

All-in-One Purification Kit **Product # 24200**

Product Insert

Norgen's All-in-One Purification Kit provides a rapid method for the isolation and purification of total RNA including microRNA, genomic DNA and proteins sequentially from a single sample of cultured animal cells, small tissue samples, blood, bacteria, yeast, fungi or plants. In addition, a microRNA Enrichment Column is provided for the optional separate purification of small RNA (<200 nt), allowing for 4-in-1 purification (large RNA, genomic DNA, microRNA and proteins). This kit is an ideal all-in-one solution for researchers studying systems biology, including those who are interested in the interactions of multiple disciplines including RNA interference, genomics, epigenomics, transcriptomics and proteomics. Norgen's All-in-One Purification Kit is especially useful for researchers who are isolating macromolecules from precious, difficult to obtain or small samples such as needle biopsies and single foci from cell cultures, as well as for mutant analysis, RNA interference studies and pathogen detection. Furthermore, analysis will be more reliable since the RNA, DNA and proteins are derived from the same sample without any fractionation, thereby eliminating inconsistent results. The purification procedure is very rapid, allowing for the isolation of large RNA, genomic DNA, microRNA and proteins from a single sample in less than 40 minutes. The isolated 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 spin column chromatography using Norgen's proprietary resin as the separation matrix. The process involves first lysing the cells or tissue of interest with the provided Lysis Solution (please see the flow charts on pages 5 and 6). The Lysis Solution contains detergents, as well as large amounts of a chaotropic denaturant that will rapidly inactivate RNases and proteases that are present. At this point, the user can decide whether they wish to isolate total RNA or separately isolate the large and small RNA. For the isolation of total RNA, a volume of ethanol is added to the lysate and the solution is loaded onto a spin-column. Norgen's resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the genomic DNA and RNA will bind to the column while the proteins are removed in the flowthrough. Next, the bound total RNA is washed with the provided RNA Wash Solution to remove impurities, and the purified total RNA is eluted with the RNA Elution Solution. The remaining bound genomic DNA is then washed with the provided gDNA Wash Buffer to remove any remaining trace levels of RNA, and the gDNA is then eluted with the gDNA Elution Buffer.

If the user wishes to separately isolate the large (>200 nt) and the small/microRNA (<200 nt), an alternative amount of ethanol is added to the lysate and again the solution is loaded onto a spin-column. In this case, the larger RNA and the genomic DNA will bind to the column while the small/microRNA and the proteins are removed in the flowthrough. Next, the bound large RNA is washed with the provided RNA Wash Solution to remove impurities, and the purified large RNA is eluted with the RNA Elution Solution. The remaining bound genomic DNA is then washed with the provided gDNA Wash Buffer to remove any remaining trace levels of RNA, and the gDNA is then eluted with the gDNA Elution Buffer. Next, the flowthrough from the binding step is then loaded onto a provided microRNA Enrichment Column, and the small RNA present will bind to the resin and again the proteins will be removed in the flowthrough. The microRNA is then washed with the provided RNA Wash Solution to remove any impurities, and the purified small RNA is eluted with the provided RNA Elution Solution.

The purified total RNA includes all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). Alternatively, the large RNA species can be isolated separately from the small RNA species (<200 nt). In all cases 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 in the flowthrough (either the initial flowthrough if total RNA was isolated, or the flowthrough from the microRNA Enrichment Column if large and small RNA were separately isolated) can now be loaded directly onto an SDS-PAGE gel for visual analysis. Alternatively, the protein samples can be further purified using the spin columns provided with the kit. After the RNA and gDNA have 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 or Western blots.

Specifications

Kit Specifications	
Maximum Column Binding Capacity	50 µg for RNA 20 µg for DNA 200 µg for protein
Maximum Column Loading Volume	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Size of DNA Purified	≥ 30 kb
Maximum Amount of Starting Material:	
Animal Cells	2 x 10 ⁶ cells
Animal Tissues	10 mg (for most tissues)
Blood	100 µL
Bacteria	1 x 10 ⁹ cells
Yeast	1 x 10 ⁸ cells
Fungi	50 mg
Plant Tissues	50 mg
Time to Complete 10 Purifications	40 minutes for all 4 molecules
Average Yields*	
HeLa Cells (1 x 10 ⁶ cells)	15 µg RNA
HeLa Cells (1 x 10 ⁶ cells)	8 µg DNA
HeLa Cells (1 x 10 ⁶ cells)	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 # 24200 (20 samples)
Lysis Solution	15 mL
RNA Wash Solution	20 mL
RNA Elution Solution	6 mL
gDNA Wash Solution	15 mL
gDNA Elution Buffer	6 mL
Protein Wash Buffer	15 mL
Protein pH Binding Buffer	4 mL
Protein Elution Buffer	6 mL
Protein Neutralizer	2 mL
Protein Loading Dye	2 mL
All-in-One Spin Columns	20
microRNA Enrichment Column	20
Collection Tubes	80
Elution tubes (1.7 mL)	80
Product Insert	1

Advantages

- Fast and easy processing using rapid spin-column format
- All columns for RNA, DNA and protein purification provided
- Sequentially isolate nucleic acids and proteins from a single lysate – no need to split the lysate
- Isolate either total RNA, from large rRNA down to microRNA (miRNA) OR separately isolate large RNA species and small RNA species including microRNA
- No phenol or chloroform extractions
- Isolate high quality RNA
- Isolate high quality genomic DNA with a molecular weight ≥ 30 kb
- High yields of isolated proteins

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.

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 All-in-One Purification Kit:

For All Protocols

- Benchtop microcentrifuge
- β -mercaptoethanol (Optional)
- 95% ethanol
- Isopropanol
- Molecular biology grade water (Milli-Q[®] water)
- Acetone (optional)

For Animal Cell Protocol

- PBS (RNase-free)

For Animal Tissue Protocol

- Liquid nitrogen
- Cell Disruption Tools such as mortar and pestle, rotor-stator homogenizer or bead mills
- 70% ethanol

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/ μ L Lyticase

For Fungi Protocol

- Liquid nitrogen
- Cell Disruption Tools such as mortar and pestle, rotor-stator homogenizer or bead mills
- 70% ethanol

For Plant Protocol

- Liquid nitrogen
- Cell Disruption Tools such as mortar and pestle, rotor-stator homogenizer or bead mills
- 70% ethanol

Working with RNA

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

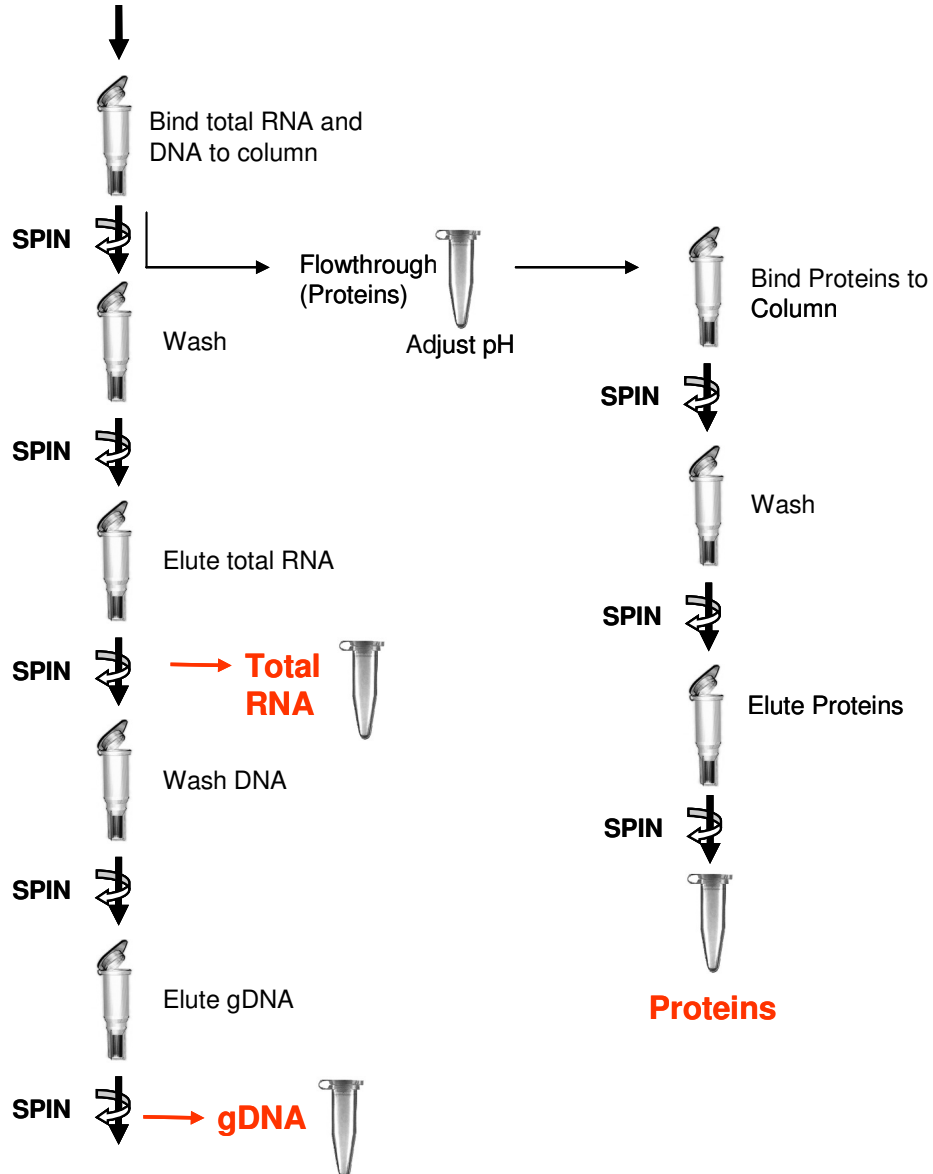
- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions,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

Flow Charts

Procedure for Purifying RNA, gDNA and Proteins using Norgen's All-in-One Purification Kit

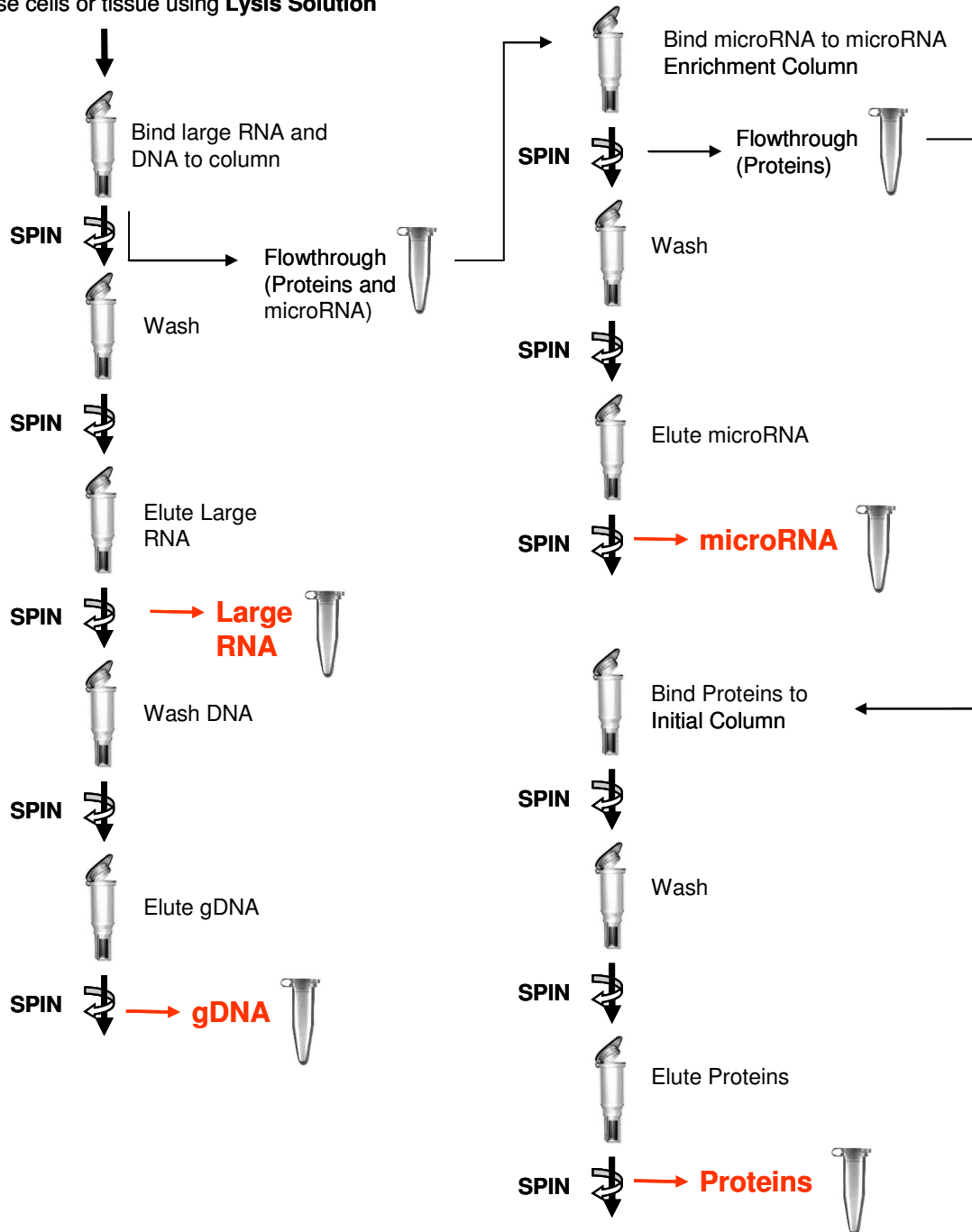
A. Purification of Total RNA, gDNA and Proteins

Lyse cells or tissue using **Lysis Solution**



B. Purification of Large RNA, gDNA, microRNA and Proteins

Lyse cells or tissue using **Lysis Solution**



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:

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

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.

IMPORTANT NOTE:

This procedure is written in three sections. Section 1 contains the protocols to lyse various types of starting materials. Section 2 contains the protocols to isolate total RNA, genomic DNA and proteins, while Section 3 contains the protocols to separately isolate large RNA, genomic DNA, microRNA (<200 nt) and proteins. Please ensure that the proper protocol is followed for your needs.

Section 1. Preparation of Lysate from Various Cell Types

Notes Prior to Use for all Purification Procedures

- 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 40 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **RNA Wash Solution**. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a working concentration of the **gDNA Wash Solution** by adding 15 mL of isopropanol (provided by the user) to the supplied bottle containing the concentrated **gDNA Wash Solution**. This will give a final volume of 30 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- **Optional:** The use of β-mercaptoethanol in lysis is highly recommended for most 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 μL of β-mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required. β-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.

1A. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use

- For optimal results, it is recommended that 1×10^6 cells be used for the input. Inputs of up to 2×10^6 cells may be used, however slight cross-contamination of RNA in the genomic DNA 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°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

- a. Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
- b. Add 350 μL of **Lysis Solution** directly to culture plate.
- c. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes.
- d. Transfer lysate to a microcentrifuge tube.
- e. Proceed to **Section 2** to isolate total RNA, genomic DNA and proteins or **Section 3** to isolate large RNA, microRNA, genomic DNA and proteins.

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

- a. Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than $200 \times g$ ($\sim 2,000$ 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 350 μL 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.
- e. Proceed to **Section 2** to isolate total RNA, genomic DNA and proteins or **Section 3** to isolate large RNA, microRNA, genomic DNA and proteins.

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. Do not allow frozen tissues to thaw

prior to homogenization 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	10 mg
Liver	10 mg
Lung	10 mg
Spleen	10 mg

1B. Cell Lysate Preparation from Animal Tissues

- a. Excise the tissue sample from the animal.
- b. Determine the amount of tissue by weighing. Please refer to Table 1 for the recommended maximum input amounts of different tissues. For tissues not included in the table, we recommend starting with an input of no more than 10 mg.
- c. Add 400 μ L of **Lysis Solution** to the tissue sample. Ensure that frozen tissue does not thaw during weighing or prior to the addition of **Lysis Solution**.
- d. Homogenize the tissues using the appropriate cell disruption tool. If needed, continue to homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.
- 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 (not provided). Note the volume of the supernatant/lysate.
- g. Proceed to **Section 2** to isolate total RNA, genomic DNA and proteins or **Section 3** to isolate large RNA, microRNA, genomic DNA and proteins.

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 350 μ L of **Lysis Solution** to the blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.
- Proceed to **Section 2** to isolate total RNA, genomic DNA and proteins or **Section 3** to isolate large RNA, microRNA, genomic DNA and proteins.

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

- Pellet bacteria by centrifuging at 14,000 $\times g$ (~14,000 RPM) for 1 minute.
- Decant supernatant, and carefully remove any remaining media by aspiration.
- 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.
- Add 300 μ L of Lysis Solution and vortex vigorously for at least 10 seconds.
- Proceed to **Section 2** to isolate total RNA, genomic DNA and proteins or **Section 3** to isolate large RNA, microRNA, genomic DNA and proteins.

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 μL of buffer is required for each preparation. The Resuspension Buffer should have the following composition: 50 mM Tris, pH 7.5, 10 mM EDTA, 1M Sorbital, 0.1% β -mercaptoethanol and 1 unit/ μL Lyticase. This solution should be prepared with sterile, RNase-free reagents, and kept on ice until needed. These reagents are to be provided by the user.
- It is recommended that no more than 10^7 yeast cells or 1 mL of culture be used for this procedure.
- For RNA isolation, yeast should be harvested in log-phase growth.
- Yeast can be stored at -70°C for later use, or used directly in this procedure.
- Frozen yeast pellets should not be thawed prior to beginning the protocol. Add the Lyticase-containing Resuspension Buffer directly to the frozen yeast pellet (**Step 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 μL of Lyticase-containing Resuspension Buffer by vortexing. Incubate at 37°C for 10 minutes.
- d. Pellet the spheroplasts at $200 \times g$ ($\sim 2,000$ RPM) for 3 minutes. Decant supernatant.
- e. Add 350 μL of Lysis Solution and vortex vigorously for at least 10 seconds.
- f. Proceed to **Section 2** to isolate total RNA, genomic DNA and proteins or **Section 3** to isolate large RNA, microRNA, genomic DNA and proteins.

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 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. Excise the tissue sample from the fungus.
- b. Determine the amount of tissue by weighing. It is recommended that no more than 50 mg of tissue be used for the protocol.
- c. Add 400 μL of **Lysis Solution** to the tissue sample. Ensure that frozen tissue does not thaw during weighing or prior to the addition of **Lysis Solution**.
- d. Homogenize the tissues using the appropriate cell disruption tool. If needed, continue to homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.

- 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.
- g. Proceed to **Section 2** to isolate total RNA, genomic DNA and proteins or **Section 3** to isolate large RNA, microRNA, genomic DNA and proteins.

1G. Lysate Preparation from Plant

Notes Prior to Use

- The maximum recommended input of plant tissue is 50 mg or 5×10^6 plant cells.
- Both fresh and frozen plant samples can be used for this protocol. Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.

1G. Cell Lysate Preparation from Plant

- a. Excise the tissue sample from the plant.
- b. Determine the amount of tissue by weighing. It is recommended that no more than 50 mg of tissue be used for the protocol.
- c. Add 400 μL of **Lysis Solution** to the tissue sample. Ensure that frozen tissue does not thaw during weighing or prior to the addition of **Lysis Solution**.
- d. Homogenize the tissues using the appropriate cell disruption tool. If needed, continue to homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.
- 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.
- g. Proceed to **Section 2** to isolate total RNA, genomic DNA and proteins or **Section 3** to isolate large RNA, microRNA, genomic DNA and proteins.

Section 2: Isolation of Total RNA, Genomic DNA and Proteins

Note: The procedure in this section should be followed if the user wishes to purify **total RNA**, genomic DNA and proteins. If you wish to separately purify large RNA (>200 nt) and small RNA (<200 nt), please proceed to **Section 3** and follow that protocol.

2A: Procedure for Total RNA Purification

1. Binding RNA to Column

- a. Add ethanol to the lysate prior to binding to the column. For lysate from cultured animal cells, blood, bacteria or yeast, add 200 μL of 95% ethanol (provided by the user) to the lysate and mix by vortexing for 10 seconds. For tissues, fungi or plants, add an equal volume of 70% ethanol (provided by the user) to the lysate (100 μL of ethanol is added to every 100 μL of lysate). Mix by vortexing for 10 seconds.

- b. Assemble an **All-in-One Spin Column** with one of the provided collection tubes.
- c. Apply up to 600 μ L of the lysate with the ethanol onto the column and centrifuge 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 2C). 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 **1c** and **1d** if necessary. The flowthroughs should be combined and retained in the same microcentrifuge tube.
- f. Reassemble the spin column with a new collection tube.

2. RNA Wash

- a. Apply 400 μ L of **RNA Wash Solution** to the column and centrifuge for 1 minute.

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

- b. Discard the flowthrough and reassemble the column with the collection tube.
- c. Wash column a second time by adding another 400 μ L of **RNA Wash Solution** and centrifuge for 1 minute.
- d. Discard the flowthrough and reassemble the spin column with its collection tube.
- e. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

3. RNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 50 μ L of **RNA Elution Solution** to the column.
- c. Centrifuge for 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, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 3b** and **3c**). For samples that are known to contain large amounts of RNA a second elution is necessary to prevent RNA carryover in the gDNA elution. Please consult Table 2 below to determine if a 2nd elution is recommended.

- d. **Retain the column for Genomic DNA Isolation and/or Protein Purification.** Proceed to Section 2B for DNA Purification. If genomic DNA isolation is not required, proceed directly to Section 2C for Protein Purification.

Table 2. Recommendations for a 2nd RNA Elution for Different Starting Materials

Starting Material	2 nd Elution Recommended?
HeLa Cells	Optional
Bacteria	Yes
Yeast	Yes
Brain Tissue	Optional
Heart Tissue	Optional
Liver Tissue	Yes
Lung Tissue	Yes
Spleen Tissue	Yes
Arabidopsis Leaves	Yes
Blood	Optional

4. Storage of RNA

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

2B: Procedure for Genomic DNA Purification

1. Genomic DNA Wash

- Reassemble the column from **Step 4d** above with a new collection tube. Apply 500 μL of **gDNA Wash Solution** to the column and centrifuge for 2 minutes.
- Discard the flowthrough and reassemble the spin column with its collection tube.
- Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

2. Genomic DNA Elution

- Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- Add 100 μL of **gDNA Elution Buffer** to the column.
- Centrifuge for **2 minutes at 200 x g (~2,000 RPM)**, followed by **1 minute at 14,000 x g (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum DNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 2b** and **2c**).

- Retain the column for Protein Purification.** Proceed to Section 2C for Protein Purification.

3. Storage of DNA

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.

2C: Procedure for Total Protein Purification

Notes Prior to Use

- At this point, the proteins that are present in the flowthrough from the RNA Binding Step (Section 2A, Step 1d) 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 1 to 4 below.
- For acetone precipitation, please refer to the supplementary protocol provided in the Appendix.

1. pH Adjustment of Lysate

- a. Transfer 300 μL of flowthrough from the RNA Binding Step (Section 2A, Step 1d) to a separate microcentrifuge tube.

Note: To purify the entire flowthrough, adjust the volume according to the note in step b below.

- b. Adjust volume to 600 μL with molecular biology grade water.

Note: To purify the entire flowthrough, adjust the volume to 1.2 mL with molecular biology grade water.

- c. Add 24 μL (or 48 μL for the entire flowthrough) of **Protein pH Binding Buffer**. Mix contents well.

2. Protein Binding

- a. Apply up to 650 μL of the pH-adjusted protein sample onto the column, and centrifuge for 2 minutes at 5,200 $\times g$ (~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 **2a** and **2b** until the entire protein sample has been loaded onto the column.

3. Column Wash

- a. Apply 500 μL 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.

4. Protein Elution and pH Adjustment

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

- a. Add 9.3 μL 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 μL of the **Protein Elution Buffer** to the column and centrifuge for 2 minutes at 5,200 x 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 μL of **Protein Elution Buffer** may be carried out. This should be collected into a different tube (to which 4.6 μL of **Neutralizer** is pre-added) to prevent dilution of the first elution.

Section 3: Isolation of Large RNA, Genomic DNA, microRNA and Proteins

Note: The procedure in this section should be followed if the user wishes to separately purify large RNA (>200 nt) and small RNA (<200 nt), genomic DNA and proteins. If you wish to purify total RNA, please follow the protocol in **Section 2** instead.

3A: Procedure for Purification of Large RNA (>200 nt)

1. Binding Large RNA to Column

- a. Add ethanol to the lysate prior to binding to the column. For lysate from cultured animal cells, blood, bacteria or yeast, add 50 μL of 95% ethanol (provided by the user) to the lysate and mix by vortexing for 10 seconds. For tissues, fungi or plants, add 12.5 μL of 95% ethanol to every 100 μL of lysate. Mix by vortexing for 10 seconds.
- b. Assemble an **All-in-One Spin Column** with one of the provided collection tubes.
- c. Apply up to 600 μL of the lysate with the ethanol onto the column and centrifuge for 1 minute.

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 microRNA Purification and Protein Purification (Sections 3C and 3D). The flowthrough contains both the microRNA and 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 **1c** and **1d** if necessary. The flowthroughs should be combined and retained in the same microcentrifuge tube.
- f. Reassemble the spin column with a new collection tube.

2. Large RNA Wash

- a. Apply 400 μ L of **RNA Wash Solution** to the column and centrifuge for 1 minute.

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

- b. Discard the flowthrough and reassemble the column with the collection tube.
- c. Wash column a second time by adding another 400 μ L of **RNA Wash Solution** and centrifuge for 1 minute.
- d. Discard the flowthrough and reassemble the spin column with its collection tube.
- e. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

3. Large RNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 50 μ L of **RNA Elution Solution** to the column.
- c. Centrifuge for 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 recovery of large RNA, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 3b** and **3c**). For samples that are known to contain large amounts of RNA a second elution is necessary to prevent RNA carryover in the gDNA elution. Please consult Table 3 below to determine if a 2nd elution is recommended.

- d. **Retain the column for Genomic DNA Isolation and/or Protein Purification.** Proceed to Section 3B for DNA Purification. If genomic DNA isolation is not required, proceed directly to Section 3C for microRNA Purification.

Table 3. Recommendations for a 2nd RNA Elution for Different Starting Materials

Starting Material	2 nd Elution Recommended?
HeLa Cells	Optional
Bacteria	Yes
Yeast	Yes
Brain Tissue	Optional
Heart Tissue	Optional
Liver Tissue	Yes
Lung Tissue	Yes
Spleen Tissue	Yes
Arabidopsis Leaves	Yes
Blood	Optional

4. Storage of RNA

The purified large 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.

3B: Procedure for Genomic DNA Purification

1. Genomic DNA Wash

- Reassemble the column from **Step 4d** above with a new collection tube. Apply 500 μL of **gDNA Wash Solution** to the column and centrifuge for 2 minutes.
- Discard the flowthrough and reassemble the spin column with its collection tube.
- Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

2. Genomic DNA Elution

- Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- Add 100 μL of **gDNA Elution Buffer** to the column.
- Centrifuge for **2 minutes at 200 x g (~2,000 RPM)**, followed by **1 minute at 14,000 x g (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum DNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 2b** and **2c**).

- Retain the column for Protein Purification.** Proceed to Section 3C for microRNA Purification, then to Section 3D for Protein Purification.

3. Storage of DNA

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.

3C: Procedure for Purification of microRNA (<200 nt)

1. Binding microRNA to Column

- Transfer the flowthrough from the RNA Binding Step (Section 3A, Step 1d) to a separate microcentrifuge tube.
- For flowthrough from cultured animal cells, blood, bacteria or yeast, add 200 μL of 95% ethanol (provided by the user) to the lysate and mix by vortexing for 10 seconds. For tissues, fungi or plants, add 60 μL of 95% ethanol to every 100 μL of original lysate. Mix by vortexing for 10 seconds.
- Assemble a **microRNA Enrichment Column** with one of the provided collection tubes.

- d. Apply up to 600 μL of the flowthrough with the ethanol onto the column and centrifuge 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.

- e. **Retain the flowthrough for Protein Purification (Section 3D). The flowthrough contains the proteins and should be stored on ice or at -20°C until the Protein Purification protocol is carried out.**
- d. Depending on your flowthrough volume, repeat steps **1a** to **1e** if necessary. The flowthroughs should be combined and retained in the same microcentrifuge tube.
- e. Reassemble the spin column with a new collection tube.

2. microRNA Wash

- a. Apply 400 μL of **RNA Wash Solution** to the column and centrifuge for 1 minute.

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

- b. Discard the flowthrough and reassemble the column with the collection tube.
- c. Wash column a second time by adding another 400 μL of **RNA Wash Solution** and centrifuge for 1 minute.
- d. Discard the flowthrough and reassemble the spin column with its collection tube.
- e. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

3. microRNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 50 μL of **RNA Elution Solution** to the column.

Note: For more concentrated RNA elution, use a minimum volume of 30 μL

- c. Centrifuge for 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.
- d. Proceed to Section 3D for Protein Purification.

4. Storage of RNA

The purified large 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.

3D: Procedure for Total Protein Purification

Notes Prior to Use

- At this point, the proteins that are present in the flowthrough from the microRNA Binding Step (Section 3C, Step 1e) 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 1 to 4 below.
- For acetone precipitation, please refer to the supplementary protocol provided in the Appendix.

1. pH Adjustment of Lysate

- a. Transfer 300 μL of flowthrough from the microRNA Binding Step (Section 3C, Step 1e) to a separate microcentrifuge tube.

Note: To purify the entire flowthrough, adjust the volume according to the note in step b below.

- b. Adjust volume to 600 μL with molecular biology grade water.

Note: To purify the entire flowthrough, adjust the volume to 1.2 mL with molecular biology grade water.

- c. Add 24 μL (or 48 μL for the entire flowthrough) of **Protein pH Binding Buffer**. Mix contents well.

2. Protein Binding

- a. Apply up to 650 μL of the pH-adjusted protein sample onto the column, and centrifuge for 2 minutes at 5,200 $\times g$ (~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 **2a** and **2b** until the entire protein sample has been loaded onto the column.

3. Column Wash

- a. Apply 500 μL of **Protein Wash Buffer** to the column and centrifuge for 2 minutes at 5,200 x 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.

4. Protein Elution and pH Adjustment

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

- d. Add 9.3 μL of **Neutralizer** to a fresh 1.7 mL Elution Tube.
- e. Transfer the spin column from the Column Wash procedure into the Elution Tube.
- f. Apply 100 μL of the **Protein Elution Buffer** to the column and centrifuge for 2 minutes at 5,200 x 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 μL of **Protein Elution Buffer** may be carried out. This should be collected into a different tube (to which 4.6 μL of **Neutralizer** is pre-added) to prevent dilution of the first elution.

Appendix: Acetone Precipitation Procedure for Proteins

- a. Add 4 volumes of ice-cold acetone to the flowthrough from the RNA Binding Step (Section 2A, Step 1d **OR** Section 3C, Step 1e).
- b. Incubate for 15 minutes on ice or at -20°C .
- c. Centrifuge for 10 minutes at 14,000 x 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 μL 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 Solution 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 flowthrough before binding to the microRNA Enrichment Column	Ensure that the appropriate amount of ethanol was added to the flowthrough from the first binding step before it is applied to the microRNA Enrichment Column if you are separately isolating large and microRNA. This is imperative in order to capture the small RNA molecules.
	Ethanol was not added to the RNA Wash Solution	Ensure that 40 mL of 95% 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.	

Problem	Possible Cause	Solution and Explanation
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.
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.
	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.

Problem	Possible Cause	Solution and Explanation
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.
Yield of Genomic DNA is Low	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Lysis Solution was used for the amount of cells or tissue. Incubate the lysis solution for an extra 5 minutes to assist in lysis.
	The DNA elution is incomplete	Ensure that centrifugation at 14,000 $\times g$ for 1 minute is performed following the 2 minute centrifugation at 200 $\times g$. Also, ensure that the entire volume of gDNA Elution Buffer passed through and is eluted from the column.
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.
Contamination of Genomic DNA with RNA	Number of cells or amount of tissue used is close to the maximum recommended amount	When the maximum recommended amount of cells or tissues is used for the procedure, some cross-contamination of RNA in the genomic DNA fraction may be observed. Reduce the input amount of cells or tissues below the maximum recommendation in order to avoid this problem. Recommended amounts of starting material to use for optimal kit performance are given in each section of the protocol.
	High levels of RNA in the starting material	Some sample types, such as bacteria, are known to contain high levels of RNA. It is recommended that a second RNA elution be performed in these cases in order to remove the large amounts of RNA from the column. An RNase treatment could also be performed in the gDNA in order to remove the RNA that is present.

Problem	Possible Cause	Solution and Explanation
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 μ L 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 μ L of Neutralizer to each 100 μ 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

Technical Support

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

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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