Product Information

GenElute™ Total RNA Purification Kit

RNB100

Product Description

The GenElute™ Universal Total RNA Purification Kit provides a rapid method for the isolation and purification of total RNA from cultured animal cells, tissue samples, blood, plasma, serum, bacteria, yeast, fungi, plants, and viruses. 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 cellular components such as proteins, 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 real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Purification is based on spin column chromatography using a 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 lysing the cells or tissue of interest with the provided **Buffer RL** (Figure 1 on page 3). Ethanol is then added to the lysate, and the solution is loaded onto a spin-column. The resin binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the column, while the contaminating proteins will be removed in the flow through or retained on the top of the resin. The bound RNA is then washed with the provided Wash Solution A in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Solution A. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Components

Component	50 preps	100 preps
Buffer RL	40 mL	2 × 40 mL
Wash Solution A	38 mL	2 × 38 mL
Elution Solution A	6 mL	2 × 6 mL
Mini Spin Columns	50	100
Collection Tubes	50	100
Elution tubes (1.7 mL)	50	100
Product Insert	1	1

Reagents and Equipment Required But Not Provided
You must have the following in order to use the Total RNA Purification Kit:

For All Protocols
- Benchtop microcentrifuge
- 96-100% ethanol
- β-mercaptoethanol (optional)

For Animal Cell Protocol
- PBS (RNase-free)

For Animal Tissue Protocol
- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

For Nasal or Throat Swabs
- Sterile, single-use cotton swabs

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 Yeast Protocol

- Resuspension Buffer with Lyticase:
  - 50 mM Tris pH 7.5
  - 10 mM EDTA
  - 1 M Sorbital
  - 1 unit/µL Lyticase

For Fungi Protocol

- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

For Plant Protocol

- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

For Plasma/Serum Protocol

- MS2 RNA (0.8 µg/µL). (Cat. No. 10165948001)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheets for information regarding hazards and safe handling practices.

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.

Working with RNA

RNases are very stable and robust enzymes that degrade RNA. The first step when preparing to work with RNA is to create an RNase-free environment. 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. 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. Care must be taken not to introduce RNAse especially during the final wash and elution.

Preparation Instructions

Before beginning the procedure, prepare the following:

1. 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.

2. The use of β-mercaptoethanol in lysis is highly recommended for most animal tissues, particularly those known to have a high RNAse content (example: pancreas), as well as for most plant tissues, and nasal and throat swabs. 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.

Storage/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.

Procedures

Note: 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:

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

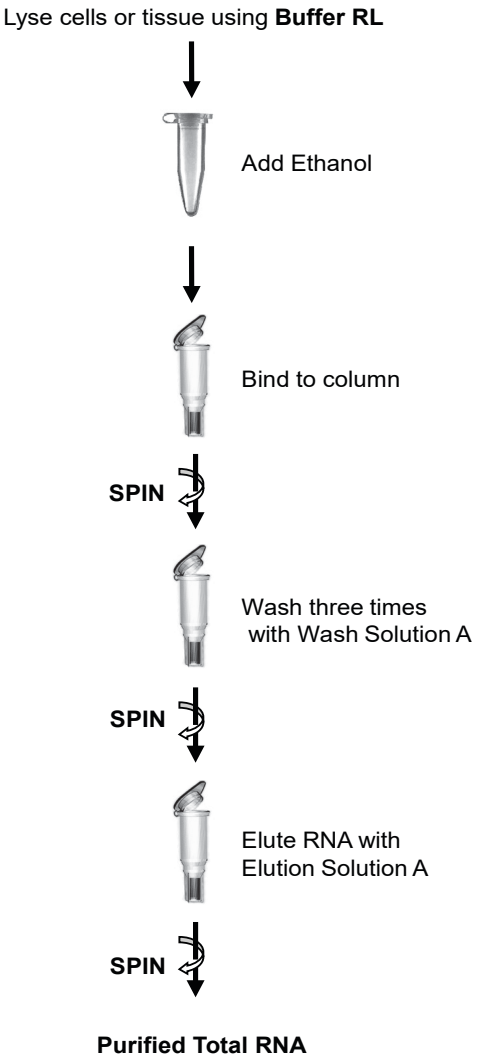
where *RCF* = required gravitational acceleration (relative centrifugal force in units of *g*); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

There are primarily 2 sections to the procedure of RNA Total extraction using GenElute™ Universal Total RNA Purification Kit:

- Preparation of Lysate
- Total RNA Purification

Figure 1.

Flowchart of Procedure for Purifying Total RNA using Total RNA Purification Kit



Section 1:

Preparation of Lysate from Various Cell Types

Sample types applicable:

- 1A. Cultured Animal Cells
  - i. Cells Growing in a Monolayer
  - ii. Cells Growing in Suspension
- 1B. Animal Tissue
- 1C. Blood
- 1D. Nasal/Throat Swabs
- 1E. Bacteria
- 1F. Yeast
- 1G. Fungi
- 1H. Plant
- 1I. Viruses
- 1J. Plasma/Serum

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

1A. Lysate Preparation from Cultured Animal Cells

Note: The maximum recommended number of cells is 3 x 10^6. 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 cell pellets should not be thawed prior to beginning the protocol. Add the **Buffer RL** directly to the frozen cell pellet.

Procedure

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

Aspirate medium and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS. Add 350 µL of **Buffer RL** directly to culture plate. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes. Transfer lysate to a microcentrifuge tube. Add 200 µL of 96-100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

For starting amounts greater than 10^6 cells, it is recommended that the lysate is passed through a 25 gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading onto the column.

Proceed to Binding RNA to Column step.

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

Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than 200 × g (~2,000 rpm) for 10 minutes to pellet cells. Carefully decant the supernatant. Add 350 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. Add 200 µL of 96-100% ethanol to the lysate. Mix by vortexing for 10 seconds.

For starting amounts greater than 10^6 cells, it is recommended that the lysate is passed through a 25 gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading onto the column.

Proceed to Binding RNA to Column step.

1B. Lysate Preparation from Animal Tissues

Note: The GenElute™ Total RNA Purification Kit is designed for isolating RNA from small amount of tissue sample (up to 10 mg). If a larger amount of starting material is desired, The GenElute™ Animal Tissue RNA Purification Kit should be used. 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. When isolating total RNA from frozen tissues, ensure that the tissue does not thaw during weighing or prior to grinding with the mortar and pestle. Tissues stored in RNA stabilization reagents are compatible with this isolation procedure. Prior to isolation, carefully remove the tissue from the storage reagent using forceps, and dry excessive liquid.

The maximum recommended weight of tissue varies depending on the type of tissue being used. Please refer to Table 1 as a guideline for maximum tissue weight. 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 Weight of Different Tissues

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

Procedure

Excise the tissue sample from the animal. Determine the amount of tissue by weighing and refer to Table 1 for maximum weight. 10 mg is optimal for tissues not mentioned in the table. 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 600 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.

Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube. 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. Add an equal volume of 70% ethanol to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

Proceed to Binding RNA to Column step.

1C. Lysate Preparation from Blood

Note: This procedure is for the isolation of RNA from whole blood. Plasma and serum are isolated using a separate protocol **(1J).** 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. Working quickly is important for this procedure.

Procedure

Transfer up to 100 µL of non-coagulating blood to an RNase-free microcentrifuge tube. Add 350 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. Add 200 µL of 96–100% ethanol to the lysate. Mix by vortexing for 10 seconds.

Proceed to Binding RNA to Column step.

1D. Lysate Preparation from Nasal or Throat Swabs

Note: Body fluid of all human and animal subjects is considered potentially infectious. It is important to work quickly during this procedure.

Procedure

Add 600 µL of **Buffer RL** to an RNase-free microcentrifuge tube. Gently brush a sterile, single-use cotton swab inside the nose or mouth of the subject. Using sterile techniques cut the cotton tip where the nasal or throat cells were collected and place into the microcentrifuge tube containing the **Buffer RL**. Close the tube. Vortex gently and incubate for 5 minutes at room temperature. Using a pipette, transfer the lysate into another RNase-free microcentrifuge tube.

Note the volume of the lysate. Add an equal volume of 70% ethanol to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

Proceed to Binding RNA to Column step.

1E. Lysate Preparation from Bacteria

Note: 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. As a general rule, an E. coli culture containing 1×10^9 cells/mL has an OD_{600} of 1.0 as per standard spectrophotometers. 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.

Procedure
Pellet bacteria by centrifuging at 14,000 × g (~14,000 rpm) for 1 minute. Decant supernatant, and carefully remove any remaining medium by aspiration. Resuspend the bacteria thoroughly in 100 µL of the appropriate lysozyme-containing TE buffer (see Table 1) by vortexing. Incubate at room temperature for the time indicated in Table 1. Add 300 µL of **Buffer RL** and vortex vigorously for at least 10 seconds. Add 200 µL of 96–100% ethanol to the lysate. Mix by vortexing for 10 seconds.

Proceed to Binding RNA to Column step.

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

1F. Lysate Preparation from Yeast

Note: Prepare the appropriate amount of Lyticase-containing Resuspension Buffer, considering that 100 µL of buffer is required for each preparation. The Resuspension Buffer should have the following composition: 50 mM Tris, pH 7.5, 10 mM EDTA, 1 M Sorbital, 0.1% β-mercaptoethanol, and 1 unit/µL Lyticase. This solution should be prepared with sterile, RNAse-free reagents, and kept on ice until needed.

It is recommended that no more than 10^7 yeast cells or 1 mL of culture be used for this procedure. For RNA isolation, yeast should be harvested in log-phase growth. Yeast can be stored at -70 °C for later use, or used directly in this procedure. Frozen yeast pellets should not be thawed prior to beginning the protocol. Add the Lyticase-containing Resuspension Buffer directly to the frozen yeast pellet.

Procedure
Pellet yeast by centrifuging at 14,000 × g (~14,000 rpm) for 1 minute. Decant supernatant, and carefully remove any remaining medium by aspiration. Resuspend the yeast thoroughly in 100 µL of Lyticase-containing Resuspension Buffer by vortexing. Incubate at 37 °C for 10 minutes. Add 300 µL of **Buffer RL** and vortex vigorously for at least 10 seconds. Add 200 µL of 96–100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

Proceed to Binding RNA to Column step.

1G. Lysate Preparation from Fungi

Note: Fresh or frozen fungi may be used for this procedure. Fungal tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70 °C freezer for long-term storage. Fungi 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.

It is recommended that no more than 50 mg of a fungus be used for this procedure in order to prevent clogging of the column.

Procedure
Determine the amount of fungus by weighing. It is recommended that no more than 50 mg be used for the protocol. Transfer the fungus into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the fungus thoroughly using a pestle. At this stage the ground fungus may be stored at –70 °C, such that the RNA purification can be performed at a later time.

Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw. Add 600 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided). 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. Add an equal volume of 70% ethanol to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

Proceed to Binding RNA to Column step.

1H. Lysate Preparation from Plant

Note: The maximum recommended starting amount of plant tissue is 50 mg or 5×10^6 plant cells. Both fresh and frozen plant samples 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.

Procedure

Transfer ≤50 mg of plant tissue or 5×10^6 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. If stored frozen samples are used, do not allow samples to thaw before transferring to the liquid nitrogen.

Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw. Add 600 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube. 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. Add an equal volume of 70% ethanol to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

Proceed to Binding RNA to Column step.

1I. Lysate Preparation from Viruses

Note: For the isolation of integrated viral RNA, follow Section 1A if the starting material is cell culture, follow Section 1B if the starting material is tissue, follow Section 1C if the starting material is blood, or follow Section 1D if the starting material is a nasal or throat swab. For the isolation of RNA from free viral particles, follow the procedure below.

It is recommended that no more than 100 µL of viral suspension be used in order to prevent clogging of the column. It is important to work quickly during this procedure.

Procedure

Transfer up to 100 µL of viral suspension to an RNase-free microcentrifuge tube (not provided). Add 350 µL of **Buffer RL**. Lyse viral cells by vortexing for 15 seconds. Add 200 µL of 96–100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

Proceed to Binding RNA to Column step.

1J. Protocol for Total RNA Purification from Plasma or Serum

Note: Plasma or Serum 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 plasma or serum.

We recommend the use of this kit to isolate RNA from plasma or serum prepared by standard protocol from non-coagulating fresh blood using EDTA or sodium citrate as the anti-coagulant. Plasma prepared from fresh blood using heparin as an anti-coagulant is not suitable for use with this protocol. For heparin-prepared samples follow the protocol in section **1C. Lysate Preparation from Blood.**

It is recommended that no more than 200 µL of plasma or serum be used in order to prevent clogging of the column. Avoid multiple freeze-thaw cycle of the plasma or serum sample. Aliquot to the appropriate volume for usage prior to freezing. It is important to work quickly during this procedure.

The yield of RNA from plasma and serum is highly variable. In general, the expected yield could vary from 1 to 100 ng per 100 µL of plasma or serum used. In addition, the expected A_{260}:A_{280} ratio as well as the A_{260}:A_{230} ratio will be lower (<1.80) than the normal acceptable range from other cells or tissues. Nonetheless, the isolated RNA could still be used effectively in different downstream applications such as RT-qPCR or microarrays.

Procedure

Transfer up to 200 µL of plasma or serum to an RNase-free microcentrifuge tube. Add 300 µL of **Buffer RL** to every 100 µL of plasma or serum. Mix by vortexing for 10 seconds.

Optional: Add 0.7 µL of 0.8 µg/µL MS2 RNA per sample. The use of MS2 RNA could increase the consistency of downstream applications such as RT-PCR. However, the use of MS2 RNA is not recommended for applications involving global gene expression analysis such as microarrays or sequencing.

Add 400 µL of 96–100% ethanol (provided by the user) to every 400 µL of the lysate (equivalent to every 100 µL of plasma or serum used). Mix by vortexing for 10 seconds.

Section 2
RNA Purification and Storage for All Types of Lysate

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

2A. Binding RNA to Column

2B. Washing Column

2C. RNA Elution

2D. RNA Storage

2A. Binding RNA to Column

Assemble a column with one of the provided collection tubes. Apply up to 600 µL of the lysate with the ethanol (from Step 1) onto the column and centrifuge for 1 minute at **≥3,500 × g** (~6,000 rpm). 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 × g** (~14,000 rpm). Discard the flowthrough. Reassemble the spin column with its collection tube. Depending on the lysate volume, repeat the above steps as necessary.

Total RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination.

Optional: On-Column DNA Removal Protocol can be performed at this step for maximum removal of residual DNA that may affect sensitive downstream applications. Please refer to separate instructions following this procedure to perform the On-Column DNA Removal (Cat. No. DNASE10) **that is not included in this kit.**

2B. Column Wash

Apply 400 µL of **Wash Solution A** to the column and centrifuge for 1 minute. Ensure the entire ethanol 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 flow through and reassemble the spin column with its collection tube. Repeat steps to wash column two additional times. After the final wash, spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

2C. RNA Elution

Place the column into a fresh 1.7 mL Elution tube provided with the kit. Add 50 µL of **Elution Solution A** to the column. Centrifuge for 2 minutes at **200 × g** (~2,000 rpm), followed by 1 minute at **14,000 × g** (~14,000 rpm). Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column at 14,000 × g (~14,000 rpm) for 1 additional minute. For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat the above steps).

2D. 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.

OPTIONAL:
Protocol for On-Column DNA Removal
(NOT included in this kit)
This is an optional protocol for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that On-Column DNase I Digestion Set (Cat. No. DNASE10) be used for this step.

For every on-column reaction to be performed, prepare a mix of 15 µL of **DNase I** and 100 µL of **Enzyme Incubation Buffer A** using the RNase-Free DNase I Kit. Mix gently by inverting the tube a few times. **DO NOT VORTEX.** If using an alternative DNase I, prepare a working stock of 0.25 Kunitz unit/µL RNase-free DNase I solution according to the manufacturer's instructions. A 100 µL aliquot is required for each column to be treated.

Perform the appropriate Total RNA Isolation Procedure for the starting material up to and including "**Binding to Column**" (Steps 1 and 2 of all protocols). Apply 400 µL of Wash Solution A to the column and centrifuge for 2 minutes. Discard the flowthrough. Reassemble the spin column with its collection tube.

Apply 100 µL of the RNase-free DNase I solution prepared in Step 1 to the column and centrifuge at 14,000 × g (~14,000 rpm) for 1 minute. Ensure the entire DNase I solution passes through the column. If needed, spin at 14,000 × g (~14,000 rpm) for an additional minute.

After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column. Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species. Incubate the column assembly at

25–30 °C for 15 minutes. Without any further centrifugation, proceed directly to the second wash step in the "**Column Wash**" section (Step 2B).

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure the appropriate amount of Buffer RL 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" 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 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 90 mL of 96-100% ethanol is added to the supplied Wash Solution A 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 the starting material.
	Cell Culture: Cell monolayer was not washed with PBS	Ensure the cell monolayer is washed with the appropriate amount of PBS in order to remove residual medium from cells.
	Yeast: Lyticase was not added to the Resuspension Buffer	Ensure the appropriate amount of lyticase is added when making the Resuspension Buffer.
	Bacteria and Yeast: All traces of medium not removed	Ensure all the medium is removed prior to the addition of the Buffer RL through aspiration.
Clogged Column	Insufficient solubilization of cells or tissues	Ensure the appropriate amount of Buffer RL 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 column.
	Centrifuge temperature too low	Ensure 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.

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 "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 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 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 the integrity of the RNA is not compromised.
	Starting material may have a high RNase content	For starting materials with high RNAase content, it is recommended that β-mercaptoethanol be added to the Buffer RL.
	Lysozyme or lyticase used may not be RNAse-free	Ensure the lysozyme and lyticase being used with this kit is RNase-free, in order to prevent possible problems with RNA degradation.
RNA does not perform well in downstream applications	RNA was not washed 3 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 3 times with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure 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 amounts of starting material used	Perform RNAse-free DNase I digestion on the RNA sample after elution to remove genomic DNA contamination. It is recommended that On-Column DNase I Digestion Set (Cat. No. DNASE10) be used for this step.

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