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microRNA Purification Kit

Product Insert

Product # 21300

Introduction

Norgen's microRNA Purification Kit provides a rapid method for the isolation and purification of small RNA molecules (< 200 nt) from cultured animal cells, small tissue samples, bacterial cells, plants and blood. These small RNAs include regulatory RNA molecules such as microRNA (miRNA) and short interfering RNA (siRNA), as well as tRNA and 5S rRNA. Small RNA molecules are often studied due to their ability to regulate gene expression. miRNAs and siRNAs are typically 20-25 nucleotides long, and regulate gene expression by binding to mRNA molecules and affecting their stability or translation. The small RNA molecules isolated using Norgen's microRNA Purification Kit can be used in various downstream applications relating to gene regulation and functional analysis, including RT-PCR, northern blotting and microarray analysis.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Norgen's resin binds RNA in a manner that depends on ionic concentrations. The small RNA molecules are preferentially purified from other cellular components such as ribosomal RNA without the use of phenol or chloroform. The process involves the use of two different spin columns: the **Large RNA Removal Column** and the **microRNA Enrichment Column** (please see flow chart on page 3). Briefly, the cells or tissues of interest are lysed using the provided Buffer RL, and ethanol is then added to the sample. The lysate is then applied to the Large RNA Removal Column, and the larger RNA molecules will bind to the resin in the spin column while the smaller RNA species will pass through into the flowthrough. Ethanol is then added to the flowthrough, and the sample is applied to the microRNA Enrichment Column. The small RNA molecules will then bind to the resin, and any impurities are removed through a series of washes with the provided Wash Solution A. The small RNA molecules are then eluted using the Elution Solution A, and are ready for use in various applications.

Specifications

Kit Specifications	
Maximum Column Binding Capacity	50 µg
Maximum Column Loading Volume	650 µL
Minimum Elution Volume	20 µL
Size of RNA Purified	< 200 nt
Maximum Amount of Starting Material: Animal Cells Animal Tissues Bacteria Plant Tissues Blood	 3×10^6 cells 5-25 mg 1×10^9 cells 50 mg 100 µL
Time to Complete 10 Purifications	25 minutes

Advantages

- Fast and easy processing using rapid spin-column format
- No phenol or chloroform extractions
- Isolate all small RNA molecules (<200 nt)
- Minimal contamination from large RNA molecules and genomic DNA
- High quality small RNA can be used in various downstream applications

Kit Components

Component	Product # 21300 (25 preps)
Buffer RL	40 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Large RNA Removal Column	25
microRNA Enrichment Column	25
Collection Tube	50
Elution tubes (1.7 mL)	50
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

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*.

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

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

For All Protocols

- Benchtop microcentrifuge
- β-mercaptoethanol (Optional)
- 96 - 100% ethanol
- RNase-free microcentrifuge tubes

For Animal Cell Protocol

- PBS (RNase-free)

For Animal Tissue Protocol

- Liquid nitrogen
- Mortar and pestle
- 25 gauge needle and syringe

For Bacterial Protocol

- Lysozyme-containing TE Buffer:
    - For Gram-negative bacteria, 1 mg/mL lysozyme in TE Buffer
    - For Gram-positive bacteria, 3 mg/mL lysozyme in TE Buffer

For Plant Protocol

- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

Working with RNA

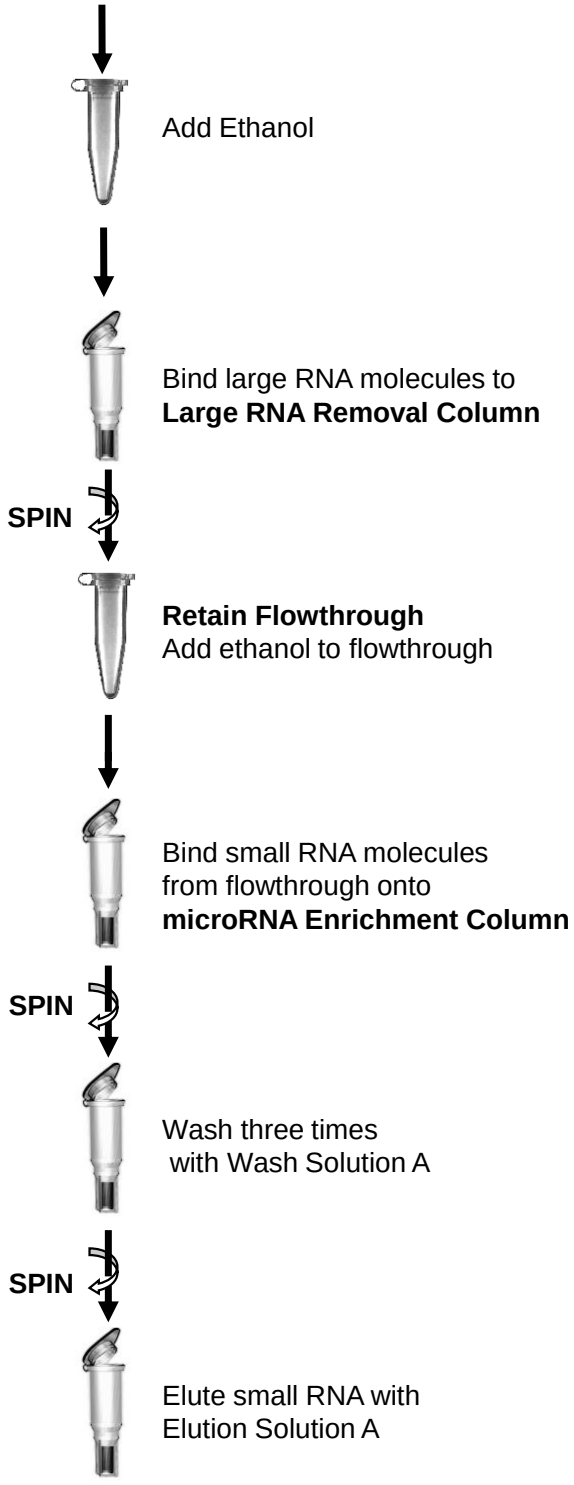
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense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

Lyse cells or tissue using **Buffer RL**



Purified Small RNA (miRNA, siRNA, tRNA, 5S rRNA, etc)

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.

Section 1. Preparation of Lysate from Various Cell Types

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.

Notes Prior to Use

- 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 – 6)**.
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed.
- 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.
- There are 2 different spin columns provided with this kit; the Large RNA Removal Column and the microRNA Enrichment Column. Ensure that the correct column is used for each step of the procedure.
- 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 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.
- **Optional:** The use of β-mercaptoethanol in lysis is highly recommended for most animal tissues, particularly those known to have a 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 RL required. β-mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the Buffer RL can be used as provided.
- It is important to work quickly during this procedure.

1A. Lysate Preparation from Cultured Animal Cells

- The maximum recommended input of cells is 3×10^6. 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°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 pellets should not be thawed prior to beginning the protocol. Add the Buffer RL directly to the frozen cell pellet **(Step A(ii) 1c).**

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 μL of **Buffer RL** 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 RNase-free microcentrifuge tube.
e. Add 150 μL of 96 – 100% ethanol (provided by the user) to the lysate**.** Mix by vortexing for 10 seconds. **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 *x g* (~2,000 RPM) for 10 minutes to pellet cells.
b. Carefully decant the supernatant. A few μL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.
c. Add 300 μL of **Buffer RL** to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
d. Add 150 μL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

1B. Lysate Preparation from Animal Tissues

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 varies depending on the type of tissue being used. Please refer to Table 1 below as a guideline for maximum tissue input amounts. If your tissue of interest is not included in the table below we recommend starting with an input of no more than 10 mg.

Table 1. Recommended Maximum Input Amounts of Different Tissues

Tissue	Maximum Input Amount
Brain	25 mg
Heart	5 mg
Kidney	10 mg
Liver	20 mg
Lung	10 mg
Spleen	10 mg

1B. Cell Lysate Preparation from Animal Tissues

a. Excise the tissue sample from the animal.
b. 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 10 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 400 µL of **Buffer RL** 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. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
h. Add a volume of 96-100% ethanol (provided by the user) that is equivalent to 50% of the lysate volume (50 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. **Proceed to Step 2.**

1C. Cell Lysate Preparation from Bacteria

Notes Prior to Use

- Prepare the appropriate lysozyme-containing TE Buffer as indicated in Table 2. This solution should be prepared with sterile, RNAse-free TE Buffer, and kept on ice until needed. These reagents are to be provided by the user.
- It is recommended that no more than 10^9 bacterial cells be used in this procedure. Bacterial growth can be measured using a spectrophotometer. As a general rule, an *E. coli* culture containing 1 x 10^9 cells/mL has an OD_{600} of 1.0.
- For RNA isolation, bacteria should be harvested in log-phase growth.
- Bacterial pellets can be stored at -70°C for later use, or used directly in this procedure.
- Frozen bacterial pellets should not be thawed prior to beginning the protocol. Add the Lysozyme-containing TE Buffer directly to the frozen bacterial pellet **(Step 1Cc)**.

1C. Cell Lysate Preparation from Bacteria

a. Pellet bacteria by centrifuging at 14,000 *x g* (~14,000 RPM) for 1 minute.
b. Decant supernatant, and carefully remove any remaining media by aspiration.
c. Resuspend the bacteria thoroughly in 100 µL of the appropriate lysozyme-containing TE buffer (see Table 2) by vortexing. Incubate at room temperature for the time indicated in Table 2.
d. Add 200 µL of Buffer RL and vortex vigorously for at least 10 seconds.
e. Add 150 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

Table 2: Incubation Time for Different Bacterial Strains

Bacteria Type	Lysozyme Concentration in TE Bufffer	Incubation Time
Gram-negative	1 mg/mL	5 min
Gram-positive	3 mg/mL	10 min

D. Lysate Preparation from Plant

Notes Prior to Use

- The maximum recommended input of plant tissue is 50 mg or 5 x 10^6 cells.
- Both fresh and frozen plant tissues can be used for this protocol. Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. 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.
- It is important to work quickly during this procedure.

1D. Cell Lysate Preparation from Plants

a. Transfer ≤50 mg of plant tissue or 5 x 10^6 plant cells into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the sample into a fine powder using a pestle in liquid nitrogen.

   Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen.

b. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
c. Add 600 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized.
d. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
e. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
f. Add a volume of 96-100% ethanol (provided by the user) that is equivalent to 50% of the lysate volume (50 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. **Proceed to Step 2.**

1E. 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 100 µL 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.
- It is important to work quickly during this procedure.

1E. Cell Lysate Preparation from Blood

a. Transfer up to 100 µL of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).
b. Add 250 µL of **Buffer RL** to the blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.
c. Add 150 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

Section 2. Purification of Small RNA from All Types of Lysate

Note: The remaining steps of the procedure for the purification of small RNA are the same from this point forward for all the different types of lysate.

2. Large RNA Removal

a. Assemble a **Large RNA Removal Column** with one of the provided collection tubes.
b. Apply the lysate with the ethanol (from step **1**) onto the column and centrifuge for 1 minute at **14,000 x *g* (~14,000 RPM). Retain the flowthrough, which contains the small RNA species.** If small RNA-depleted RNA is to be isolated, retain the column and proceed to the Optional **Large RNA Purification Protocol (Appendix A)**. Otherwise, discard the column.
c. Transfer the flowthrough to an RNase-free microcentrifuge tube (not provided). The flowthrough contains the small RNA, thus ensure that this fraction is not discarded.

3. Small RNA Capture

a. **For animal cells, bacteria or blood**: Add 350 µL of 96 -100% ethanol (provided by the user) to the flowthrough collected in **Step 2b**. Mix by vortexing for 10 seconds.
For animal tissues or plant: Based on the lysate volume determined in **Steps 1Bg** or **1De**, add 1 volume of 96 – 100% ethanol (provided by the user) to the flowthrough collected in **Step 2b** (100 µL of ethanol is added to every 100 µL of flowthrough)**.** Mix by vortexing for 10 seconds.
b. Assemble a **microRNA Enrichment Column** with one of the provided collection tubes.
c. Apply half of the lysate mix with ethanol onto the column and centrifuge for 1 minute at **≥ 3,500 x 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 and reassemble the spin column with the collection tube.
e. Repeat steps **3c** and **3d** to complete the capture of the small RNA.

4. Column Wash

a. Apply 400 µL of **Wash Solution A** to the **microRNA Enrichment Column** and centrifuge for 1 minute at **14,000 x *g* (~14,000 RPM).**

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 spin column with the collection tube.
c. Repeat steps **4a** and **4b** to wash column a second time.
d. Wash column a third time by adding another 400 µL of **Wash Solution A** and centrifuging for 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. Small RNA Elution

a. Place the **microRNA Enrichment Column** into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 µL of **Elution Solution A** to the column.

Note: For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 20 µL 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 5b and 5c**).

6. 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 Large RNA Purification Protocol

1. Column Wash

a. Reassemble the **Large RNA Removal Column** with the collection tube used in Step **2b**
b. Apply 400 µL of **Wash Solution A** to the **Large RNA Removal Column** and centrifuge for 1 minute at **14,000 x *g* (~14,000 RPM).**

Note: Ensure that 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 spin column with the collection tube.
d. Repeat steps **1b** and **1c** to wash the column a second time.
e. Wash column a third time by adding another 400 µL of **Wash Solution A** and centrifuging for 1 minute.
f. Discard the flowthrough and reassemble the spin column with its collection tube.
g. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

2. Large RNA Elution

a. Place the **Large RNA Removal Column** into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 µL 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, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 2b and 2c**).

3. 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.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Buffer RL was used for the amount of cells or tissue.
	Large RNA Removal 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.
	Low RNA content	Different tissues and cells have different RNA contents. Some tissues may not contain small RNA at detectable levels when processing the small sample sizes required for this procedure.
	Flowthrough from the first binding step was discarded	The flowthrough from the binding step with the Large RNA Removal Column contains the small RNA molecules, thus ensure that it is not inadvertently discarded.
	Ethanol was not added to the flowthrough before binding to the microRNA Enrichment Column	Ensure that the appropriate amount of ethanol was added to the flowthrough from the first binding step before it is applied to the microRNA Enrichment Column. This is imperative in order to capture the small RNA molecules.
	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.
	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.
	Bacteria: All traces of media not removed	Ensure that all media is removed prior to the addition of the Buffer RL through aspiration.

Problem	Possible Cause	Solution and Explanation
Clogged Column	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of Lysis Buffer was used for the amount of cells or tissue.
	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.
	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 Large RNA Removal 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 "*Working with RNA"* 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.
	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 pellets were allowed to thaw prior to disruption	Tissue samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. 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.

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 microRNA Enrichment Column is not washed three times with 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.
Genomic DNA contamination	Large amount of starting material used	Perform RNase-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination.
Large RNA species present in elution	Improper amount of ethanol added to the lysate before binding to the Large RNA Removal Column	Ensure that the appropriate amount of ethanol was added to the lysate before it is applied to the Large RNA Removal Column. This is imperative in order to capture the large RNA molecules onto the column.
	Large amount of starting material used	Repeat purification using less starting material. Alternatively, the isolation procedure can be repeated using the elution as the input. The elution volume should first be adjusted to 300 µL using the provided Buffer RL. The procedure can then be followed as written in the manual, starting with the addition of ethanol, centrifuging the lysate in order to pellet any debris, and applying the clarified lysate to the Large RNA Removal Column. Repeating the procedure should result in the removal of the large, contaminating RNA species.

Related Products	Product #
100b RNA Ladder	15002
1kb RNA Ladder	15003
Total RNA Purification Kit	17200
Cytoplasmic & Nuclear RNA Purification Kit	21000
Leukocyte RNA Purification Kit	21200
RNA/Protein Purification Kit	24100
RNA/DNA/Protein Purification Kit	24000

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) or through email at techsupport@norgenbiotek.com.

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