal separation of RNA and DNA is 5 mg. For other fibrous tissues, we recommend starting with an input of no more than 3 mg.

### 1C. Cell Lysate Preparation from Animal Tissues (Fibrous)

- a. Excise the tissue sample from the animal.
- b. Determine the amount of tissue by weighing. For tissues other than heart or muscle, we recommend starting with an input of no more than 3 mg.
- c. Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.
- d. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
- e. Add 300  $\mu$ L of **Buffer SKP** to the tissue sample and continue to grind until the sample has been homogenized. Homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.
- f. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
- g. Add 300  $\mu$ L of **RNase-Free water**. Mix by vortexing
- h. Add 20  $\mu$ L of reconstituted Proteinase K to the lysate, and incubate at 55°C for 15 minutes. Vortex the tubes occasionally during incubation.
- i. Spin lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube (not provided). Note the volume of the supernatant/lysate. **Proceed to Step 2.**

## 1D. Lysate Preparation from Blood

### Notes Prior to Use

- Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.
- It is recommended that no more than 50  $\mu$ L of blood be used in order to prevent clogging of the column.
- We recommend the use of this kit to isolate RNA from non-coagulating fresh blood using EDTA as the anti-coagulant.

### 1D. Cell Lysate Preparation from Blood

- a. Transfer up to 50  $\mu$ L of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).



- b. Add 250  $\mu\text{L}$  of **Buffer SKP** to every 50  $\mu\text{L}$  of blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent (with a dark red color) before proceeding to the next step.

**Optional:** The lysate could be heated at 55°C for 10 minutes to enhance lysis

- c. **Proceed to Step 2.**

## Section 2: Genomic DNA Purification from All Types of Lysate

**Note:** The following steps of the procedure for the purification of genomic DNA are the same for all the different types of lysate.

### 2. Binding DNA to gDNA Purification Micro Column

- a. Assemble a **gDNA Purification Micro Column** with one of the provided collection tubes.
- b. Apply up to 600  $\mu\text{L}$  of the lysate onto the column and centrifuge at **5,200 x g (~8,000 RPM)** for 2 minutes

**Note:** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **14,000 x g (~14,000 RPM)**.

- c. **Retain the flowthrough for RNA Purification (Section 3). The flowthrough contains the RNA and should be stored on ice or at -20°C until the RNA Purification protocol is carried out.**
- d. Reassemble the spin column with the collection tube.

### 3. Genomic DNA Wash

- a. Apply 500  $\mu\text{L}$  of **Wash Solution A** to the column and centrifuge at at  $\geq$  **3,500 x g (~6,000 RPM)** for 1 minute. Discard the flowthrough.
- b. Apply 500  $\mu\text{L}$  of **Wash Solution A** to the column and centrifuge at at  $\geq$  **3,500 x g (~6,000 RPM)** for 1 minute. Discard the flowthrough.
- c. Spin the column at **14,000 x g (~14,000 RPM)** for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

### 4. Genomic DNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 20 – 50  $\mu\text{L}$  of **Elution Buffer F** to the column and let stand at room temperature for 2 minutes.
- 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 DNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 4b** and **4c**).

### 5. Storage of DNA

The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at  $\leq$ -20°C for long term storage.

## Section 3: Total RNA Purification from All Types of Lysate

### 6. Binding RNA to Column

- a. To every 100  $\mu\text{L}$  of flowthrough from Step 2c, add 60  $\mu\text{L}$  of 96 – 100 % Ethanol. Mix by vortexing.

**Note:** For example, for 300  $\mu\text{L}$  of flowthrough, add 180  $\mu\text{L}$  of 96 – 100 % Ethanol

- b. Assemble an **RNA Purification Micro Column** with one of the provided collection tubes.
- c. Apply up to 600  $\mu\text{L}$  of the lysate with the ethanol onto the column and centrifuge for 1 minute at  $\geq 3,500 \times g$  (~6,000 RPM).

**Note:** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **14,000 x g (~14,000 RPM)**.

- d. Discard the flowthrough. Reassemble the spin column with the collection tube.

### 7. RNA Wash

- a. Apply 400  $\mu\text{L}$  of **Wash Solution A** to the column and centrifuge at  $\geq 3,500 \times g$  (~6,000 RPM) for 1 minute.

**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 column with the collection tube.
- c. Wash column a second time by adding another 400  $\mu\text{L}$  of **Wash Solution A** and centrifuge at  $\geq 3,500 \times g$  (~6,000 RPM) for 1 minute.
- d. Discard the flowthrough and reassemble the spin column with its collection tube.
- e. Wash column a third time by adding another 400  $\mu\text{L}$  of **Wash Solution A** and centrifuge at  $\geq 3,500 \times g$  (~6,000 RPM) for 1 minute.
- f. Discard the flowthrough and reassemble the spin column with its collection tube.
- g. Spin the column at **14,000 x g (~14,000 RPM)** for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

### 8. RNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 20 – 50  $\mu\text{L}$  of **Elution Solution A** to the column.
- 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, particularly for samples that are known to contain large amounts of RNA, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 8b** and **8c**).

### 9. 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.

Related Products	Product #
1kb RNA Ladder	15003
UltraRanger 1kb DNA Ladder	12100
RNA/Protein Purification Plus Kit	48200
RNA/DNA Purification Kit	48700
RNA/DNA/Protein Purification Plus Kit	47700

## Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of <b>Buffer SKP</b> was used for the amount of cells or tissue.
	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”
	An alternative elution solution was used	It is recommended that the <b>Elution Solution A</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 A</b>	Ensure that 96 – 100 % ethanol is added to the supplied <b>Wash Solution A</b> prior to use.
	Low RNA content in cells or tissues used	Different tissues and cells have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA content of your starting material.
	Cell Culture: Cell monolayer was not washed with PBS	Ensure that the cell monolayer is washed with the appropriate amount of PBS in order to remove residual media from cells.

Clogged Column	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20°C may cause precipitates to form that can cause the columns to clog.
Problem	Possible Cause	Solution and Explanation
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. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Also, after the DNA binding step, the flowthrough should be kept on ice or –20°C if the RNA purification step is not carried out immediately.
	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.
	Frozen tissues or cell pellets were allowed to thaw prior to RNA isolation	Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
	Tissue samples were frozen improperly	Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage.
RNA does not perform well in downstream applications	RNA was not washed twice with the provided <b>Wash Solution A</b>	Traces of salt from the binding step may remain in the sample if the column is not washed twice with <b>Wash Solution A</b> . Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the RNA Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.

**Technical Support**

Contact our Technical Support Team between the hours of 8:30 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

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