

RNA/DNA/Protein Purification 96-Well Plus Kit Product Insert

Product # 51700

Norgen's RNA/DNA/Protein Purification 96-Well Plus Kit provides a rapid method for the high throughput isolation and purification of total RNA, genomic DNA and proteins simultaneously from a single sample of cultured animal cells, small tissue samples, blood, bacteria, yeast, fungi or plants. This 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/DNA/Protein Purification 96-Well 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, DNA 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 and DNA Purification

Purification is based on 96-well column chromatography. The process involves first lysing the cells or tissue of interest with the provided Lysis Buffer Q. The DNA is then captured and purified on a gDNA Purification 96-Well Plate. Ethanol is then added to the flowthrough of the DNA purification step, and the solution is loaded onto a RNA/Protein Purification 96-Well Plate. 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 Wash Solution A to remove impurities, and the purified RNA is eluted with the Elution Solution A. 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. The genomic DNA is of the highest quality, and can be used in PCR reactions, sequencing, Southern blotting and SNP analysis.

Protein Purification

The proteins that are present from the flowthrough during the RNA purification 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 96-Well Plate that was used for purifying the RNA. After the RNA has been eluted from the RNA/Protein 96-Well Plate, the flowthrough is then pH adjusted and loaded back onto the plate 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 either a vacuum manifold or centrifugation
- All 96-Well Plates for RNA, DNA and protein purification provided
- Sequentially isolate nucleic acids and proteins from a single lysate – no need to split the lysate
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions
- Isolate high quality total RNA and Genomic DNA
- High yields of isolated proteins

Specifications

Kit Specifications	
Binding Capacity Per Well	50 µg for RNA 20 µg for DNA 150 µg for protein
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Size of DNA Purified	≥ 30 kb
Maximum Amount of Starting Material:	
Animal Cells	1 x 10 ⁶ cells
Animal Tissues	10 mg
Blood	100 µL
Bacteria	1 x 10 ⁹ cells
Yeast	1 x 10 ⁸ cells
Fungi	40 mg
Plant Tissues	40 mg
Time to Complete 10 Purifications	35 minutes
Average Yields*	
Liver (5 mg)	12.5 µg RNA 2 µg DNA 55 µg protein

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

Kit Components

Component	Used For	Product # 51700 (96 samples)
Lysis Buffer Q	RNA Lysis	40 mL
Wash Solution A	RNA Wash gDNA Wash	2 x 38 mL 1 x 18 mL
Elution Solution A	RNA Elution	20 mL
Elution Buffer F	gDNA Elution	2 x 15 mL
Wash Solution C	Protein Wash	60 mL
Binding Buffer A	Protein Binding	8 mL
Elution Buffer C	Protein Elution	30 mL
Protein Neutralizer	Protein Eluent Neutralization	4 mL
Protein Loading Dye	SDS-PAGE Gel Loading	3 x 2 mL
gDNA Purification 96-Well Plate	gDNA Purification	1
RNA/Protein Purification 96-Well Plate	RNA/Protein Purification	1
Collection Plate		5
Elution Plate		3
Lysate Preparation Plate		2
Adhesive Tape		4
Product Insert		1

Storage Conditions and Product Stability

The **Protein Loading Dye** should be stored at -20°C after the addition of DL-Dithiothreitol (DTT). 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.

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.

Lysis Buffer Q contains guanidine salts, and should be handled with care. Guanidine salt forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the RNA/DNA/Protein Purification Plus 96-Well Kit:

For All Protocols

- For **Vacuum Format**:
 - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -650 mbar or -25 in. Hg (such as Whatman UniVac 3 Vacuum to Collect Manifold)
 - Sealing tape or pads
- For **Centrifuge Format**:
 - Centrifuge with rotor for 96-well plate assembly (such as Thermo Fisher IEC Centra CL3 series or Beckman GS-15R)
- 96 - 100% ethanol
- Isopropanol
- β -mercaptoethanol (optional)
- Molecular Biology Grade Water
- DL-Dithiothreitol (DTT)

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 Sorbital
 - 1 unit/ μ L Lyticase

For Fungi Protocol

- Liquid nitrogen
- Mortar and pestle

For Plant Protocol

- Liquid nitrogen
- Mortar and pestle

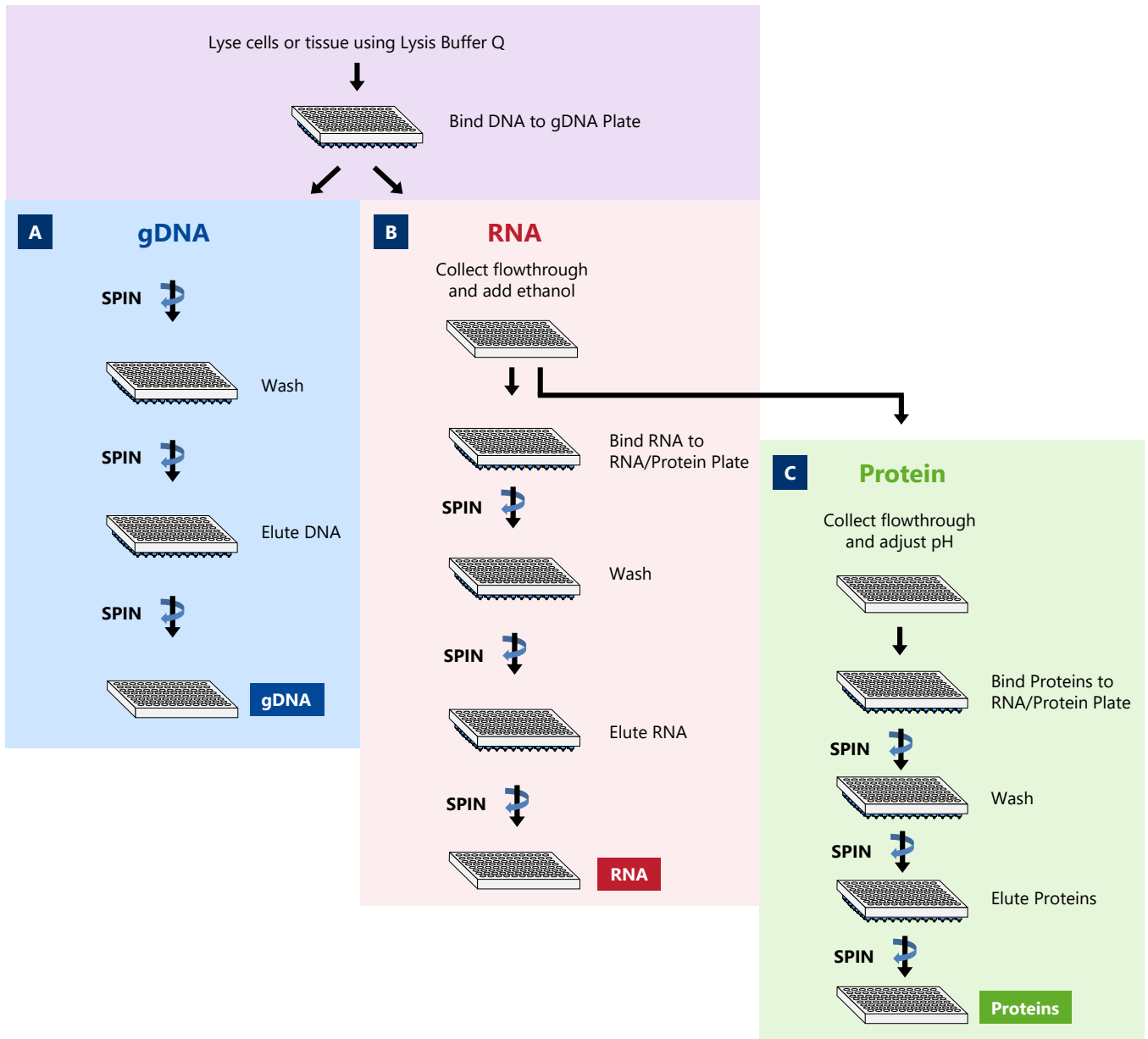
Working with RNA

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

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

Flow Chart

Procedure for Purifying Total RNA, gDNA and Proteins using Norgen's RNA/DNA/Protein Purification Plus 96-Well Kit



Procedures

For Vacuum Manifold: All vacuum steps are performed at room temperature. The correct pressure can be calculated using the conversions:

$$1 \text{ mbar} = 100 \text{ Pa} = 0.0394 \text{ in. Hg} = 0.75 \text{ mm Hg} = 0.0145 \text{ psi}$$

For Centrifugation: All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

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

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.

NOTE: This procedure is written in two steps. Section 1 contains the protocols for preparation of lysate from different types of starting materials. Please ensure that the proper protocol is followed for your sample. The user then carry out total RNA, DNA and total protein purification using either Section 2 with vacuum or Section 3 with centrifugation.

Section 1. Preparation of Lysate From Various Cell Types

Notes Prior to Use for all RNA/DNA/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 – 13**).
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed.
- 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 bottles containing 38 mL of concentrated **Wash Solution A**. This will give a final volume of 128 mL.
 - 42 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing 18 mL concentrated **Wash Solution A**. This will give a final volume of 60 mL.The labels on the bottles have a box that may be checked to indicate that the ethanol has been added. The **Wash Solution A** is used for both RNA and DNA Purification
- The volumes stated in each procedure for lysate preparation are the volumes required to prepare samples for each well of the 96-well plate.
- **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. It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 μ L of β -mercaptoethanol (provided by the user) to each 1 mL of **Lysis Buffer Q** required. β -mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the **Lysis Buffer Q** can be used as provided.
- It is important to work quickly during this procedure.

Section 1A. Preparation of Lysate From Various Cell Types

1A. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use

- The recommended input is 5×10^5 cells per well. However, up to 1×10^6 cells may be processed for most cell lines.
- 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 **Lysis Buffer Q** directly to the frozen cell pellet (**Step 1A(ii) d**).

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

- a. Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
- b. Add 300 μL of **Lysis Buffer Q** 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. **Proceed to Step 2.**

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

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 in a microcentrifuge at no more than $200 \times g$ ($\sim 2,000$ RPM) for 10 minutes to pellet cells.
- b. Carefully decant the supernatant to ensure that the pellet is not dislodged. Wash the cell pellet with an appropriate amount of PBS. Centrifuge at $200 \times g$ ($\sim 2,000$ RPM) for another 5 minutes.
- c. Carefully decant the supernatant. A few μL of PBS may be left behind with the pellet in order to ensure that the pellet is not dislodged.
- d. Add 300 μL of **Lysis Buffer Q** 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: At this stage the lysate may be stored at -70°C , such that the RNA purification can be performed at a later time.

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 homogenization.

- The optimal amount of non-fibrous tissue be used per well is up to 8 mg. However, for most tissues (except tissues with high cell number such as liver and spleen), up to 10 mg could be processed. For fibrous tissue such as heart, a maximum of 2 mg is recommended

1B. Cell Lysate Preparation from Animal Tissues

- Excise the tissue sample from the animal.
- Determine the amount of tissue by weighing. It is recommended that no more than 10 mg of tissue be used for each well.
- Transfer the tissue samples to appropriate vessels for the desired disruption method.
- Add 300 μ L of **Lysis Buffer Q** to each tissue sample.

Note: Ensure that frozen tissues do not thaw during weighing or prior to the addition of **Lysis Buffer Q**. For maximum RNA recovery, homogenize frozen tissues to fine powder in liquid nitrogen prior to the addition of **Lysis Buffer Q**.

- Homogenize the tissues using the appropriate cell disruption tool.

Note: Thorough homogenization is required for optimal performance. For tissue inputs of ≤ 8 mg, it is not required to perform centrifugation to remove cell debris if the homogenization is complete. For tissue inputs larger than 8 mg, or if incomplete cell disruption is suspected, centrifuge the lysate at maximum speed for 2 minutes in an appropriate centrifuge. Transfer the supernatant to a new 96-well microplate.

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

- 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 100 μ 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

- Transfer up to 100 μ L of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).
- Add 300 μ L of **Lysis Buffer Q** to the blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step. **Proceed to Step 2.**

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

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 1×10^9 cells/mL has an OD₆₀₀ 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 1Dc**).

1D. Cell Lysate Preparation from Bacteria

- a. Pellet bacteria by centrifugation of a 96-well block at $3,000 \times g$ (3,000 RPM) for 5 minutes or centrifugation of microcentrifuge tubes at $14,000 \times g$ (~14,000 RPM) for 1 minute.
- b. Decant supernatant, and carefully remove any remaining media by aspiration.
- c. Resuspend the bacteria thoroughly in 100 μ L 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 μ L of **Lysis Buffer Q** and vortex vigorously for at least 10 seconds. **Proceed to Step 2.**

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

Table 1: 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 μ 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 Sorbitol, 0.1% β -mercaptoethanol and 1 unit/ μ 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°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 centrifugation of a 96-well block at 3,000 $\times g$ (3,000 RPM) for 5 minutes or centrifugation of microcentrifuge tubes at 14,000 $\times g$ (~14,000 RPM) for 1 minute.
- b. Decant supernatant, and carefully remove any remaining media by aspiration.
- c. Resuspend the yeast thoroughly in 500 μL of Lyticase-containing Resuspension Buffer by vortexing. Incubate at 37°C for 10 minutes.
- d. Pellet the spheroplasts. Centrifuge a 96-well block at 650 $\times g$ (~800 - 1,000 RPM) for 3 minutes or microcentrifuge tubes at 200 $\times g$ (~2,000 RPM) for 3 minutes and remove the supernatant by pipetting.
- e. Add 300 μL of **Lysis Buffer Q** and vortex vigorously for at least 10 seconds. **Proceed to Step 2.**

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

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°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 40 mg of fungi be used for this procedure in order to prevent clogging of the column.

1F. Cell Lysate Preparation from Fungi

- a. Determine the amount of fungi by weighing. It is recommended that no more than 40 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.
- c. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
- d. Add 300 μL of **Lysis Buffer Q** 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. **Proceed to Step 2.**

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

1G. Lysate Preparation from Plant

Notes Prior to Use

- The maximum recommended input of plant tissue is 40 mg or 4×10^6 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.

1G. Cell Lysate Preparation from Plant

- a. Transfer ≤ 40 mg of plant tissue or 4×10^6 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 300 μ L of **Lysis Buffer Q** to the tissue sample and continue to grind until the sample has been homogenized.
- d. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
- e. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. **Proceed to Step 2.**

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

Note: The purification of total RNA from the lysate prepared in Section 1 could be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section 2A. For purification using centrifugation, please follow the procedure outlined in Section 2B

Section 2: RNA/DNA/Protein Purification from All Types of Lysate using Vacuum

Note: The remaining steps of the procedure for the purification of total RNA/DNA/Proteins using a vacuum manifold are the same from this point forward for all the different types of lysate.

A. Genomic DNA Purification Using Vacuum Manifold

2. Binding DNA to gDNA Purification 96-Well Plate

- a. Assemble the **gDNA Purification 96-Well Plate** and the vacuum manifold according to manufacturer's recommendations.

Note: The provided **Lysate Preparation Plate** can be used to collect the lysate/flowthrough and subsequent mixing with ethanol.

- b. Apply the lysate (from **Step 1**) into each well of the **gDNA Purification 96-Well Plate**. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.
- c. Turn off vacuum and ventilate the manifold. **Retain the Lysate Preparation Plate containing the flowthrough for RNA Purification (Section 2B). The flowthrough contains the RNA and proteins and should be stored on ice or at -20°C until the RNA and Protein Purification protocol is carried out.** Reassemble the **gDNA Purification 96-Well Plate** and the vacuum manifold. Use one of the provided **Collection Plates** or any 96-well collection plate (not provided) for waste collection.

Note: Ensure that all of the lysate from each well has passed through into the **Lysate Preparation Plate**. If the entire lysate volume has not passed, apply vacuum for an additional 2 minutes.

3. gDNA Wash

- a. Apply 400 μL of **Wash Solution A** to each well of the **gDNA Purification 96-Well Plate**. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire wash solution has passed through into the collection/waste tray by inspecting the **gDNA Purification 96-Well Plate**. If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

- b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
- c. Reassemble the **gDNA Purification 96-Well** and the vacuum manifold.
- d. Apply 400 μL of **Wash Solution A** to each well of the **gDNA Purification 96-Well Plate**. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.
- e. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
- f. Pat the bottom of the **gDNA Purification 96-Well Plate** dry. Reassemble the **gDNA Purification 96-Well Plate** and the vacuum manifold. Apply vacuum for an additional 5 minutes in order to completely dry the plate.
- g. Turn off vacuum and ventilate the manifold.

4. gDNA Elution

- a. Replace the collection/waste tray in the vacuum manifold with the provided 96-Well **Elution Plate**. Complete the vacuum manifold assembly with the **gDNA Purification 96-Well Plate**.
- b. Add 100 μL of **Elution Buffer F** to each well of the plate and let stand at room temperature for 2 minutes.
- c. Apply vacuum for 2 minutes.

Note: Ensure the entire **Elution Buffer F** has passed through into the elution plate by inspecting the 96-Well Plate. If the entire elution volume has not passed, apply vacuum for an additional 2 minutes.

5. Storage of DNA

Use the provided adhesive tape to seal the 96-Well **Elution Plate**. The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at $\leq -20^\circ\text{C}$ for long term storage.

B. Total RNA Purification Using Vacuum Manifold

6. Binding RNA to RNA/Protein Purification 96-Well Plate

- a. To every 100 μL of flowthrough from Step 2c collected in the **Lysate Preparation Plate**, add 60 μL of 96 – 100 % Ethanol. Mix by pipetting up and down.

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

- b. Assemble the **RNA/Protein Purification 96-Well Plate** and the vacuum manifold according to manufacturer's recommendations.

Note: Use the second provided **Lysate Preparation Plate** to collect the lysate/flowthrough

and subsequent adjustment for protein binding.

- c. Apply the lysate with the ethanol (from **Step 6a**) into each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application of the mixture to the wells.

- d. Turn off vacuum and ventilate the manifold. **Retain the Lysate Preparation Plate containing the flowthrough for Protein Purification (Section 2C). The flowthrough contains the proteins and should be stored on ice or at -20°C until the Protein Purification protocol is carried out.** Reassemble the **RNA/Protein Purification 96-Well Plate** and the vacuum manifold. Use one of the provided **Collection Plates** or any 96-well collection plate (not provided) for waste collection..

Note: Ensure that all of the lysate from each well has passed through into the collection/waste tray. If the entire lysate volume has not passed, apply vacuum for an additional 2 minutes.

7. RNA Wash

- a. Apply 400 μ L of **Wash Solution A** to each well of the **RNA/Protein Purification 96-Well Plate**. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire wash solution has passed through into the collection/waste tray by inspecting the **RNA/Protein Purification 96-Well Plate**. If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

- b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
- c. Reassemble the **RNA/Protein Purification 96-Well Plate** and the vacuum manifold. Repeat steps **7a** and **7b** to wash column for a second time.
- d. Reassemble the **RNA/Protein Purification 96-Well Plate** and the vacuum manifold. Repeat steps **7a** and **7b** to wash column for a third time.
- e. Pat the bottom of the **RNA/Protein Purification 96-Well Plate** dry. Reassemble the **RNA/Protein Purification 96-Well Plate** and the vacuum manifold. Apply vacuum for an additional 5 minutes in order to completely dry the plate.
- f. Turn off vacuum and ventilate the manifold.

8. RNA Elution

- a. Replace the collection/waste tray in the vacuum manifold with the provided 96-Well **Elution Plate**. Complete the vacuum manifold assembly with the **RNA/Protein Purification 96-Well Plate**.
- b. Add 75 μ L of **Elution Solution A** to each well of the plate and let stand at room temperature for 2 minutes
- c. Apply vacuum for 2 minutes.

Note: Ensure the entire **Elution Solution A** has passed through into the elution plate by inspecting the **RNA/Protein Purification 96-Well Plate**. If the entire elution volume has not passed, apply vacuum for an additional 2 minutes.

- d. **Retain the RNA/Protein Purification 96-Well Plate for Protein Purification.**

9. Storage of RNA

Use the provided adhesive tape to seal the 96-Well **Elution Plate**. The purified RNA samples may be stored at -20°C for a few days. It is recommended that samples be placed at -70°C for long term storage.

C. Total Protein Purification from All Cell Types Using Vacuum

Notes Prior to Use

- At this point, the proteins that are present in the flowthrough from the RNA Binding Step (**Step 6d above**) can be loaded directly onto an SDS-PAGE gel for visual analysis, or the proteins can be further purified using the protocol below.
- Add 93 mg of DL-Dithiothreitol (DTT, not provided) to each bottle of **Protein Loading Dye**. The **Protein Loading Dye** should be stored at -20°C after the addition of DTT. The label on the bottle has a box that may be checked to indicate that DTT has been added
- 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.

10. pH Adjustment of Lysate

- a. Measure or estimate the volume of flowthrough collected in the **Lysate Preparation Plate** from the RNA Binding Step (**Step 6d above**).
- b. For every 100 μL of flowthrough, dilute with 200 μL of molecular biology grade water.

Note: For example, to purify the entire flowthrough of 480 μL , dilute with 960 μL molecular biology grade water.

- c. For every 100 μL of flowthrough, add 14.5 μL (or 70 μL for an entire flowthrough of 480 μL) of **Binding Buffer A**. Mix contents well.

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

11. Protein Binding

- a. Apply the pH-adjusted protein samples into each well of the **RNA/Protein Purification 96-Well Plate**. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.
- b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
- c. Depending on your sample volume, repeat steps **11a** and **11b** until the entire protein sample has been loaded onto the column.

12. Column Wash

- a. Apply 400 μL of **Wash Solution C** to each well of the **RNA/Protein Purification 96-Well Plate**. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.
- b. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the **RNA/Protein Purification 96-Well Plate** and the vacuum manifold.
- c. Turn off vacuum and ventilate the manifold. Pat the bottom of the **RNA/Protein Purification 96-Well Plate** dry. Reassemble the **RNA/Protein Purification 96-Well Plate** and the vacuum manifold. Apply vacuum for an additional 5 minutes in order to completely dry the plate.
- d. Turn off vacuum and ventilate the manifold.

13. Protein Elution and pH Adjustment

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

- a. Add 9.3 μL of **Protein Neutralizer** to the appropriate wells of a new 96-Well **Elution Plate**.
- b. Stack the **RNA/Protein Purification 96-Well Plate** on top of the 96-Well **Elution Plate** containing the **Protein Neutralizer** to complete the vacuum manifold set up.
- c. Apply 100 μL of the **Elution Buffer C** to the wells of the **RNA/Protein Purification 96-Well Plate**.
- d. Apply vacuum for 2 minutes to elute the proteins.

Note: Ensure the entire **Elution Buffer C** has passed through into the elution plate by inspecting the **RNA/Protein Purification 96-Well Plate**. If the entire elution volume has not passed, apply vacuum for an additional 2 minutes

- e. Gently agitate the elution plate to mix the eluent with the **Protein Neutralizer**.

Section 3: RNA/DNA/Protein Purification from All Types of Lysate using Centrifugation

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

A. Genomic DNA Purification Using Centrifugation

2. Binding DNA to gDNA Purification 96-Well Plate

- a. Place the **gDNA Purification 96-Well Plate** on top of a provided 96-Well **Collection Plate**.
- b. Apply up to 150 μL of the lysate (from **Step 1**) into each well of the **gDNA Purification 96-Well Plate**. Centrifuge the assembly at maximum speed or 3,000 $\times g$ (~3,000 RPM) for 2 minutes.
- c. **Retain the flowthrough for RNA and Protein Purification (Section 3B) by transferring to the provided Lysate Preparation Plate. The flowthrough contains the RNA and proteins and should be stored on ice or at -20°C until the RNA and Protein Purification protocol is carried out. Reassemble the gDNA Purification 96-Well Plate and the Collection Plate.**

Note: Ensure that all of the lysate from each well has passed through into the bottom plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

- d. Repeat Steps **2b** and **2c** to bind all lysate to the **gDNA Purification 96-Well Plate**.

3. gDNA Wash

- a. Apply 400 μL of **Wash Solution A** to each well of the **gDNA Purification 96-Well Plate**. Centrifuge the assembly at maximum speed or 3,000 $\times g$ (~3,000 RPM) for 2 minutes.

Note: Ensure the entire wash solution has passed through into the **Collection Plate** by inspecting the **gDNA Purification 96-Well Plate**. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

- b. Discard the flowthrough. Reassemble the **gDNA Purification 96-Well Plate** and the **Collection Plate**.

- c. Apply 400 μL of **Wash Solution A** to each well of the **gDNA Purification 96-Well Plate**. Centrifuge the assembly at maximum speed or $3,000 \times g$ (~3,000 RPM) for 2 minutes.

Note: Ensure the entire wash solution has passed through into the **Collection Plate** by inspecting the **gDNA Purification 96-Well Plate**. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

- d. Discard the flowthrough.
- e. Pat the bottom of the **gDNA Purification 96-Well Plate** dry. Reassemble the **gDNA Purification 96-Well Plate** and the **Collection Plate**. Centrifuge the assembly at maximum speed or $3,000 \times g$ (~3,000 RPM) for 5 minutes in order to completely dry the plate. **Retain the 96-Well Collection Plate for RNA/Protein Purification.**

4. gDNA Elution

- a. Stack the **gDNA Purification 96-Well Plate** on top of the **96-Well Elution Plate**.
- b. Add 100 μL of **Elution Buffer F** to each well of the **gDNA Purification 96-Well Plate** and let stand at room temperature for 2 minutes.
- c. Centrifuge the assembly at maximum speed or $3,000 \times g$ (~3,000 RPM) for 2 minutes.

Note: Ensure the entire **Elution Buffer F** has passed through into the elution plate by inspecting the **gDNA Purification 96-Well Plate**. If the entire elution volume has not passed, apply centrifugation for an additional 2 minutes

5. Storage of DNA

Use the provided adhesive tape to seal the 96-Well **Elution Plate**. The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at $\leq -20^{\circ}\text{C}$ for long term storage.

B. RNA Purification Using Centrifugation

6. Binding RNA to RNA/Protein Purification 96-Well Plate

- a. To every 100 μL of flowthrough from Step **2c** collected in the **Lysate Preparation Plate**, add 60 μL of 96 – 100 % Ethanol. Mix by pipetting up and down.

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

- b. Place the **RNA/Protein Purification 96-Well Plate** on top of a provided 96-Well **Collection Plate**.
- c. Apply the lysate with ethanol (from **Step 6a**) into each well of the **RNA/Protein Purification 96-Well Plate**. Centrifuge the assembly at maximum speed or $3,000 \times g$ (~3,000 RPM) for 2 minutes.

Note: Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application to the wells

- c. **Retain the flowthrough for Protein Purification (Section 3C) by transferring to the provided Lysate Preparation Plate. The flowthrough contains the proteins and should be stored on ice or at -20°C until the Protein Purification protocol is carried out.** Reassemble the the **RNA/Protein Purification 96-Well Plate** and the **Collection Plate**.

Note: Ensure that all of the lysate from each well has passed through into the bottom plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

7. RNA Wash

- a. Apply 400 μL of **Wash Solution A** to each well of the **RNA/Protein Purification 96-Well Plate**. Centrifuge the assembly at maximum speed or $3,000 \times g$ ($\sim 3,000$ RPM) for 2 minutes.

Note: Ensure the entire wash solution has passed through into the bottom plate by inspecting the **RNA/Protein Purification 96-Well Plate**. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

- b. Discard the flowthrough. Reassemble the **RNA/Protein Purification 96-Well Plate** and the **Collection Plate**.
- c. Repeat steps **7a** and **7b** to wash column for a second time.
- d. Repeat steps **7a** and **7b** to wash column for a third time.
- e. Pat the bottom of the **RNA/Protein Purification 96-Well Plate** dry. Reassemble **RNA/Protein Purification 96-Well Plate** and the **Collection Plate**. Centrifuge the assembly at maximum speed or $3,000 \times g$ ($\sim 3,000$ RPM) for 5 minutes in order to completely dry the plate.

8. RNA Elution

- a. Stack the **RNA/Protein Purification 96-Well Plate** on top of the 96-Well **Elution Plate**.
- b. Add 75 μL of **Elution Solution A** to each well of the **RNA/Protein Purification 96-Well Plate** and let stand at room temperature for 2 minutes.
- c. Centrifuge the assembly at maximum speed or $3,000 \times g$ ($\sim 3,000$ RPM) for 2 minutes.

Note: Ensure the entire **Elution Solution A** has passed through into the elution plate by inspecting the **RNA/Protein Purification 96-Well Plate**. If the entire elution volume has not passed, apply centrifugation for an additional 2 minutes

- d. **Retain the RNA/Protein Purification 96-Well Plate for Protein Purification.**

9. Storage of RNA

Use the provided adhesive tape to seal the 96-Well **Elution Plate**. 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.

C. Total Protein Purification from All Cell Types Using Centrifugation

Notes Prior to Use

- At this point, the proteins that are present in the flowthrough from the RNA Binding Step (**Step 2 above**) can be loaded directly onto an SDS-PAGE gel for visual analysis, or the proteins can be further purified using the protocol below.
- Add 93 mg of DL-Dithiothreitol (DTT, not provided) to each bottle of **Protein Loading Dye**. The **Protein Loading Dye** should be stored at -20°C after the addition of DTT. The label on the bottle has a box that may be checked to indicate that DTT has been added
- 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.

10. pH Adjustment of Lysate

- a. Measure or estimate the volume of flowthrough collected in the **Lysate Preparation Plate** from the RNA Binding Step (**Step 6d above**).
- b. For every 100 μL of flowthrough, dilute with 200 μL of molecular biology grade water.

Note: For example, to purify the entire flowthrough of 480 μL , dilute with 960 μL molecular biology grade water.

- c. For every 100 μL of flowthrough, add 14.5 μL (or 70 μL for an entire flowthrough of 480 μL) of **Binding Buffer A**. Mix contents well.

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

11. Protein Binding

- a. Apply the pH-adjusted protein samples into each well of the **RNA/Protein Purification 96-Well Plate** assembled with a **Collection Plate**. Centrifuge the assembly at maximum speed or 3,000 $\times g$ (~3,000 RPM) for 2 minutes.
- b. Discard the flowthrough. Reassemble the **RNA/Protein Purification 96-Well Plate** with the **Collection Plate**.
- c. Depending on your sample volume, repeat steps **11a** and **11b** until the entire protein sample has been loaded onto the column.

12. Column Wash

- a. Apply 400 μL of **Wash Solution C** to each well of the **RNA/Protein Purification 96-Well Plate**. Centrifuge the assembly at maximum speed or 3,000 $\times g$ (~3,000 RPM) for 2 minutes.
- b. Discard the flowthrough and reassemble the **RNA/Protein Purification 96-Well Plate** with the **Collection Plate**.
- c. Inspect each well to ensure that the liquid has passed through into the **Collection Plate**. There should be no liquid in the well. If necessary, spin for an additional minute to dry.

13. Protein Elution and pH Adjustment

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

- a. Add 9.3 μL of **Protein Neutralizer** to the appropriate wells of a new 96-Well **Elution Plate**.
- b. Stack the **RNA/Protein Purification 96-Well Plate** on top of the 96-Well **Elution Plate** containing the **Protein Neutralizer**.
- c. Apply 100 μL of the **Elution Buffer C** to the wells of the **RNA/Protein Purification 96-Well Plate**.
- d. Centrifuge the assembly at maximum speed or 3,000 $\times g$ (~3,000 RPM) for 2 minutes to elute bound proteins.

Note: Ensure the entire **Elution Buffer C** has passed through into the elution plate by inspecting the **RNA/Protein Purification 96-Well Plate**. If the entire elution volume has not passed, apply centrifugation for an additional 2 minutes

- e. Gently agitate the elution plate to mix the eluent with the **Protein Neutralizer**.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Lysis Buffer Q was used for the amount of cells or tissue.
	Well 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 Well” 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.
	Alcohol was not added to the lysate	Ensure that the appropriate amount of isopropanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that the proper amount 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 Lysis Buffer Q through aspiration.
Clogged Well	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of lysis buffer was used for the amount of cells or tissue.
	Insufficient Vacuum	Ensure that a vacuum pressure of at least -650 mbar or -25 in. Hg is developed
	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

Problem	Possible Cause	Solution and Explanation
Clogged Well	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 20°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 “ <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.
	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.
	DNase I used may not be RNase-free	Ensure that the optional DNase I being used with this kit is RNase-free in order to prevent possible problems with RNA degradation. Norgen’s RNase-Free DNase I Kit (Cat# 25710) is recommended for this step.
	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 is Degraded	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 Lysis Buffer Q .
	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.
RNA does not perform well in downstream applications	RNA was not washed three times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the well is not washed three times with Wash Solution. Salt may interfere with downstream applications, and thus must be washed from the well.
	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.

Problem	Possible Cause	Solution and Explanation
Yield of Genomic DNA is Low	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Lysis Buffer Q was used for the amount of cells or tissue. Incubate the Lysis Buffer Q for an extra 5 minutes to assist in lysis.
Genomic DNA is Sheared	Sample is old	Ensure that the sample is not too old, as old samples often yield only degraded DNA
	Sample repeatedly frozen and thawed	Samples should not be repeatedly frozen and thawed, as this tends to increase the likelihood of isolating degraded DNA.
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 Binding Buffer A has been added and prior to binding to the column. If necessary, add additional Binding Buffer A .
	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
Proteins are degraded	Eluted protein solution was not neutralized.	Add 9.3 µL of Protein Neutralizer to each 100 µL 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 Protein Neutralizer in order to lower the pH.

Related Products	Product #
Total RNA Purification Kit	17200
Total RNA Purification 96-Well Kit	24300
RNase-Free DNase I Kit	25710
1kb RNA Ladder	15003
UltraRanger 1kb DNA Ladder	12100
RNA/DNA/Protein Purification Plus Kit	47700

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

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