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Total RNA Purification Maxi Kit — Product Insert

Product # 26800

Norgen's Total RNA Purification Maxi Kit provides a rapid method for the isolation and purification of total RNA from cultured animal cells, tissue samples, blood, 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.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's 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 (please see the flow chart on page 4). Ethanol is then added to the lysate, and the solution is loaded onto a maxi spin-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.

Specifications

Kit Specifications	
Column Binding Capacity	1.5 mg
Maximum Column Loading Volume	20 mL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Animal Cells Animal Tissues Blood Bacteria Yeast Fungi Plant Tissues	 5×10^7 cells 50 - 250 mg 2 – 10 mL* 2.5×10^{10} cells 2×10^8 cells 1 g 1 g
Time to Complete 4 Purifications	40 minutes
Average Yields HeLa Cells (5×10^7 cells) *E. coli* (2.5×10^{10} cells)	 750 µg 1.5 mg

***** *for isolating total RNA from purified leukocytes*

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

Kit Components

Component	Product # 26800 (8 preps)
Buffer RL	2 x 40 mL
Wash Solution A	4 x 38 mL
Elution Solution A	3 x 20 mL
RNA Maxi Spin Columns (with collection tubes)	8
Elution tubes (50 mL)	8
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years 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 Maxi Kit:

For All Protocols

- Centrifuge with a swinging bucket rotor capable of 3000 x g
- 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 Leukocytes

- RBC Lysis Buffer (Norgen Cat. # 21201)

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

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

Flowchart

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

Lyse cells or tissue using **Buffer RL**

Add Ethanol

Bind to column

SPIN

Wash three times
with Wash Solution A

SPIN

Elute RNA with
Elution Solution A

SPIN

Purified Total RNA

Procedures

All centrifugation steps are carried out in a centrifuge with a swinging bucket. Various speeds are required for different steps, so please check your centrifuge 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

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.
- All centrifugation steps are carried out in a centrifuge with a swinging bucket at 3,000 x g (~ 3,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96-100% ethanol (provided by the user) to each of the four supplied bottles containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL per bottle. The label on the bottles 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.
- 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. For the isolation of RNA from free viral particles, follow Section **1I**.
- Pre-warm an appropriate amount of **Elution Solution A** to 70°C prior to isolation.
- 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 5 x 10^7. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a confluent 15 cm plate of HeLa cells will contain 2 x10^7 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 a total of 8 mL of **Buffer RL** directly to culture plate(s). A minimum volume of 3 mL of **Buffer RL** should be added to each 15 cm plate.
c. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes.
d. Transfer lysate to a 50 mL centrifuge tube.
e. Add 4.5 mL of 96 – 100% ethanol (provided by the user) to the 8 mL lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

Note: For input amounts greater than 3 x 10^7 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 300 *x g* (~800 to 1,000 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 8 mL 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 4.5 mL of 96 - 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

Note: For input amounts greater than 3 x 10^7 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

- 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 100 mg. **To isolate RNA from larger amounts of tissue, particularly from those with high connective tissue content such as muscle and heart, please follow the specialized protocol with the use of Proteinase K in Appendix B.**

Table 1. Recommended Maximum Input Amounts of Different Tissues

Tissue	Maximum Input Amount
Brain	250 mg
Heart	50 mg
Kidney	100 mg
Liver	100 mg
Lung	100 mg
Spleen	100 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 100 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 8 mL 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 50 mL centrifuge tube (not provided).

g. Spin the lysate for 10 minutes at 3,000 *x g* (~3,000 RPM) to pellet any cell debris. Transfer the supernatant to another RNase-free 50 mL centrifuge tube. Note the volume of the supernatant/lysate.
h. Add an equal volume of 70% ethanol (provided by the user) to the lysate volume collected (1 mL of 70% ethanol is added to every 1 mL of clarified lysate). Vortex to mix. **Proceed to Step 2.**

1C. 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 2 mL of blood be used in order to prevent clogging of the column. If you wish to isolate RNA from greater than 2 mL, please follow the protocol in Section 1D below.
- 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 2 mL of non-coagulating blood to an RNase-free 50 mL centrifuge tube (not provided).
b. Add 6 mL 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 4 mL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

1D. Lysate Preparation from Leukocytes

Notes Prior to Use

- The maximum recommended input for the following protocol is 10 mL of blood. Please note that the RBC Lysis Buffer (Cat. # 21201) is to be provided by the user.
- The maximum recommended input of leukocytes is 5×10^7. As a general guideline, blood from a healthy individual contains about 5×10^6 leukocyte per mL.
- If the leukocytes were purified by other methods, ensure that either the cells are used fresh (proceed to **Step 1D f**) or flash-frozen by liquid nitrogen before use.
- 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 1D f).**

1D. Lysate Preparation from Leukocytes

a. Add 5 volumes of **RBC Lysis Buffer** (provided by the user) to blood samples collected with EDTA (i.e.: Add 5 mL of **RBC Lysis Buffer** to 1 mL of blood). Up to 10 mL of blood from a healthy individual may be used as the input.
b. Incubate at room temperature for 3 to 5 minutes, with brief vortexing during the incubation to mix.

Note: Ensure that the solution changes from a milky, opaque pink to clear red before proceeding to the next step.

c. Centrifuge at 300 x g (~800 to 1,000 RPM) for 5 minutes and decant the supernatant.
d. Add 2 additional volumes of **RBC Lysis Buffer** to pelleted white blood cells and mix by gentle vortexing for 10 seconds. (i.e. Add 2 mL of **RBC Lysis Buffer** to every 1 mL of input blood volume)
e. Centrifuge at 300 x g (~800 to 1,000 RPM) for 5 minutes and decant supernatant. A few μL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.
f. Add 8 mL of **Buffer RL** directly to pelleted leukocytes.
g. Lyse cells by gentle vortexing until homogeneity is reached.
h. Add 4.5 mL of 96 – 100% ethanol (provided by the user) to the mixture and mix by vortexing for 10 seconds.

Note: For input amounts greater than 3×10^7 leukocytes, 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.

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 2.5×10^{10} bacterial cells (~ 25 mL *E. coli*) be used in this procedure. Bacterial growth can be measured using a spectrophotometer. As a general rule, an *E. coli* culture containing 1×10^9 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 3,000 *x g* (~3,000 RPM) for 5 minutes.
b. Decant supernatant, and carefully remove any remaining media by aspiration.
c. Resuspend the bacteria thoroughly in 2 mL of the appropriate lysozyme-containing TE buffer (see Table 2) by vortexing. Incubate at room temperature for the time indicated in Table 1.
d. Add 6 mL of Buffer RL and vortex vigorously for at least 10 seconds.
e. Add 4 mL 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

1F. Lysate Preparation from Yeast

Notes Prior to Use

- Prepare the appropriate amount of Lyticase-containing Resuspension Buffer, considering that 2 mL 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 2 x 10^8 yeast cells or 20 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 3,000 *x g* (~3,000 RPM) for 5 minutes.
b. Decant the supernatant, and carefully remove any remaining media by aspiration.
c. Resuspend the yeast thoroughly in 2 mL of Lyticase-containing Resuspension Buffer by vortexing. Incubate at 37ºC for 10 minutes.
d. Add 6 mL of Buffer RL and vortex vigorously for at least 10 seconds.
e. Add 4 mL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

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 1 g 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 1 g 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 8 mL 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 50 mL centrifuge tube (not provided).
f. Spin the lysate for 5 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free 50 mL centrifuge tube. Note the volume of the supernatant/lysate.
g. Add an equal volume of 70% ethanol (provided by the user) that is equivalent to the lysate volume collected (1 mL of ethanol is added to every 1 mL of lysate). Vortex to mix. **Proceed to Step 2.**

1H. Lysate Preparation from Plant

Notes Prior to Use

- The maximum recommended input of plant tissue is 1 g or 1 x 10^8 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 ≤1 g of plant tissue or 1 x 10^8 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 8 mL 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 50 mL centrifuge tube (not provided).
d. Spin the lysate for 5 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free 50 mL centrifuge tube. Note the volume of the supernatant/lysate.
e. Add an equal volume of 70% ethanol (provided by the user) that is equivalent to the lysate volume collected (1 mL of 70% ethanol is added to every 1 mL of lysate). Vortex to mix. **Proceed to Step 2.**

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.
- For the isolation of RNA from free viral particles, follow the procedure below.
- It is recommended that no more than 2 mL 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 2 mL of viral suspension to an RNase-free 50 mL centrifuge tube (not provided).
b. Add 6 mL of **Buffer RL**. Lyse viral cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.
c. Add 4 mL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

Section 2. Total RNA Purification from All Types of Lysate

Notes:

- 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.
- Preheat an appropriate amount of **Elution Solution A** to 70°C prior to isolation

2. Binding RNA to Column

a. Apply all of the lysate with the ethanol (from **Step 1**) onto the RNA maxi spin column assembly. Place the cap loosely onto the column assembly so as not to impede liquid flow. Centrifuge at 3,000 x g (~3,000 RPM) for 5 minutes.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional 5 minutes.

b. Discard the flowthrough. Reassemble the spin column with its collection tube.

Optional Step:

Norgen's Total RNA Purification Maxi Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step. This step should be performed at this point in the protocol

3. Column Wash

a. Apply 15 mL of **Wash Solution A** to the column assembly. Place the cap loosely onto the column assembly so as not to impede liquid flow. Centrifuge at 3,000 x g (~3,000 RPM) for 5 minutes.

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 **3a** and **3b** to wash column a second time.
d. Spin the column for 5 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. RNA Elution

a. Ensure that the **Elution Solution A** is heated to 70°C.
b. Place the column into a fresh 50 mL Elution Tube provided with the kit.
c. Add 2 mL of warm **Elution Solution A** to the column. Place the cap loosely onto the column assembly so as not to impede liquid flow.
d. Centrifuge for 5 minutes at **3,000 x *g* (~3,000 RPM)**. Note the volume eluted from the column. If the entire 2 mL has not been eluted, spin the column at 3,000 x *g* (~3,000 RPM) for an additional 5 minutes.

Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate 50 mL centrifuge tube (Repeat **Steps 4c** and **4d**).

5. Storage of RNA

The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Appendix A

Protocol for Optional On-Column DNA Removal

Norgen's Total RNA Purification Maxi Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-free DNase I Kit (Product # 25710) be used for this step. Each RNase-free DNase I Kit will be sufficient to perform four on-column DNA removals.

1. For every on-column reaction to be performed, prepare a mix of 150 µL of **DNase I** and 1 mL of **Enzyme Incubation Buffer A** using Norgen's RNase-Free DNase I Kit (Product # 25710). Mix gently by inverting the tube a few times. **DO NOT VORTEX**.

   Note: 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 1 mL aliquot is required for each column to be treated.

2. Perform the appropriate Total RNA Isolation Procedure for your starting material up to and including "**Binding to Column**" (Steps 1 and 2 of all protocols)
3. Apply 7.5 mL of **Wash Solution A** to the column and centrifuge at 3,000 x g (~3,000 RPM) for 5 minutes. Discard the flowthrough. Reassemble the spin column with its collection tube.
4. Apply 1 mL of the RNase-free DNase I solution prepared in Step 1 to the column and centrifuge at 3,000 x g (~3,000 RPM) for 5 minutes.

   Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at 3,000 x g (~3,000 RPM) for an additional two minutes.

5. After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column.

   Note: 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.

6. Incubate the column assembly at 25 - 30°C for 15 minutes.
7. Without any further centrifugation, apply 7.5 mL of Wash Solution A to the column and centrifuge at 3,000 x g (~3,000 RPM) for 5 minutes. Discard the flowthrough. Reassemble the spin column with its collection tube.
8. Apply 15 mL of **Wash Solution A** to the column and centrifuge for 5 minute.
9. Discard the flowthrough and reassemble the spin column with its collection tube.
10. Spin the column for 5 minutes in order to thoroughly dry the resin. Discard the collection tube. Proceed to Step 4 (RNA Elution)

Appendix B

Lysate Preparation from Animal Tissues with the use of Proteinase K

Customer-Supplied Reagent

- RNase-Free Proteinase K
- RNase-Free Water

Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- All enzymes provided should remain at the storage temperature indicated on each vial until use.
- Reconstitute each of the **Proteinase K** vials in an appropriate amount of molecular biology grade water or 10 mM Tris.HCl pH 7.5 (RNase-Free) to give a 20 mg/mL final concentration. Aliquot into small fractions and store the unused portions at -20°C until needed.
- 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.
- 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 any excessive liquid.
- This protocol is particularly suitable for isolating RNA from > 50 mg of connective tissues. However, it is recommended that the maximum tissue input does not exceed 250 mg.

Cell Lysate Preparation

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 100 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.

   Note: The use of liquid nitrogen is recommended. However, if homogenization without flash-freezing is preferred, proceed to Step **e**.

d. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
e. Add 4 mL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized.

   Note: Maximum homogenization may be achieved 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 50 mL tube (not provided).
g. Add 8 mL of **RNase-Free Water** (not provided) to the lysate. Vortex to mix.
h. Add 250 µL of reconstituted Proteinase K to the lysate, and incubate at 55°C for 15 minutes. Vortex the tubes occasionally during incubation.
i. Spin the lysate for 10 minutes at 3,000 *x g* (~3,000 RPM) to pellet any cell debris. Transfer the supernatant to another RNase-free 50 mL centrifuge tube.
j. Add 6 mL of 96-100% ethanol (provided by the user) to the lysate. Vortex to mix.

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	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 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 each of the supplied Wash Solution A bottle 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	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.

Problem	Possible Cause	Solution and Explanation
Clogged 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 Maxi Column Assembly was tightly capped	Ensure that the cap is loosely placed onto the column assembly so as not to impede liquid flow.
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 2 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 2 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.

Problem	Possible Cause	Solution and Explanation
Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination. 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
Total RNA Purification Kit	17200
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 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.

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

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