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Total RNA Purification Plus Kit — Product Insert

Product # 48400

Norgen's Total RNA Purification Plus 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). Genomic DNA is removed from the sample using a Genomic DNA Removal column, and 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.

Norgen's Purification Technology

Purification is based on spin column chromatography. 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 (please see the flow chart on page 4). The DNA is then captured on a Genomic DNA Removal Column. Ethanol is then added to the flowthrough of the DNA removal step, and the solution is loaded onto an RNA Purification Column. Norgen's resin binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the column, while the contaminating proteins will be removed in the flowthrough 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.

Kit Components

Component	Product # 48300 (50 preps)	Product # 48400 (100 preps)
Buffer RL	40 mL	2 x 40 mL
Wash Solution A	38 mL	2 x 38 mL
Elution Solution A	6 mL	2 x 6 mL
RNA Purification Columns	50	100
Genomic DNA Removal Columns	50	100
Collection Tubes	100	200
Elution tubes (1.7 mL)	50	100
Product Insert	1	1

Advantages

- Fast and easy processing using rapid spin-column format
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions
- Isolate high quality total RNA from a variety of sources
- RNA can be isolated and detected from as little as a single animal cell
- Rapidly remove contaminating genomic DNA without the use of enzymes

Specifications

Kit Specifications	
Maximum Column Binding Capacity	50 µg
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 Plasma/Serum Bacteria Yeast Fungi Plant Tissues	 3 x 10^6 cells 25 mg (for most tissues) 100 µL 200 µL 1 x 10^9 cells 1 x 10^8 cells 50 mg 50 mg
Time to Complete 10 Purifications	22 minutes
Average Yields HeLa Cells (1 x 10^6 cells) *E. coli* (1 x 10^9cells)	 15 µg 50 µg

Storage Conditions and Product Stability
All 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*.

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
You must have the following in order to use the Total RNA Purification Plus 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

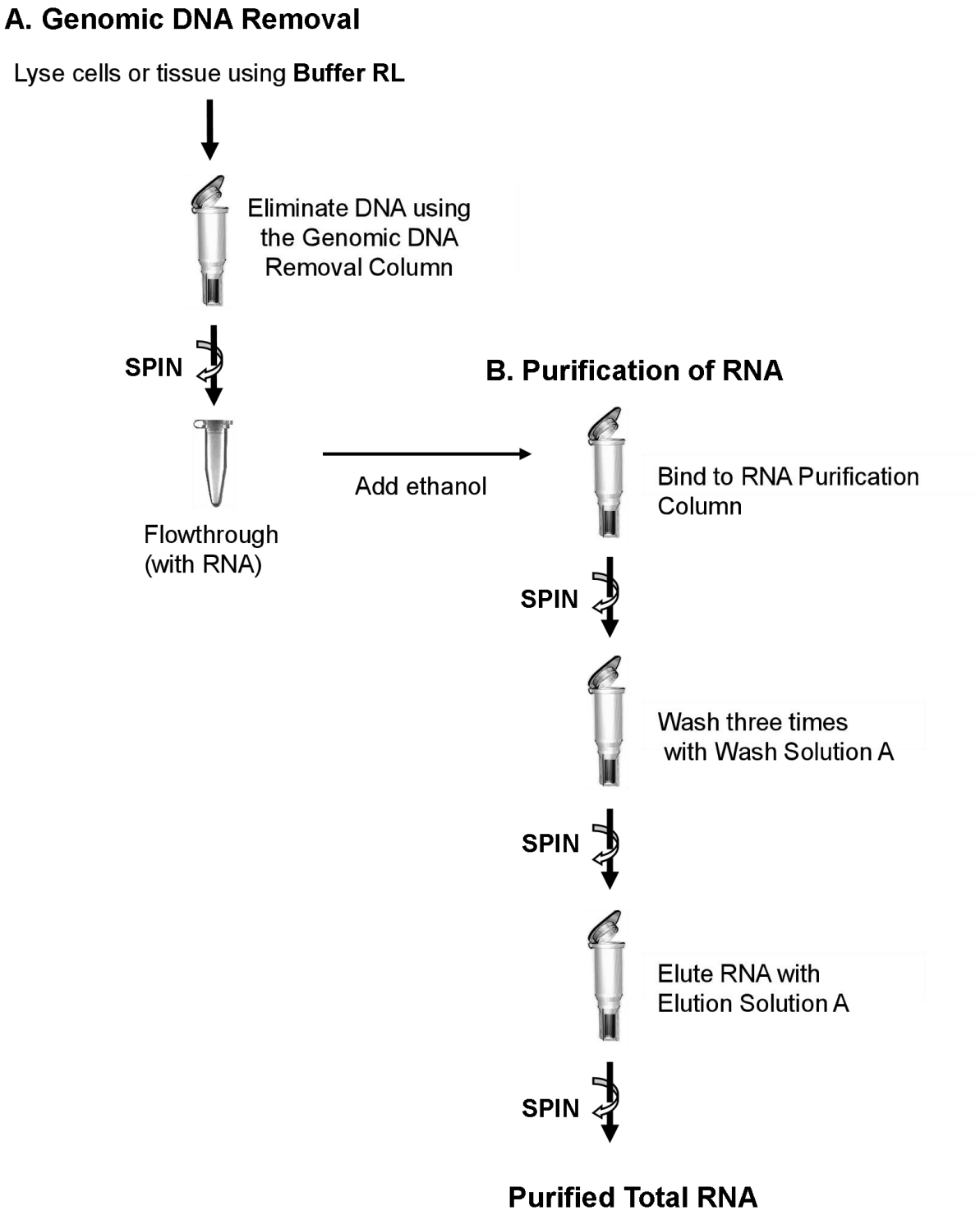
- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

For Plasma/Serum Protocol

- MS2 RNA (0.8 µg/µl). (Roche, Cat. No. 10165948001)

Flowchart

Procedure for Purifying Total RNA using Norgen's Total RNA Purification Plus Kit



Procedures

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

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

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

Working with RNA

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

Section 1. Preparation of Lysate From Various Cell Types

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), with the exception of the protocol for plasma/serum. A separate protocol for the isolation of total RNA from plasma/serum samples is located in Appendix B.***
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed, as indicated in the table below:

Sample Type	Lysate Preparation Page #
Cultured Cells	6
Animal Tissue	7
Blood	8
Plasma/Serum	12
Nasal / Throat Swabs	8
Bacteria	8
Yeast	9
Fungi	10
Plant	10
Viruses	11

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96-100% ethanol (provided by the user) to each 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), 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.

1A. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use

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

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 350 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 microcentrifuge tube.
e. **Proceed to Step 2 (page 11).**

Note: For input 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.

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 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.

d. **Proceed to Step 2 (page 11).**

Note: For input 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.

1B. Lysate Preparation from Animal Tissues

Notes Prior to Use

- Norgen's Total RNA Purification Plus Kit is designed for isolating RNA from small amount of tissue sample (up to 20 mg in most cases). If a larger amount of starting material is desired, Norgen's Animal Tissue RNA Purification Kit (Cat.# 25700) 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 such as RNA*later*® 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 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	20 mg
Liver	20 mg
Lung	20 mg
Spleen	20 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 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.
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. **Proceed to Step 2 (page 11).**

1C. Lysate Preparation from Blood

Notes Prior to Use

- This procedure is for the isolation of RNA from whole blood. ***For the isolation of RNA from plasma or serum samples, please see Appendix A.***
- 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.

1C. 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 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.
c. **Proceed to Step 2 (page 11).**

1D. Lysate Preparation from Nasal or Throat Swabs

Notes Prior to Use

- Body fluid 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 these samples.
- It is important to work quickly during this procedure.

1D. Cell Lysate Preparation from Nasal or Throat Swabs

a. Add 600 µL of **Buffer RL** to an RNase-free microcentrifuge tube (not provided).
b. Gently brush a sterile, single-use cotton swab inside the nose or mouth of the subject.
c. 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.
d. Using a pipette, transfer the lysate into another RNase-free microcentrifuge tube (not provided). Note the volume of the lysate.
e. **Proceed to Step 2 (page 11).**

1E. 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 1Ec)**.

1E. 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 1) by vortexing. Incubate at room temperature for the time indicated in Table 1.
d. Add 300 μL of Buffer RL and vortex vigorously for at least 10 seconds.
e. **Proceed to Step 2 (page 11).**

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

Notes Prior to Use

- 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, 1M 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. These reagents are to be provided by the user.
- 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 **(Step 1Fc)**.

1F. Cell Lysate Preparation

a. Pellet yeast 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 yeast thoroughly in 100 μL of Lyticase-containing Resuspension Buffer by vortexing. Incubate at 37°C for 10 minutes.
d. Add 300 μL of **Buffer RL** and vortex vigorously for at least 10 seconds.
e. **Proceed to Step 2 (page 11).**

1G. Lysate Preparation from Fungi

Notes Prior to Use

- 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 fungi be used for this procedure in order to prevent clogging of the column.

1G. Cell Lysate Preparation from Fungi

a. Determine the amount of fungi by weighing. It is recommended that no more than 50 mg of fungi be used for the protocol.
b. 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.

   Note: At this stage the ground fungus may be stored at -70°C, such that the RNA purification can be performed at a later time.

c. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
d. Add 600 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized.
e. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
f. 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.
g. **Proceed to Step 2(page 11).**

1H. Lysate Preparation from Plant

Notes Prior to Use

- The maximum recommended input of plant tissue is 50 mg or 5 x 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.

1H. Cell Lysate Preparation from Plant

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).
d. 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.

e. **Proceed to Step 2 (page 11).**

1I. Lysate Preparation from Viruses

Notes Prior to Use

- 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 **1H** 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.

1I. Cell Lysate Preparation from Viral Suspension

a. Transfer up to 100 µL of viral suspension to an RNase-free microcentrifuge tube (not provided).
b. Add 350 µL of **Buffer RL**. Lyse viral cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.
c. **Proceed to Step 2 (page 11).**

Section 2. Genomic DNA Removal from All Types of Lysate

Notes

- The following steps of the procedure for the removal of genomic DNA are the same for all of the different types of lysate.
- 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 Removal Columns - column has blue and white contents
    - RNA Purification Columns – column has grey and white contents

2. Genomic DNA Removal

a. Assemble a gDNA Removal Column with one of the provided collection tubes.
b. Apply up to 600 µL of the lysate prepared from Section 1 onto the column and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

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.

c. Retain the flowthrough for RNA Purification (Section 3). The flowthough contains the RNA and should be stored on ice or at -20°C until the RNA Purification protocol is carried out.
d. Dispose of the gDNA Removal Column with the bound gDNA.

Section 3. Total RNA Purification from All Types of Lysate

3. Binding RNA to Column

a. To every 100 µL of flowthrough from Step 2c, add 60 µL of 96 – 100 % Ethanol. Mix by vortexing.

Note: For example, for 300 µL of flowthrough, add 180 µL of 96 – 100 % Ethanol

b. Assemble an RNA Purification Column with one of the provided collection tubes.
c. Apply up to 600 µL of the lysate with the 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. Depending on your lysate volume, repeat Step **3b** and **3c** as necessary.

4. Column Wash

a. Apply 400 µL of **Wash Solution A** to the column and centrifuge 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 spin column with its 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. RNA Elution

a. Place the 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 50 µL 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

Protocol for Total RNA Purification from Plasma or Serum

Notes Prior to Use

- 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**.
- Due to the relatively low DNA content in plasma, the Genomic DNA Removal column is not necessary for this procedure.
- 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 plasma or serum used. In addition, the expected A260:A280 ratio as well as the A260:A230 ratio will be lower (<1.80) than the normal acceptable range from other cells or tissues. Nonetheless, these isolated RNA could still be used effectively in different downstream applications such as RT-qPCR or microarrays.

1. Cell Lysate Preparation from Plasma/Serum

a. Transfer up to 200 µL of plasma or serum to an RNase-free microcentrifuge tube (not provided).
b. Add 300 µL of Buffer RL to every 100 µL of plasma or serum. Mix by vortexing for 10 seconds.
c. **Optional:** Add 0.7 µL of 0.8 µg/µl MS2 RNA per sample.

Note: 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.

d. Add 400 µL of 96 – 100% ethanol (provided by the user) to every 400 µL of the lysate (equivalent to every 100 µL plasma or serum used). Mix by vortexing for 10 seconds. Proceed to Step 2 below.

2. Binding RNA to Column

a. Assemble an RNA Purification Column with one of the provided collection tubes
b. 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 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)**.

c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Depending on your lysate volume, repeat Step 2b and 2c as necessary.

3. Column Wash

a. Apply 400 µL of 96 – 100% ethanol (provided by the user) to the column and centrifuge for 1 minute.

Note: 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.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps 3a and 3b to wash column a second time.
d. Wash column a third time by adding another 400 µL of 96 – 100% ethanol (provided by the user) 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.

4. RNA Elution

a. Place the 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 50 µL 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.

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.
	Column has become clogged	Either the Genomic DNA Removal Column or the RNA Purification Column may become clogged if the recommended amount of starting materials is exceeded. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.
	An alternative elution solution was used	It is recommended that the Elution Solution A supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use.
	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.
	Yeast: Lyticase was not added to the Resuspension Buffer	Ensure that the appropriate amount of lyticase is added when making the Resuspension Buffer.
	Bacteria and Yeast: All traces of media not removed	Ensure that all media is removed prior to the addition of the Buffer RL through aspiration.
Clogged Column (either Genomic DNA Removal Column or RNA Purification Column)	Insufficient solubilization of cells or tissues	Ensure that 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 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 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.
	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 that 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 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	Amount of genomic DNA in sample exceeds capacity of Genomic DNA Removal Column	Perform additional DNase treatment post-isolation. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

Related Products	Product #
RNase-Free DNase I Kit	25710
Animal Tissue RNA Purification Kit	25700
Plant/Fungi Total RNA Purification Kit	25800
RNA/Protein Purification Kit	24100
RNA/DNA/Protein Purification Kit	24000
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) or through email at support@norgenbiotek.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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