

## RNA/Protein Purification Plus Kit

Product # 48100, 48200

## Product Insert

Norgen's RNA/Protein Purification Plus Kit provides a rapid method for the isolation and purification of total RNA and proteins sequentially from a single sample of cultured animal cells, tissue samples, blood, bacteria, yeast, fungi or plants. The total RNA and proteins are all column purified in less than 30 minutes. This kit allows for rapid, efficient, non-enzymatic removal of contaminating DNA using the provided gDNA Removal Columns. The kit is ideal for researchers who are interested in studying the genome, proteome and transcriptome of a single sample, such as for studies of microRNA profiling, gene expression including gene silencing experiments or mRNA knockdowns, studies involving biomarker discovery, and for characterization of cultured cell lines. Norgen's RNA /Protein Purification Plus Kit is especially useful for researchers who are isolating macromolecules from precious, difficult to obtain or small samples such as biopsy materials or single foci from cell cultures, as it eliminates the need to fractionate the sample. Furthermore, analysis will be more reliable since the RNA and proteins are derived from the same sample, thereby eliminating inconsistent results. The purified macromolecules are of the highest purity and can be used in a number of different downstream applications.

### Norgen's Purification Technology

#### ***RNA Purification***

Purification is based on spin column chromatography. The process involves first lysing the cells or tissue of interest with the provided Lysis Solution. The DNA is then captured and eliminated using a Genomic DNA Removal Column. Ethanol is then added to the flowthrough of the DNA elimination step, and the solution is loaded onto a RNA/Protein Purification Column. Norgen's resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the RNA including microRNAs will bind to the column while the proteins are removed in the flowthrough. Next, the bound RNA is washed with the provided RNA Wash Solution to remove impurities, and the purified RNA is eluted with the RNA Elution Solution. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). 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.

#### ***Protein Purification***

The proteins that are present from the RNA binding flowthrough can now be loaded directly onto an SDS-PAGE gel for visual analysis. Alternatively, the protein samples can be further purified using the same RNA/Protein Purification Column that was used for purifying the RNA. After the RNA has been eluted from the column, the flowthrough is then pH adjusted and loaded back onto the column in order to bind the proteins that are present. The bound proteins are washed with the provided wash buffer, and are then eluted such that they can be used in downstream applications. The purified proteins can be used in a number of downstream applications including SDS-PAGE analysis, Western blots and mass spectrometry.

### Advantages

- Fast and easy processing using rapid spin-column format
- Column purification of both RNA and proteins
- Rapid and efficient non-enzymatic gDNA removal using gDNA Removal Columns
- Sequentially isolate RNA and proteins from a single lysate – no need to split the lysate
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- Superior performance with tissues and other complex samples
- Isolate high quality total RNA
- High yields of isolated proteins

## Specifications

| Kit Specifications  |   |
|---|---|
| Maximum Column Binding Capacity   | 50 µg for RNA<br>200 µg for protein   |
| Maximum Column Loading Volume   | 650 µL  |
| Size of RNA Purified  | All sizes, including small RNA (<200 nt)  |
| Maximum Amount of Starting Material:<br>Animal Cells<br>Animal Tissues<br>Blood<br>Bacteria<br>Yeast<br>Fungi<br>Plant Tissues              | 5 x 10 <sup>6</sup> cells<br>Up to 25 mg (for most tissues)<br>200 µL<br>1 x 10 <sup>9</sup> cells<br>1 x 10 <sup>8</sup> cells<br>50 mg<br>50 mg |
| Time to Complete 10 Purifications   | 30 minutes  |
| Average Yields*<br>HEK 293 Cells (1 x 10 <sup>6</sup> cells)<br>HEK 293 Cells (1 x 10 <sup>6</sup> cells)<br>Liver (15 mg)<br>Liver (15 mg) | 10 - 15 µg RNA<br>70 - 100 µg protein<br>30 - 35 µg RNA<br>100 - 150 µg protein   |

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

## Kit Components

| Component                        | Product # 48100<br>(20 samples) | Product # 48200<br>(50 samples) |
|----------------------------------|---------------------------------|---------------------------------|
| Lysis Solution                   | 15 mL                           | 40 mL                           |
| RNA Wash Solution                | 10 mL                           | 22 mL                           |
| RNA Elution Solution             | 6 mL                            | 6 mL                            |
| Protein Wash Buffer              | 15 mL                           | 30 mL                           |
| Protein pH Binding Buffer        | 4 mL                            | 8 mL                            |
| Protein Elution Buffer           | 6 mL                            | 8 mL                            |
| Protein Neutralizer              | 2 mL                            | 2 mL                            |
| Protein Loading Dye              | 2 mL                            | 2 mL                            |
| gDNA Removal Columns             | 20                              | 50                              |
| RNA/Protein Purification Columns | 20                              | 50                              |
| Collection Tubes                 | 40                              | 100                             |
| Elution tubes (1.7 mL)           | 40                              | 100                             |
| Product Insert                   | 1                               | 1                               |

### **Storage Conditions and Product Stability**

The Protein Loading Dye should be stored at -20°C upon arrival. All other solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers

### **Precautions and Disclaimers**

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at [www.norgenbiotek.com](http://www.norgenbiotek.com).

The **Lysis Solution** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions

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

### **Customer-Supplied Reagents and Equipment**

You must have the following in order to use the RNA/Protein Purification Plus Kit:

#### *For All Protocols*

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

#### *For Animal Cell Protocol*

- PBS (RNase-free)

#### *For Animal Tissue Protocol*

- Liquid nitrogen
- Mortar and pestle

#### *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 Sorbitol
  - 1 unit/ $\mu$ L Lyticase

#### *For Fungi Protocol*

- Liquid nitrogen
- Mortar and pestle

#### *For Plant Protocol*

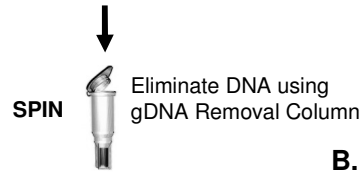
- Liquid nitrogen
- Mortar and pestle

# Flow Chart

Procedure for Purifying Total RNA and Proteins using Norgen's RNA/Protein Purification Plus Kit

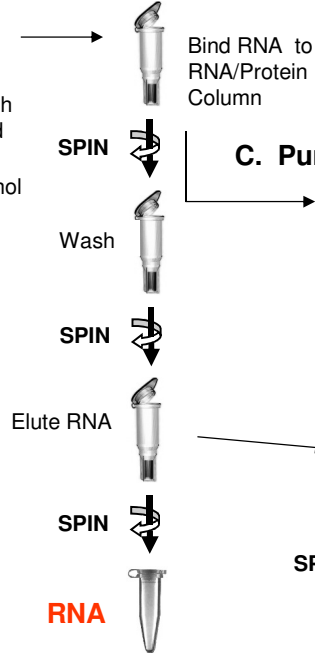
## A. Genomic DNA Removal

Lyse cells or tissue using **Lysis Solution**

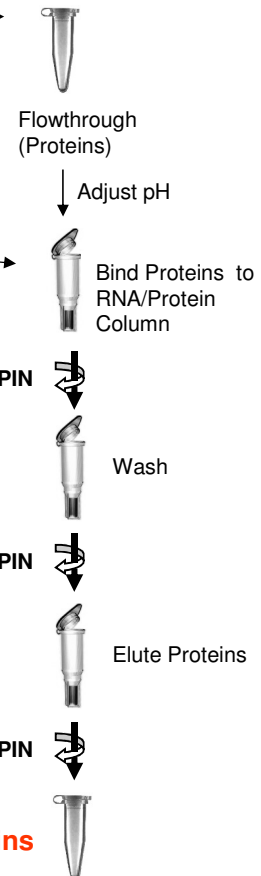


## B. Purification of RNA

Flowthrough (RNA and Proteins)  
- Add ethanol



## C. Purification of Proteins



## 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

## Procedures

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

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

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

## IMPORTANT NOTE:

This procedure is written in four steps. Section 1 contains the lysate preparation protocols from different types of starting materials. Please ensure that the proper protocol is followed for your sample. Section 2 contains the protocol to eliminate genomic DNA from the sample. Section 3 contains the protocol to isolate total RNA and Section 4 contains the protocol to isolate total proteins from the sample. The same protocols for Section 2 to Section 4 will apply to all the different starting materials.

### **Notes Prior to Use for all RNA/Protein Purification Procedures**

- The steps for preparing the lysate are different depending on the starting material (**Step 1**). However, the subsequent steps are the same in all cases (**Steps 2 – 10**).
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed.
- 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 **RNA Wash Solution** by adding 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated **RNA Wash Solution**:
  - Cat # 48100: Add 20 mL of 96 – 100% ethanol for a final volume of 30 mL
  - Cat# 48200: Add 50 mL of 96 – 100% ethanol for a final volume of 72 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  $\beta$ -mercaptoethanol in lysis is highly recommended for most tissues, particularly those known to have high RNase content (ex: pancreas). It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10  $\mu$ L of  $\beta$ -mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required.  $\beta$ -mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the lysis solution can be used as provided.
- It is important to work quickly when purifying RNA.
- This kit is provided with 2 separate columns. When columns are removed from the labelled bags they are supplied in they can easily be identified as follows:
  - gDNA Removal Columns - provided with collection tubes attached, column has predominately white contents
  - RNA/Protein Purification Columns – collection tubes provided separately, column has predominately black contents

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

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

#### *Notes Prior to Use*

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

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

- Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
- Add 300  $\mu$ L of **Lysis Solution** 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. **Proceed to Step 2.**

**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 reduce the viscosity of the lysate prior to loading onto the column.

### 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  $200 \times g$  (~2,000 RPM) for 10 minutes to pellet cells.
- Carefully decant the supernatant to ensure that the pellet is not dislodged. Wash the cell pellet with an appropriate amount of PBS. Centrifuge at  $200 \times g$  (~2,000 RPM) for another 5 minutes.
- Carefully decant the supernatant. A few  $\mu\text{L}$  of PBS may be left behind with the pellet in order to ensure that the pellet is not dislodged.
- Add 300  $\mu\text{L}$  of **Lysis Solution** to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step.  
**Proceed to Step 2.**

**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 reduce the viscosity of the lysate 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  $-70^\circ\text{C}$  freezer for long-term storage. Tissues may be stored at  $-70^\circ\text{C}$  for several months. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
- The maximum recommended input of tissue varies depending on the type of tissue being used. Please refer to Table 1 below as a guideline for maximum tissue input amounts. If your tissue of interest is not included in the table below we recommend starting with an input of no more than 10 mg.

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

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

### 1B. Cell Lysate Preparation from Animal Tissues

- Excise the tissue sample from the animal.
- Determine the amount of tissue by weighing. Please refer to Table 1 for the recommended maximum input amounts of different tissues. For tissues not included in the table, we recommend starting with an input of no more than 10 mg.
- Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.

- d. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
- e. Add 300  $\mu\text{L}$  of **Lysis Solution** 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 lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube (not provided). Note the volume of the supernatant/lysate. **Proceed to Step 2.**

## 1C. Lysate Preparation from 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 200  $\mu\text{L}$  of blood be used in order to prevent clogging of the column.
- We recommend the use of this kit to isolate RNA from non-coagulating fresh blood using EDTA as the anti-coagulant.

### 1C. Cell Lysate Preparation from Blood

- a. Transfer up to 200  $\mu\text{L}$  of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).
- b. Add 200  $\mu\text{L}$  of **Lysis Solution** to every 100  $\mu\text{L}$  of blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step. **Proceed to Step 2.**

## 1D. Lysate Preparation from Bacteria

### Notes Prior to Use

- Prepare the appropriate lysozyme-containing TE Buffer as indicated in Table 1. 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  $1 \times 10^9$  cells/mL has an  $\text{OD}_{600}$  of 1.0.
- For RNA isolation, bacteria should be harvested in log-phase growth.
- Bacterial pellets can be stored at  $-70^\circ\text{C}$  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 1Dc**).

### 1D. Cell Lysate Preparation from Bacteria

- a. Pellet bacteria by centrifuging at  $14,000 \times g$  ( $\sim 14,000$  RPM) for 1 minute.
- b. Decant supernatant, and carefully remove any remaining media by aspiration.
- c. Resuspend the bacteria thoroughly in 100  $\mu\text{L}$  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 300  $\mu\text{L}$  of Lysis Solution and vortex vigorously for at least 10 seconds. **Proceed to Step 2.**



**Table 2: Incubation Time for Different Bacterial Strains**

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

## 1E. Lysate Preparation from Yeast

### *Notes Prior to Use*

- Prepare the appropriate amount of Lyticase-containing Resuspension Buffer, considering that 500  $\mu$ L 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%  $\beta$ -mercaptoethanol and 1 unit/ $\mu$ L 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  $-70^{\circ}\text{C}$  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 1Ec**).

### 1E. Cell Lysate Preparation from Yeast

- a. Pellet yeast by centrifuging at  $14,000 \times g$  ( $\sim 14,000$  RPM) for 1 minute.
- b. Decant supernatant, and carefully remove any remaining media by aspiration.
- c. Resuspend the yeast thoroughly in 500  $\mu$ L of Lyticase-containing Resuspension Buffer by vortexing. Incubate at  $37^{\circ}\text{C}$  for 10 minutes.
- d. Pellet the spheroplasts at  $200 \times g$  ( $\sim 2,000$  RPM) for 3 minutes. Decant supernatant.
- e. Add 300  $\mu$ L of Lysis Solution and vortex vigorously for at least 10 seconds. **Proceed to Step 2.**

## 1F. Lysate Preparation from Fungi

### *Notes Prior to Use*

- Fresh or frozen fungi may be used for this procedure. Fungal tissue should be flash-frozen in liquid nitrogen and transferred immediately to a  $-70^{\circ}\text{C}$  freezer for long-term storage. Fungi may be stored at  $-70^{\circ}\text{C}$  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.
- It is important to work quickly during this procedure.

### 1F. 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  $-70^{\circ}\text{C}$ , 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 300  $\mu\text{L}$  of **Lysis Solution** 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 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. **Proceed to Step 2.**

## 1G. Lysate Preparation from Plant

### *Notes Prior to Use*

- The maximum recommended input of plant tissue is 50 mg or  $5 \times 10^6$  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  $-70^{\circ}\text{C}$  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.

### 1G. Cell Lysate Preparation from Plant

- a. Transfer  $\leq 50$  mg of plant tissue or  $5 \times 10^6$  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  $\mu\text{L}$  of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized.
- d. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
- e. Spin 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. **Proceed to Step 2.**

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

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

### 2. Genomic DNA Removal

- a. Retrieve a **gDNA Removal Column** pre-assembled with a collection tube.
- b. Apply up to 600  $\mu\text{L}$  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 and Protein Purification (Section 3). The flowthrough contains the RNA and proteins 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  $\mu\text{L}$  of flowthrough from Step 2c, add 60  $\mu\text{L}$  of 96 – 100 % Ethanol. Mix by vortexing.

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

- b. Assemble an **RNA/Protein Purification Column** with one of the provided collection tubes.
- c. Apply up to 600  $\mu\text{L}$  of the lysate with the ethanol onto the column and centrifuge for 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.

- d. **Retain the flowthrough for Protein Purification (Section 4). The flowthrough contains the proteins and should be stored on ice or at -20°C until the Protein Purification protocol is carried out.**
- e. Depending on your lysate volume, repeat steps **3c** and **3d** if necessary. The flowthroughs should be combined and retained in the same microcentrifuge tube.
- f. Reassemble the spin column with the collection tube.

### 4. RNA Wash

- a. Apply 400  $\mu\text{L}$  of **RNA Wash Solution** to the column and centrifuge at **14,000 x g (~14,000 RPM)** for 1 minute.

**Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

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

### 5. RNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 50  $\mu\text{L}$  of **RNA Elution Solution** to the column.

- c. Centrifuge for 2 minutes at **200 x g**, followed by 1 minute at **14,000 x g (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

**Note:** For maximum RNA recovery, particularly for samples that are known to contain large amounts of RNA, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b** and **5c**).

- d. **Retain the column for Protein Purification.** Proceed to Section 4 below for Protein Purification.

## 6. Storage of RNA

The purified RNA sample may be stored at  $-20^{\circ}\text{C}$  for a few days. It is recommended that samples be placed at  $-70^{\circ}\text{C}$  for long term storage.

## Section 4. Procedure to Isolate Total Proteins from All Cell Types

### Notes Prior to Use

- At this point, the proteins that are present in the flowthrough from the RNA Binding Step (Section 3, Step 3d) can be processed by one of the following three options:
  - Direct running on an SDS-PAGE gel with the provided loading dye for visual analysis
  - Column purification (recommended)
  - Acetone precipitation
- For direct running on a gel, the provided **Protein Loading Dye** should be used instead of regular SDS-PAGE Loading Buffer in order to prevent any precipitates from forming. Add 1 volume of the **Protein Loading Dye** to the sample and boil for 2 minutes before loading.
- Column purification of the proteins is recommended. For column purification please follow steps 10 to 14 below.
- For acetone precipitation, please refer to the supplementary protocol provided in the Appendix A below

## 7. pH Adjustment of Lysate

- a. Transfer the flowthrough from the RNA Binding Step (Section 3, Step 3d) to a separate microcentrifuge tube.
- b. For every 100  $\mu\text{L}$  of flowthrough, dilute with 100  $\mu\text{L}$  of molecular biology grade water.

**Note:** For example, to purify the entire flowthrough of 480  $\mu\text{L}$ , dilute with 480  $\mu\text{L}$  molecular biology grade water.

- c. For every 100  $\mu\text{L}$  of flowthrough, add 8  $\mu\text{L}$  (or 40  $\mu\text{L}$  for an entire flowthrough of 480  $\mu\text{L}$ ) of **Protein pH Binding Buffer**. Mix contents well.

**Note:** Depends on the type and amount of input, slight precipitation may occur which will not affect the purification procedure

## 8. Protein Binding

- a. Apply up to 600  $\mu\text{L}$  of the pH-adjusted protein sample onto the column, and centrifuge for 2 minutes at  $5,200 \times g$  ( $\sim 8,000$  RPM). Inspect the column to ensure that the entire sample has passed through into the collection tube. If necessary, spin for an additional 3 minutes.
- b. Discard the flowthrough. Reassemble the spin column with its collection tube.

**Note:** You can save the flowthrough in a fresh tube for assessing your protein's binding efficiency.

- c. Depending on your sample volume, repeat steps **8a** and **8b** until the entire protein sample has been loaded onto the column.

## 9. Column Wash

- a. Apply 500  $\mu\text{L}$  of **Protein Wash Buffer** to the column and centrifuge for 2 minutes at  $5,200 \times g$  (8000 RPM).
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Inspect the column to ensure that the liquid has passed through into the collection tube. There should be no liquid in the column. If necessary, spin for an additional minute to dry.

## 10. Protein Elution and pH Adjustment

The supplied **Protein Elution Buffer** consists of 10 mM sodium phosphate pH 12.5.

- a. Add 9.3  $\mu\text{L}$  of **Neutralizer** to a fresh 1.7 mL Elution Tube.
- b. Transfer the spin column from the Column Wash procedure into the Elution Tube.
- c. Apply 100  $\mu\text{L}$  of the **Protein Elution Buffer** to the column and centrifuge for 2 minutes at  $5,200 \times g$  (8000 RPM) to elute bound proteins.

**Note:** Approximately 95% of bound protein is recovered in the first elution. If desired, a second elution using 50  $\mu\text{L}$  of **Protein Elution Buffer** may be carried out. This should be collected into a different tube (to which 4.6  $\mu\text{L}$  of **Neutralizer** is pre-added) to prevent dilution of the first elution.

## Appendix A: Acetone Precipitation Procedure for Proteins

- a. Add 4 volumes of ice-cold acetone to the flowthrough from the RNA Binding Step (Section 3, Step 3d).
- b. Incubate for 15 minutes on ice or at  $-20^{\circ}\text{C}$ .
- c. Centrifuge for 10 minutes at  $14,000 \times g$  ( $\sim 12,000$  RPM). Discard the supernatant and allow the pellet to air-dry.

**Note:** At this point the pellet can be washed with 100  $\mu\text{L}$  of ice cold ethanol and again air-dried.

- d. Resuspend the pellet in the buffer of your choice that is suited to your downstream application.

## Troubleshooting Guide

| Problem           | Possible Cause   | Solution and Explanation   |
|-------------------|--|--|
| Poor RNA Recovery | Incomplete lysis of cells or tissue                                    | Ensure that the appropriate amount of Lysis Solution 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 RNA Elution Buffer 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 RNA Wash Solution                         | Ensure that 20 mL (Cat# 48100) or 50 mL (Cat #48200) of 96 – 100 % ethanol is added to the supplied Wash Solution 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 lysis solution through aspiration.   |
| Clogged Column    | Insufficient solubilization of cells or tissues                        | Ensure that the appropriate amount of lysis buffer was used for the amount of cells or tissue.   |
|                   | Maximum number of cells or amount of tissue exceeds kit specifications | Refer to specifications to determine if amount of starting material falls within kit specifications.   |
|                   | Centrifuge temperature too low   | Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20 °C may cause precipitates to form that can cause the columns to clog.   |

| Problem  | Possible Cause   | Solution and Explanation   |
|--|--|--|
| RNA is Degraded                                      | RNase contamination  | RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to “ <i>Working with RNA</i> ” at the beginning of this user guide.  |
|  | Procedure not performed quickly enough                                     | In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Also, after the DNA binding step, the flowthrough should be kept on ice or –20 °C if the RNA purification step is not carried out immediately. |
|  | Improper storage of the purified RNA                                       | For short term storage RNA samples may be stored at –20 °C for a few days. It is recommended that samples be stored at –70 °C for longer term storage.   |
|  | Frozen tissues or cell pellets were allowed to thaw prior to RNA isolation | Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.  |
|  | Lysozyme or lyticase used may not be RNase-free                            | Ensure that the lysozyme and lyticase being used with this kit are RNase-free, in order to prevent possible problems with RNA degradation.   |
|  | Tissue samples were frozen improperly                                      | Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage.  |
| RNA does not perform well in downstream applications | RNA was not washed twice with the provided RNA Wash Solution               | Traces of salt from the binding step may remain in the sample if the column is not washed twice with RNA Wash Solution. Salt may interfere with downstream applications, and thus must be washed from the column.  |
|  | Ethanol carryover  | Ensure that the dry spin under the RNA Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.  |
| Poor Protein Recovery                                | Incorrect pH adjustment of sample.   | Ensure that the pH of the starting protein sample is adjusted to pH 3.5 or lower after the pH Binding Buffer has been added and prior to binding to the column. If necessary, add additional pH Binding Buffer.  |
|  | Low protein content in the starting materials                              | Run a 20 µL fraction from the flowthrough (after RNA binding) on a SDS-PAGE gel to estimate the amount of protein present in the sample. In addition, use the entire flowthrough in protein purification procedure   |

| Problem               | Possible Cause                                     | Solution and Explanation   |
|-----------------------|--|--|
| Proteins are Degraded | Eluted protein solution was not neutralized.       | Add 9.3 $\mu$ L of Neutralizer to each 100 $\mu$ L of eluted protein in order to adjust the pH to neutral. Some proteins are sensitive to high pH, such as the elution buffer at pH 12.5 |
|                       | Eluted protein was not neutralized quickly enough. | If eluted proteins are not used immediately, degradation will occur. We strongly suggest adding Neutralizer in order to lower the pH.  |

| Related Products                      | Product # |
|---------------------------------------|-----------|
| 1kb RNA Ladder                        | 15003     |
| UltraRanger 1kb DNA Ladder            | 12100     |
| RNA/Protein Purification Kit          | 23000     |
| RNA/DNA/Protein Purification Kit      | 23500     |
| RNA/DNA/Protein Purification PLUS Kit | 47700     |

### Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website ([www.norgenbiotek.com](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

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