

## Product Information

### GenElute™ RNA/DNA/Protein Plus Purification Kit

Catalog Number **E5163**

## TECHNICAL BULLETIN

### Product Description

Sigma's GenElute™ RNA/DNA/Protein Plus Purification Kit provides a rapid method for the isolation and purification of total RNA, genomic DNA, and proteins sequentially from a single sample of cultured animal cells, small tissue samples, blood, bacteria, yeast, fungi, or plants. The total RNA, genomic DNA, and proteins are all column purified in less than 30 minutes.

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. This 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.

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.

### Product Overview

DNA and RNA purification is based on spin column chromatography. The process involves first lysing the cells or tissue of interest with the provided Lysis Solution. The DNA is then captured and purified on a DNA Purification Column. Ethanol is then added to the flowthrough of the DNA purification step and the solution is loaded onto a RNA/Protein Purification Column. The resin in the column binds nucleic acids in a manner that depends on ionic concentrations, thus only the RNA including microRNAs will bind to the column while the proteins are removed in the flowthrough. Next, the bound RNA is washed with the provided RNA Wash Solution to remove impurities and the purified RNA is eluted with the RNA Elution Solution.

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

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

This kit contains solutions that may contain irritants. Wear gloves and safety glasses when handling any reagent provided in this kit.

**Storage/Stability**

This kit is shipped in two parts: 1) Cat. No. E5164-1KT, includes reagents shipped at room temperature and 2) Cat. No. 23506NB, Protein Loading Dye, is shipped on dry ice. The Protein Loading Dye should be stored at  $-20\text{ }^{\circ}\text{C}$  upon arrival. All other solutions should be kept tightly sealed and stored at room temperature.

Reagents Provided	Catalog Number	E5163 50 preps
Lysis Solution	17209NB	40 mL
RNA Wash Solution	47705NB	22 mL
RNA Elution Solution	23503NB	6 mL
gDNA Wash Solution I	47802NB	10 mL
gDNA Wash Solution II	47803NB	15 mL
gDNA Elution Buffer	24005NB	15 mL
Protein Wash Buffer	24006NB	30 mL
Protein pH Binding Buffer	47702NB	8 mL
Protein Elution Buffer	24008NB	8 mL
Protein Neutralizer	21105NB	2 mL
Protein Loading Dye	23020NB	2 mL
gDNA Purification Columns	47703NB	50
RNA/Protein Purification Columns	47704NB	50
Collection Tubes	10113NB	100
Elution tubes (1.7 mL)	10102NB	150

**Reagents and equipment required, not provided***For All Protocols*

- Benchtop microcentrifuge
- 2-mercaptoethanol, Cat. No. M3148
- 96-100 % ethanol, Cat. No. E7148, E7023, or 459836
- Isopropanol, Cat. No. I9516 or 59304
- Molecular biology grade water, Cat. No. W4502
- DL-Dithiothreitol (DTT)

*For Animal Cell Protocol*

- PBS (RNase-free), Cat. No. P5493

*For Animal Tissue Protocol*

- Liquid nitrogen
- Mortar and pestle

*For Bacterial Protocol*

- Lysozyme-containing TE Buffer:
  - For Gram-negative bacteria, 1 mg/mL lysozyme (Cat. No. L6876) in  $1\times$  TE Buffer (Cat. No. T9285)
  - For Gram-positive bacteria, 3 mg/mL lysozyme in TE Buffer

*For Yeast Protocol*

- Resuspension Buffer with Lyticase:
  - 50 mM Tris, pH 7.4
  - 10 mM EDTA
  - 1 M Sorbitol
  - 1 unit/ $\mu\text{L}$  Lyticase (Cat. No. L2524)

*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 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 they remain on ice during downstream applications

## Preparation Instructions

Before beginning the procedure, complete the following:

1. Ensure all solutions are at room temperature prior to use.
2. RNA Wash Solution: Add 50 mL of 96–100% ethanol to the concentrated RNA Wash Solution. This will give a final volume of 72 mL. Mark the label of the bottle to indicate ethanol was added.
3. gDNA Wash Solution I: Add 20 mL of 96–100% ethanol to the concentrated gDNA Wash Solution I. This will give a final volume of 30 mL. Mark the label of the bottle to indicate ethanol was added.
4. gDNA Wash Solution II: Add 15 mL of 96–100% ethanol to the concentrated gDNA Wash Solution II. This will give a final volume of 30 mL. Mark the label of the bottle to indicate ethanol was added.
5. Protein Loading Dye: Add 93 mg of DL-Dithiothreitol (DTT, not provided) to the Protein Loading Dye. The Protein Loading Dye should be stored at  $-20\text{ }^{\circ}\text{C}$  after the addition of DTT. The label on the bottle has a box that may be checked to indicate DTT was added.
6. Optional: The use of  $\beta$ -mercaptoethanol in lysis is highly recommended for most tissues, particularly those known to have high RNase content (example: pancreas). It is also recommended for researchers who wish to isolate RNA for sensitive downstream applications. Add 10  $\mu\text{L}$  of 2-mercaptoethanol to each 1 mL of Lysis Solution. Alternatively, the lysis solution can be used as provided.

## Procedure

### I. Sample Preparation

#### Cell Preparation

For optimal results, it is recommended that  $1 \times 10^6$  cells be used for the input. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain  $10^6$  cells. Inputs of up to  $5 \times 10^6$  cells may be used; however, slight cross-contamination of genomic DNA in the RNA fraction may be observed in input ranges greater than  $10^6$  cells.

#### 1. Harvest cells

- **Attached cell cultures:** Attached cells can be lysed directly in culture vessel. Wash the cells in PBS.
- **Suspension and lifted cells:** Pellet up to to  $5 \times 10^6$  cells for 10 minutes at  $\sim 200 \times g$ . Carefully, decant the supernatant to ensure 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. Carefully decant the supernatant. Several microliters ( $\mu\text{L}$ ) of PBS may be left behind with the pellet in order to ensure the pellet is not dislodged.

Note: Cell pellets can be stored at  $-70\text{ }^{\circ}\text{C}$  for later use or used directly in the procedure. Determine the number of cells present before freezing. Frozen pellets should be stored for no longer than 2 weeks to ensure 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 (**Lysis, step 2**).

#### 2. Lyse cells

- **Attached cell cultures:** Add 300  $\mu\text{L}$  of Lysis Solution directly to culture plate. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes. Transfer lysate to a microcentrifuge tube. **Proceed to Section II.**
- **Suspension and lifted cells:** Add 300  $\mu\text{L}$  of Lysis Solution to the pellet. Lyse cells by vortexing for 15 seconds. Ensure the entire pellet is completely dissolved before proceeding to the next step. **Proceed to Section II.**

Note: For input amounts greater than  $10^6$  cells, it is recommended the lysate is passed through a 25 gauge needle attached to a syringe 5–10 times at this point, in order to reduce the viscosity of the lysate prior to loading onto the column

### Animal Tissues Preparation

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  $-70\text{ }^{\circ}\text{C}$  freezer for long-term storage. Tissues may be stored at  $-70\text{ }^{\circ}\text{C}$  for several months. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure 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 as a guideline for maximum tissue input amounts. If your tissue of interest is not included in the table below, we recommend starting with an input of no more than 10 mg.

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

Tissue	Maximum Input Amount
Brain	25 mg
Heart	5 mg
Kidney	20 mg
Liver	20 mg
Lung	20 mg
Spleen	20 mg

1. Prepare tissue.
  - a. Excise the tissue sample from the animal.
  - b. Determine the amount of tissue by weighing. Please refer to Table 1 for the recommended maximum input amounts of different tissues. For tissues not included in the table, we recommend starting with an input of no more than 10 mg.

- c. Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.
  - d. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.

### 2. Lyse tissue.

- a. Add 300  $\mu\text{L}$  of Lysis Solution to the tissue sample and continue to grind until the sample has been homogenized. Homogenize by passing the lysate 5–10 times through a 25 gauge needle attached to a syringe.
- b. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
- c. Spin lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube (not provided). Note the volume of the supernatant/lysate. **Proceed to Section II**

### Lysate Preparation from Blood

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

1. Transfer up to 100  $\mu\text{L}$  of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).
2. Add 300  $\mu\text{L}$  of Lysis Solution to the blood. Lyse cells by vortexing for 15 seconds. Ensure the mixture becomes transparent before proceeding to the next step. **Proceed to Section II**

### Lysate Preparation from Bacteria

Prepare the appropriate lysozyme-containing TE Buffer as indicated in Table 2. This solution should be prepared with sterile, RNase-free TE Buffer and kept on ice until needed. These reagents are to be provided by the user.

It is recommended that no more than  $10^9$  bacterial cells be used in this procedure. Bacterial growth can be measured using a spectrophotometer. As a general rule, an *E. coli* culture containing  $1 \times 10^9$  cells/mL has an OD<sub>600</sub> 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.

1. Bacteria harvest
  - a. Pellet bacteria by centrifuging at  $14,000 \times g$  ( $\sim 14,000$  rpm) for 1 minute.
  - b. Decant supernatant and carefully remove any remaining medium by aspiration. The bacterial pellet can be frozen at  $-70$  °C for later use or used immediately.
2. Bacterial cell lysis
  - a. Resuspend the bacteria thoroughly in  $100 \mu\text{L}$  of the appropriate lysozyme-containing TE Buffer (see Table 2) by vortexing. Incubate at room temperature for the time indicated in Table 2. If using frozen bacterial pellets, do not thaw prior to beginning the protocol. Add the Lysozyme-containing TE Buffer directly to the frozen bacterial pellet.
  - b. Add  $300 \mu\text{L}$  of Lysis Solution and vortex vigorously for at least 10 seconds. **Proceed to Section II.**

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

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

### Lysate Preparation from Yeast

Prepare the appropriate amount of Lyticase-containing Resuspension Buffer, considering that  $500 \mu\text{L}$  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%  $\beta$ -mercaptoethanol, and 1 unit/ $\mu\text{L}$  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 (**Lysis, step 2**).

1. Yeast cell harvest
  - 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 medium by aspiration.
  - c. Resuspend the yeast thoroughly in  $500 \mu\text{L}$  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.
2. Lysis of spheroplasts
 

Add  $300 \mu\text{L}$  of Lysis Solution and vortex vigorously for at least 10 seconds. **Proceed to Section II.**

### Lysate Preparation from Fungi

Fresh or frozen fungi may be used for this procedure. Fungal tissue should be flash-frozen in liquid nitrogen and transferred immediately to a  $-70\text{ }^{\circ}\text{C}$  freezer for long-term storage. Fungi may be stored at  $-70\text{ }^{\circ}\text{C}$  for several months. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised. It is recommended that no more than 50 mg of fungi be used for this procedure in order to prevent clogging of the column. It is important to work quickly during this procedure.

#### 1. Fungi harvest and cell preparation

- a. Determine the amount of fungi by weighing. It is recommended that no more than 50 mg of fungi be used for the protocol.
- b. Transfer the fungus into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the fungus thoroughly using a pestle.

Note: At this stage the ground fungus may be stored at  $-70\text{ }^{\circ}\text{C}$ , such that the RNA purification can be performed at a later time.

- c. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.

#### 2. Lyse cells

- a. Add 300  $\mu\text{L}$  of Lysis Solution to the tissue sample and continue to grind until the sample has been homogenized.
- b. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
- c. Spin lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate. **Proceed to Section II**

### Lysate Preparation from Plant

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

#### 1. Plant cell preparation

- a. Transfer  $\leq 50$  mg of plant tissue or  $5 \times 10^6$  plant cells into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the sample into a fine powder using a pestle in liquid nitrogen.

Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen.

- b. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.

#### 2. Lyse plant cells

- a. Add 600  $\mu\text{L}$  of Lysis Solution to the tissue sample and continue to grind until the sample has been homogenized.
- b. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
- c. Spin lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate. **Proceed to Section II.**

## II: Genomic DNA Purification

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

### 1. Binding DNA to gDNA Purification Column

- Retrieve a gDNA Purification Column pre-assembled with a collection tube.
- Apply up to 600  $\mu\text{L}$  of the lysate prepared from Section 1 onto the column and centrifuge at  $14,000 \times g$  for 1 minute. 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.
- Retain the flowthrough for RNA Purification (Section III).** The flowthrough contains the RNA and proteins, and should be stored on ice or at  $-20\text{ }^{\circ}\text{C}$  until the RNA Purification protocol is carried out.
- Reassemble the spin column with the collection tube.

### 2. Genomic DNA Wash

- Apply 500  $\mu\text{L}$  of gDNA Wash Solution I to the column and centrifuge at  $14,000 \times g$  for 1 minute. Discard the flowthrough.
- Apply 500  $\mu\text{L}$  of gDNA Wash Solution II to the column and centrifuge at  $14,000 \times g$  for 1 minute. Discard the flowthrough.
- Spin the column at  $14,000 \times g$  for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

### 3. Genomic DNA Elution

- Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- Add 100  $\mu\text{L}$  of gDNA Elution Buffer to the column.

- Centrifuge for 2 minutes at  $200 \times g$ , followed by 1 minute at  $14,000 \times g$ . Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at  $14,000 \times g$  for 1 additional minute.  
**Note:** For maximum DNA recovery, it is recommended a second elution be performed into a separate microcentrifuge tube (Repeat Steps 3b and 3c).

### 4. Storage of DNA

The purified DNA sample may be stored at  $4\text{ }^{\circ}\text{C}$  for a few days. It is recommended that samples be placed at  $\leq -20\text{ }^{\circ}\text{C}$  for long term storage.

## III: Total RNA Purification

**Note:** For sensitive applications that require the complete removal of genomic DNA, an optional on-column DNase I treatment could be performed after completion of Step 1f.

### 1. Binding RNA to Column

- To every 100  $\mu\text{L}$  of flowthrough from Step 2c, add 60  $\mu\text{L}$  of 96–100 % ethanol. Mix by vortexing.  
**Note:** For example, for 300  $\mu\text{L}$  of flowthrough, add 180  $\mu\text{L}$  of 96–100 % ethanol
- Assemble an RNA/Protein Purification Column with one of the provided collection tubes.
- Apply up to 600  $\mu\text{L}$  of the lysate with the ethanol onto the column and centrifuge for at  $14,000 \times g$  for 1 minute. 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.
- Retain the flowthrough for Protein Purification (Section IV).** The flowthrough contains the proteins and should be stored on ice or at  $-20\text{ }^{\circ}\text{C}$  until the Protein Purification protocol is carried out.
- Depending on your lysate volume, repeat steps 1c and 1d if necessary. The flowthroughs should be combined and retained in the same microcentrifuge tube.
- Reassemble the spin column with the collection tube.

## 2. RNA Wash

- a. Apply 400  $\mu\text{L}$  of RNA Wash Solution to the column and centrifuge at  $14,000 \times g$  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 the column a second time by adding another 400  $\mu\text{L}$  of RNA Wash Solution and centrifuge at  $14,000 \times g$  for 1 minute.
- d. Discard the flowthrough and reassemble the spin column with its collection tube.
- e. Wash the column a third time by adding another 400  $\mu\text{L}$  of RNA Wash Solution and centrifuge at  $14,000 \times g$  for 1 minute.
- f. Discard the flowthrough and reassemble the spin column with its collection tube.
- g. Spin the column at  $14,000 \times g$  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  $\mu\text{L}$  of RNA Elution Solution to the column.
- c. Centrifuge for 2 minutes at  $200 \times g$ , followed by 1 minute at  $14,000 \times g$ . Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at  $14,000 \times g$  for 1 additional minute.  
Note: For maximum RNA recovery, particularly for samples that are known to contain large amounts of RNA, it is recommended a second elution be performed into a separate microcentrifuge tube (Repeat Steps 3b and 3c).
- d. **Retain the column for Protein Purification.** Proceed to Section IV for Protein Purification.

## 4. Storage of RNA

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

## IV. Procedure to Isolate Total Protein from All Cell Types

### Notes Prior to Use

- At this point, the proteins that are present in the flowthrough from the RNA Binding Step (Section III, 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 acetone precipitation, please refer to the supplementary protocol provided in Appendix A
- Add 93 mg of DL-Dithiothreitol (DTT, not provided) to the **Protein Loading Dye**. The Protein Loading Dye should be stored at  $-20\text{ }^{\circ}\text{C}$  after the addition of DTT. The label on the bottle has a box that may be checked to indicate that DTT was added.

### 1. pH Adjustment of Lysate

- a. Transfer the flowthrough from the RNA Binding Step (Section III, step 1d) to a separate microcentrifuge tube.
- b. For every 100  $\mu\text{L}$  of flowthrough, dilute with 100  $\mu\text{L}$  of molecular biology grade water.  
Note: For example, to purify the entire flowthrough of 480  $\mu\text{L}$ , dilute with 480  $\mu\text{L}$  molecular biology grade water.
- c. For every 100  $\mu\text{L}$  of flowthrough, add 8  $\mu\text{L}$  (or 40  $\mu\text{L}$  for an entire flowthrough of 480  $\mu\text{L}$ ) of Protein pH Binding Buffer. Mix contents well.  
Note: Depends on the type and amount of input, slight precipitation may occur which will not affect the purification procedure.



## 2. Protein Binding

- a. Apply up to 600  $\mu\text{L}$  of the pH-adjusted protein sample onto the column and centrifuge for 2 minutes at  $5,200 \times g$  ( $\sim 8,000$  RPM). Inspect the column to ensure 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  $\mu\text{L}$  of Protein Wash Buffer to the column and centrifuge for 2 minutes at  $5,200 \times g$ .
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Inspect the column to ensure 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  $\mu\text{L}$  of Neutralizer to a fresh 1.7 mL Elution tube.
- b. Transfer the spin column from the Column Wash procedure into the Elution tube.
- c. Apply 100  $\mu\text{L}$  of the Protein Elution Buffer to the column and centrifuge for 2 minutes at  $5,200 \times g$  to elute bound proteins.  
Note: Approximately 95% of bound protein is recovered in the first elution. If desired, a second elution using 50  $\mu\text{L}$  of Protein Elution Buffer may be carried out. This should be collected into a different tube (to which 4.6  $\mu\text{L}$  of Neutralizer is pre-added) to prevent dilution of the first elution.

## Appendix A: Acetone Precipitation Procedure for Proteins

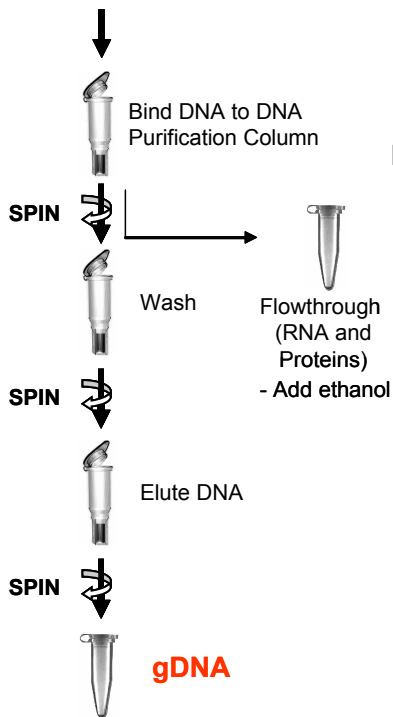
- Add 4 volumes of ice-cold acetone to the flowthrough from the RNA Binding Step (Section 3, step 6d).
- Incubate for 15 minutes on ice or at  $-20\text{ }^{\circ}\text{C}$ .
- Centrifuge for 10 minutes at  $14,000 \times g$  ( $\sim 12,000$  rpm). Discard the supernatant and allow the pellet to air-dry.  
Note: At this point the pellet can be washed with  $100\text{ }\mu\text{L}$  of ice cold ethanol and again air-dried.
- Resuspend the pellet in the buffer of your choice that is suited to your downstream application.

### Flow Chart

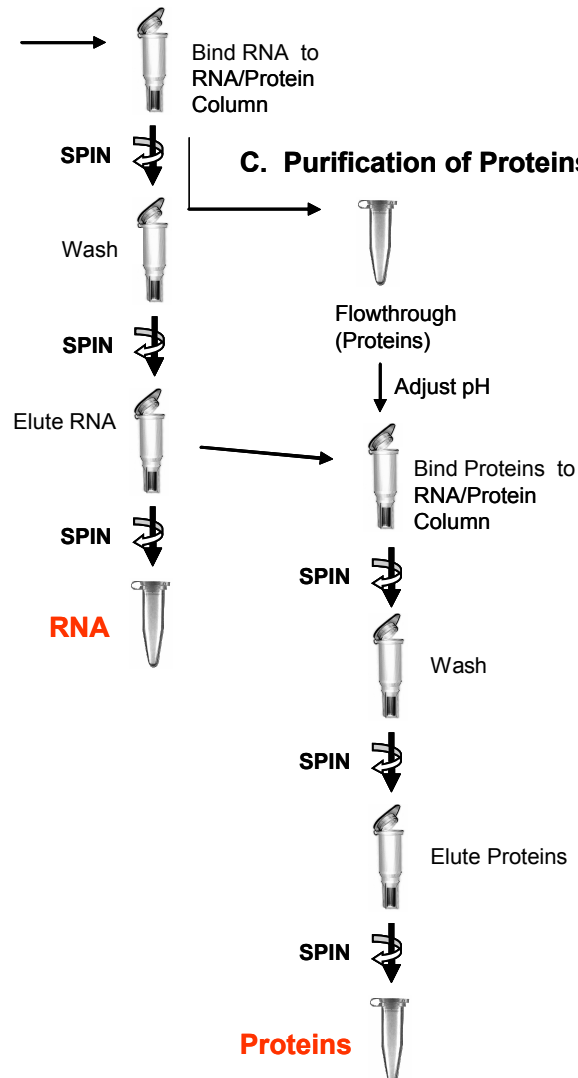
Procedure for Purifying gDNA, Total RNA and Proteins using GenElute RNA/DNA/Protein Plus Purification Kit

#### A. Purification of DNA

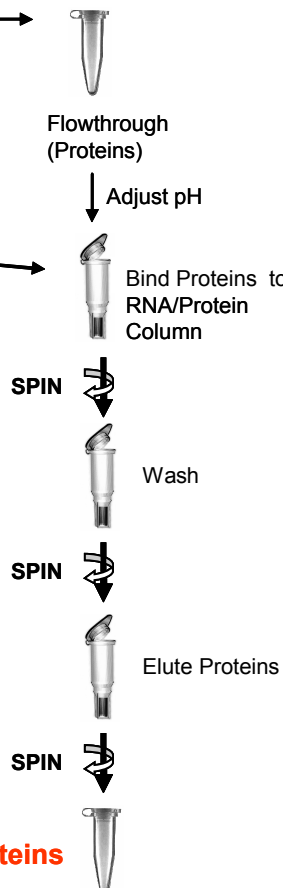
Lyse cells or tissue using **Lysis Solution**



#### B. Purification of RNA



#### C. Purification of Proteins



## Troubleshooting Guide

Problem	Cause	Solution
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure the appropriate amount of Lysis Solution was used for the amount of cells or tissue.
	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.
	An alternative elution solution was used	It is recommended that the RNA Elution Buffer supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the RNA Wash Solution	Ensure 50 mL of 96–100% ethanol is added to the supplied Wash Solution prior to use.
	Low RNA content in cells or tissues used	Different tissues and cells have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA content of your starting material.
	Cell Culture: Cell monolayer was not washed with PBS	Ensure the cell monolayer is washed with the appropriate amount of PBS in order to remove residual medium from cells.
	Yeast: Lyticase was not added to the Resuspension Buffer	Ensure the appropriate amount of lyticase is added when making the Resuspension Buffer.
Clogged Column	Bacteria and Yeast: All traces of medium not removed	Ensure all medium is removed prior to the addition of the lysis solution through aspiration.
	Insufficient solubilization of cells or tissues	Ensure the appropriate amount of lysis buffer was used for the amount of cells or tissue.
	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20 °C may cause precipitates to form that can cause the columns to clog.

Problem	Cause	Solution
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 the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Also, after the DNA binding step, the flow through should be kept on ice or $-20\text{ }^{\circ}\text{C}$ if the RNA purification step is not carried out immediately.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at $-20\text{ }^{\circ}\text{C}$ for a few days. It is recommended samples be stored at $-70\text{ }^{\circ}\text{C}$ 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 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 $-70\text{ }^{\circ}\text{C}$ freezer for long-term storage.
RNA does not perform well in downstream applications	RNA was not washed twice with the provided RNA Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed twice with RNA Wash Solution. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure 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 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 the entire volume of gDNA Elution Buffer passed through and is eluted from the column.

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
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 RNA with genomic DNA	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 genomic DNA in the RNA 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. Alternatively, carry out the on-column DNase I treatment as described in Appendix B
Poor Protein Recovery	Incorrect pH adjustment of sample.	Ensure 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 $\mu$ 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 $\mu$ L of Neutralizer to each 100 $\mu$ L of eluted protein in order to adjust the pH to neutral. Some proteins are sensitive to high pH, such as the elution buffer at pH 12.5
	Eluted protein was not neutralized quickly enough.	If eluted proteins are not used immediately, degradation will occur. We strongly suggest adding Neutralizer in order to lower the pH.

GenElute is a trademark of Sigma-Aldrich Co. LLC.

JG,IDC,JC,PHC,MAM 06/14-1