User Guide

Catalog Nos.

RTN10

RTN70

RTN350

GenElute™ Mammalian Total RNA Miniprep Kit

sigma.com

Ordering Information

Catalog No.	Product Description	Pkg Size
RTN10	GenElute Mammalian Total RNA Miniprep Kit	10 preps
RTN70	GenElute Mammalian Total RNA Miniprep Kit	70 preps
RTN350	GenElute Mammalian Total RNA Miniprep Kit	350 preps

Related Products

Related Products	Catalog No.	Related Products	Catalog No.
Ethanol, absolute, Molecular Biology Grade	**E7023**	Deoxyribonuclease I, Amplification Grade	**AMPD1**
Proteinase K	**P4850**	*Taq* DNA Polymerase	**D1806**
Trypsin-EDTA Solution (1×)	**T3924**	Deoxynucleotide (dNTP) Mix	**D7295**
Precast Agarose Gels, 1.25%, 8 well	**P6222**	Enhanced Avian Reverse Transcriptase	**A4464**
MOPS-EDTA-Sodium Acetate Buffer	**M5755**	Enhanced Avian HS RT-PCR Kit	**HSRT100**
RNA Sample Loading Buffers	**R1386** **R4268**	Ethidium Bromide Aqueous Solution, 10 mg/mL	**E1510**
RNA Markers, 0.2–10 kb	**R7020**	PerfectHyb™ Plus Hybridization Buffer	**H7033**

To reorder product call 1-800-325-3010, visit our Web site at sigma-aldrich.com, or contact your local sales representative.

GenElute™ Mammalian Total RNA Miniprep Kit

Table of Contents

Product Description

Sigma's GenElute Mammalian Total RNA Miniprep Kit provides a simple and convenient way to isolate total RNA from mammalian cells and tissues. Protocols are provided for cells, tissues, and fibrous tissues. These protocols differ in their cell lysis and disruption conditions. Once the RNA is bound to the GenElute Binding Column, the purification procedure is the same for all starting materials. For fibrous tissues the kit must be used with proteinase K (Cat. No. **P4850**) to ensure effective cell disruption.

The kit combines the advantages of a silica-based system with a microspin format and eliminates the need for cesium chloride gradients, alcohol precipitation, and hazardous organic compounds such as phenol and chloroform. Cells or tissues are lysed and homogenized in a buffer containing guanidine thiocyanate to ensure thorough denaturation of macromolecules and inactivation of RNases. Addition of ethanol causes RNA to bind when the lysate is spun through a silica membrane in a microcentrifuge tube.

After washing to remove contaminants, RNA is eluted in 50–100 µL of Elution Solution. Up to 150 µg of total RNA can be isolated in less than 30 minutes. The purified RNA is ready for reverse transcription and PCR, labeling and microarray analysis, and other common applications. Note that RNA shorter than 200 nucleotides in length, such as tRNA, 5S rRNA, and 5.8S rRNA, is not recovered efficiently under the conditions used with this kit.

If all traces of DNA contamination must be eliminated, further treatment with DNase I is recommended. DNase I digestion can be performed while the RNA is bound to the GenElute Binding Column using the On-Column DNase I Digestion Set (Catalog Nos. **DNASE10** or **DNASE70**). Alternatively, for more stringent removal of contaminating DNA, the final RNA preparation can be treated with Amplification Grade DNase I (Catalog No. **AMPD1**).

Reagents Provided	Catalog No.	RTN10 10 Preps	RTN70 70 Preps	RTN350 350 Preps
Lysis Solution	**L8285**	10 mL	70 mL	350 mL
2-Mercaptoethanol (2-ME)	**M3148**	0.15 mL	0.9 mL	2 × 2 mL
Wash Solution 1	**W3136**	6 mL	40 mL	200 mL
Wash Solution 2 Concentrate	**W3261**	2.5 mL	15 mL	75 mL
Elution Solution	**E8024**	1.5 mL	10 mL	50 mL
GenElute Filtration Columns	**CP9346***	10 each	70 each	5 × 70 each
GenElute Binding Columns	**CP9471***	10 each	70 each	5 × 70 each
Collection Tubes, 2.0 mL capacity	**T5449**	4 × 10 each	4 × 70 each	20 × 70 each

On-Column DNase I Digestion Set (Not included with these kits)	Catalog No.	DNASE10 10 Preps	DNASE70 70 Preps
DNase Digest Buffer	**D1566**	1.5 mL	4 × 1.5 mL
Deoxyribonuclease I	**D2816**	0.15 mL	0.9 mL

*MilliporeSigma continually seeks ways to improve our products. Please note that the product codes for the GenElute Nucleic Acid Binding Columns and GenElute Filtration Columns have changed from C9471 to CP9471 and C9346 to CP9346, respectively. These changes have been made to streamline and make more consistent all the GenElute products across the line. The performance and functionality of the C9471 & CP9471 binding columns and the C9346 & CP9346 filtration columns are equivalent.

Reagents and Equipment Required But Not Provided

- Ethanol (200 proof), Cat. No. **E7023**
- 70% Ethanol solution
- Proteinase K, Cat. No. **P4850** (fibrous tissues only)
- 55 °C Water bath or heating block (fibrous tissues only)
- Molecular Biology Reagent Water, Cat. No. **W4502** (fibrous tissues only)
- RNase-free pipette tips (aerosol barrier recommended)
- Microcentrifuge tubes
- Microcentrifuge (2 mL tube, rotor equipped)**
- On-Column DNase I Digestion Set, Cat Nos. **DNASE10** and **DNASE70** (optional)
- Amplification Grade DNase I, Cat. No. **AMPD1** (optional)
- RNA*later*™, RNA Stabilization Solution for Tissue, Cat. No. **R0901** (optional)
- Tissue homogenizer and vessels for tissue samples

Note: To ensure proper fit of all tubes, a 24-place rotor is recommended. If you are using a 36-place rotor, we recommend using every other place for proper tube fit.

Precautions and Disclaimer

The GenElute Mammalian Total RNA Miniprep Kit is for laboratory use only, not for drug, household, or other uses. Lysis Solution and Wash Solution 1 contain guanidine thiocyanate, which is harmful. Wear gloves, safety glasses, and suitable protective clothing when handling these solutions or any reagent provided with the kit. Consult the MSDS for information regarding hazards and safe handling practices.

RNases are ubiquitous and very stable proteins, which are a concern for any researcher attempting to isolate RNA. The prepared Lysis Solution contains guanidine thiocyanate and 2-mercaptoethanol, both of which inactivate RNases. Conditions during column binding and the first wash remove RNases.

Care must be taken not to introduce RNase, especially during the final wash and elution. Use RNase-free pipette tips, preferably those having an aerosol barrier. Wear latex or vinyl gloves and change them frequently. Keep bottles and tubes closed when not adding or removing their contents. The references given at the end of this bulletin are good sources of additional information on working with RNA.

Storage and Stability

Store the GenElute Mammalian Total RNA Miniprep Kit at room temperature. If any reagent forms a precipitate, warm at 65 °C until the precipitate dissolves and allow to cool to room temperature before use. The On-Column DNase I Digestion Set may be stored at 2–8 °C for up to 6 months. For longer term, storage at –20 °C is recommended. All reagents are stable for at least 1 year when stored properly.

Preparation Instructions

Before beginning the procedure, prepare the following:

1. Prepare the Lysis Solution/2-ME Mixture

Add 2-mercaptoethanol (2-ME) (**M3148**) to a volume of Lysis Solution (**L8285**) sufficient for that day's use, usually 250 or 500 µL of Lysis Solution/2-ME Mixture per RNA preparation (see Table 1). 2-ME is required to fully inactivate RNases. Add 10 µL of 2-ME for each 1 mLl of Lysis Solution.

Table 1. Lysis Solution/2-ME Mixture Volumes for Sample Preparation

Sample Size*	Lysis Solution/2-ME Mixture Volume
Pelleted Cells	
Up to 5×10^6 cells	250 µL
5×10^6 to 1×10^7 cells	500 µL
Attached Cells	
Up to 10 cm^2 of surface area	250 µL
10 to 25 cm^2 of surface area	500 µL
Mammalian Tissue	
Up to 40 mg of tissue	500 µL

***Note**: For larger amounts of cells or tissue, scale up the volume of the Lysis Solution/2-ME Mixture proportionally. Divide the lysate into 500 to 700 µL aliquots and process through separate filtration and binding columns.

2. Dilute Wash Solution 2 Concentrate

Dilute the Wash Solution 2 Concentrate (**W3261**) with 10 mL (10 prep package), 60 mL (70 prep package), or 300 mL (350 prep package) of 200 proof ethanol (**E7023**). After each use, tightly cap the diluted Wash Solution 2 to prevent evaporation of the ethanol.

Procedure

All steps are carried out at room temperature except where otherwise noted. Please note that centrifugation speeds are given in units of *g*. Convert to rpm depending on size of centrifuge rotor, according to the formula:

$$RCF = 1.118 \times 10^{-5} \times \text{radius (in cm)} \times rpm^2$$

where RCF = gravitational acceleration in units of *g*. If centrifuges/rotors for the required *g*-forces are not available, use the maximum *g*-force possible and increase the spin time proportionally. Spin until all liquid passes through column.

Important: Depending on the starting material being used, choose between sections A, B, or C below.

A. Cell Preparation

For best yields of intact RNA use only rapidly growing cells before they reach their maximum density.

1a. Harvest cells

- **Suspension cell cultures**: Pellet up to 1 $\times 10^7$ cells for 5 minutes at 300 × *g*. Remove the culture medium completely and discard. Continue with step 2a.
- **Attached cell cultures**: Attached cells can be lysed directly in culture vessels or released with trypsin and pelleted before lysis. To lyse cells directly, remove culture medium completely, then continue with step 2a. Alternatively, trypsinize and pellet cells using standard methods and proceed as for suspension cultures.

Note: Cells can be flash-frozen in liquid nitrogen and stored at –70 °C for several months before preparing RNA. However, do not allow frozen cells to thaw before disruption in the Lysis Solution/2-ME Mixture.

2a. Lyse cells and inactivate RNase

This is a critical step that must be accomplished quickly and thoroughly. The resulting lysate may be stored at –70 °C for several months.

- **Pelleted cells**: Vortex pellet to loosen cells. Add 250 µL of the Lysis Solution/2-ME Mixture for up to 5 × 10^6 cells or 500 µL of the Lysis Solution/2-ME Mixture for 5 × 10^6 to 1 × 10^7 cells (see Table 1). Vortex or pipette thoroughly until all clumps disappear. Continue with step 3a.
- **Attached cells**: Add 250 µL of the Lysis Solution/2-ME Mixture for up to 10 cm^2 of surface area or 500 µL of the Lysis Solution/2-ME Mixture for 10 to 25 cm^2 of surface area (see Table 1). Rock the culture vessel while tapping the side for a few seconds to completely cover the cells. Let the culture vessel sit for 1 to 2 minutes with the Lysis Solution/2-ME Mixture covering the cells. Repeat the rocking and tapping, and then tilt the culture vessel to one side to collect the lysate. Continue with step 3a.

3a. Filter lysate

This step removes cellular debris and shears DNA, and may be omitted with fewer than 10^6 cultured cells. Assemble a filtration column (**CP9346**) with a 2 mL collection tube (**T5449**). Carefully pipette the lysed cells from step 2a onto the filtration column. Centrifuge at maximum speed (12,000–16,000 × *g*) for 2 minutes. Discard the filtration column.

4a. Prepare for binding

Add an equal volume of 70% ethanol solution (250 or 500 µL) to the filtered lysate. Vortex or pipette thoroughly to mix. Continue to Section D.

B. Fresh, Frozen, and RNA*later* Stabilized Mammalian Tissue Preparation

For best yields of intact RNA, harvest tissue as quickly as possible from a freshly sacrificed animal and proceed with the procedure below immediately. Alternatively, tissue may be immediately flash-frozen in liquid nitrogen and stored at –70 °C for several months prior to RNA extraction, or the tissue may be stabilized in RNAlater Stabilization Solution. RNA*later* stabilized tissue can be stored for up to 1 day at 37 °C, 1 week at 25 °C, 1 month at 4 °C, or at –20 °C or colder temperatures for longer term storage prior to RNA preparation.

Up to 40 mg of tissue may be used per preparation, with the exception of spleen and thymus, which require no more than 20 mg per preparation. Unless you have experience preparing total RNA from your tissue type with a silica bind, wash, and elute method, it is recommended to start with no more than 25 mg of tissue or 10 mg of spleen or thymus and then empirically determining the maximum amount of tissue that can be processed.

Skeletal muscle, heart, and skin contain large amounts of contractile fibers, connective tissue, and/or collagen. Therefore, these tissues require a specialized protocol that includes a proteinase K digestion step (see Section C).

1b. Prepare tissue

Quickly slice and weigh a piece of fresh, frozen, or RNA*later* stabilized tissue. Up to 40 mg may be used per preparation, with the exception of spleen and thymus, which require no more than 20 mg. Transfer to an appropriate vessel for homogenization.

Note: It is essential to disrupt fresh tissue in the Lysis Solution/2-ME Mixture as quickly as possible to minimize RNA degradation. Do not allow frozen tissue to thaw before disruption in the Lysis Solution/2-ME Mixture.

2b. Lyse tissue and inactivate RNase

This is a critical step that must be accomplished quickly and thoroughly. The resulting lysate may be stored at –70 °C for several months. Add 500 µL of the Lysis Solution/2-ME Mixture and homogenize immediately until no visible pieces remain.

Note: Approximately 60 seconds of homogenization with a Brinkman Polytron PT 1200 rotor-stator homogenizer or equivalent is sufficient for most tissues.

3b. Filter lysate

This step removes debris and shears DNA. Assemble a filtration column (**CP9346**) with a 2 mL collection tube (**T5449**). Carefully pipette the homogenized tissue from step 2b onto the filtration column. Centrifuge at maximum speed (12,000–16,000 × *g*) for 2 minutes. Discard the filtration column.

4b. Prepare for binding

Add 500 µL of 70% ethanol solution to the filtered lysate. Vortex or pipette thoroughly to mix. Continue to Section D.

C. Fibrous Tissue Preparation (e.g., Heart, Skeletal Muscle, and Skin)

To effectively release RNA from fibrous tissues, the kit must be used with proteinase K (**P4850**), which is not included in this kit. The most difficult step in extracting RNA from fibrous tissue is completely disrupting the tissue. These tissues are polynucleate, have a low cell density, and contain an abundance of contractile proteins, connective tissue, and collagen. Tissues with these characteristics typically give low RNA yields. To overcome these problems, the protocol must be modified to include a proteinase K digestion to facilitate cell disruption. This procedure has been used for the isolation of total RNA from heart, skeletal muscle, and skin. In general, other tissue should be processed using the protocol for Fresh, Frozen, and RNA*later* Stabilized Mammalian Tissue Preparation (See Section B). If working with other tissues that may benefit from a proteinase K digestion step, it is recommended that the two protocols be compared. Avoid using this procedure with nuclease-rich tissues such as spleen, pancreas, thymus, and intestine as the RNase-inactivating Lysis Solution/2-ME Mixture must be diluted to facilitate proteinase K digestion.

Up to 40 mg of tissue may be used per preparation. Unless you have experience preparing total RNA from your tissue type with a silica bind, wash, and elute method, it is recommended to start with no more than 25 mg of tissue and then empirically determining the maximum amount of tissue that can be processed.

1c. Prepare tissue

Preheat a water bath or heat block to 55 °C for use in step 3c. Quickly slice and weigh a piece of fresh, frozen, or RNA*later* stabilized tissue. Up to 40 mg may be used per preparation. Transfer to an appropriate vessel for homogenization.

Note: It is essential to disrupt fresh tissue in the Lysis Solution/2-ME Mixture as quickly as possible to minimize RNA degradation. Do not allow frozen tissue to thaw before disruption in the Lysis Solution/2-ME Mixture.

2c. Lyse tissue and inactivate RNase

This is a critical step that must be accomplished quickly and thoroughly. Add 300 µL of the Lysis Solution/2-ME Mixture and homogenize immediately until no visible pieces remain. The resulting lysate may be stored at –70 °C for several months.

Note: Fibrous tissues require 90 seconds or longer of homogenization with a Brinkman Polytron PT 1200 rotor-stator homogenizer or equivalent to completely disrupt the tissue.

3c. Proteinase K digestion

Add 590 µL of RNase-free water and 10 µL of the proteinase K solution (**P4850**) to the homogenized tissue. Vortex or pipette thoroughly to mix and incubate at 55 °C for 10 minutes.

Note: The proteinase K solution must be added directly to each sample preparation every time. Do not combine the proteinase K and lysis solution or dilute with water for storage. For multiple preparations, a mix of RNase-free water and the proteinase K solution can be prepared, if desired, and aliquoted for each preparation.

4c. Filter lysate

This step removes debris and shears DNA. Assemble a filtration column (**CP9346**) with a 2 mL collection tube (**T5449**). Carefully transfer 700 µL the digested tissue from step 3c onto the filtration column. Centrifuge at maximum speed (12,000–16,000 × *g*) for 2 minutes. Transfer the filtrate to a new 2 mL microcentrifuge tube.

5c. Repeat step 4c with the remainder of the digested tissue and pool the filtrates.

6c. Prepare for binding

Add 450 µL of 200 proof ethanol to the filtered lysate. Vortex or pipette thoroughly to mix. Continue to Section D.

D. RNA Isolation

This is a continuation of the procedure from the samples prepared in Sections A, B, or C.

1d. Load lysate into binding column

Assemble a binding column (**CP9471**) with a 2 mL collection tube (**T5449**). Pipette 700 uL of the lysate/ethanol mixture from step 4a, 4b or 6c onto the binding column. If the volume of lysate/ethanol mixture is greater than 700 µL, the RNA must be bound to the column in 2 steps. Centrifuge at maximum speed for 15 seconds. Discard the flow-through liquid, but retain the collection tube. Return the binding column to the collection tube and apply any remaining lysate/ethanol mixture to the column. Repeat the centrifugation as described above. Discard the flow-through liquid, but retain the collection tube.

Optional: For on-column DNase I digestion continue with the procedure described in Appendix I (page 14): On-Column DNase I Digestion.

2d. First column wash

Pipette 500 µL of Wash Solution I (**W3136**) into the column and centrifuge at maximum speed for 15 seconds.

3d. Second column wash

Transfer the binding column into a fresh 2 mL collection tube. Discard the flow-through liquid and the original collection tube. Ensure that Wash Solution 2 Concentrate (**W3261**) has been diluted with ethanol as described in the Preparation Instructions. Pipette 500 µL of this ethanol containing Wash Solution 2 into the column and centrifuge at maximum speed for 15 seconds. Discard the flow-through liquid, but retain the collection tube.

4d. Third column wash

Pipette a second 500 µL volume of Wash Solution 2 into the column and centrifuge at maximum speed for 2 minutes to dry the binding column.

Optional spin to further dry the column. The binding column must be free of ethanol before eluting the RNA. Centrifuge the column for an additional 1 minute at maximum speed if any residual Wash Solution 2 is seen on the surface of the binding column. Empty and re-use the collection tube if you need this additional centrifugation step.

5d. Elute RNA

Transfer the binding column to a fresh 2 mL collection tube. Pipette 50 µL of Elution Solution (**E8024**) into the binding column and centrifuge at maximum speed for 1 minute. If greater than 50 µg of RNA is expected (see Expected Yield under Results), repeat the elution with a second 50 µL volume of Elution Solution, collecting both eluates in the same tube. Purified RNA is now in the flow-through eluate (~45 or 90 µL total), and is ready for immediate use or storage at –70 °C. Keep the RNA on ice whenever it is thawed for use.

Results

Analysis of RNA

The concentration and quality of total RNA prepared can be determined by spectro-photometric analysis. Dilute the RNA in TE buffer (10 mM Tris-HCl, pH 7-8, with 1 mM EDTA) and measure the absorbance at 260 and 280 nm using a quartz microcuvette. For best results, absorbance readings should be between 0.1 and 1.0 absorbance units (or within the linear range of your spectrophotometer). An absorbance of 1.0 at 260 nm corresponds to approximately 40 µg/mL of RNA. The ratio of absorbance at 260 to 280 nm (A_{260}/A_{280}) should be between 1.8 and 2.1.

RNA quality can also be evaluated by agarose gel electrophoresis, analysis on an Agilent Bioanalyzer, or by capillary electrophoresis. The 28S and 18S rRNA should appear as discrete bands or peaks at approximately 5.3 and 2.0 kb, respectively. The mRNA should appear as a light smear or series of peaks, primarily between the 28S and 18S rRNA bands.

Expected Yield

The yield of total RNA from cells and tissues varies with growth conditions and developmental stage. In general, younger and more rapidly growing cultures or animals will contain more RNA. Approximately 10-30 µg of total RNA per million cultured cells have been obtained with the GenElute Mammalian Total RNA Miniprep Kit (15–30 µg per million HeLa cells and 10–15 µg per million NIH-3T3 cells). Yields of 2–50 µg of total RNA per 10 mg of mouse tissue have been obtained with this kit (2–5 µg from brain, heart, and skeletal muscle; 5–10 µg from lung; 10–20 µg from kidney and pancreas; 25–30 µg from spleen; and 30–50 µg from liver).

Troubleshooting Guide

Binding column is clogged	**Cause** — Sample size is too large. **Solution** — For future preparations, use fewer cells or smaller tissue samples. To salvage the current preparation, spin longer than 15 seconds in step 1d until the lysate/ethanol mixture passes through the binding column. Yield of RNA will likely be reduced.
	Cause — Cells or tissue are insufficiently disrupted. **Solution** — In step 2a, vortex or pipette lysate until no cell clumps remain. In steps 2b or 2c, homogenize tissues in the Lysis Solution/2-ME Mixture until no visible particles remain.
	Cause — Spin through Filtration Column was omitted. **Solution** — If using ; 10^6 cells or any amount of tissue, the lysed cells or homogenized tissue must be filtered through a Filtration Column to remove debris and shear DNA (steps 3a, 3b, and 4c).
Low yield or RNA degraded	**Cause** — Starting cells or tissue contain small amounts of total RNA. **Solution** — Yields will vary greatly between different types of cells and tissues (see Expected Yield under Results).
	Cause — Tissue or culture is too old. **Solution** — Use cultures before they reach maximum density or become fully confluent, and harvest tissues as rapidly as possible.
	Cause — Cells or tissue are RNase-rich. **Solution** — Cells such as monocytes and macrophages, and tissues such as pancreas, spleen, and thymus are rich in RNases and require immediate and thorough disruption in the Lysis Solution/2-ME Mixture to prevent degradation of RNA.

Low yield or RNA degraded	**Cause** — Cells or tissues are insufficiently disrupted. **Solution** — Vortex or pipette lysate until no cell clumps remain. Homogenize tissues in the Lysis Solution/2-ME Mixture until no visible particles remain.
	Cause — Improper storage. **Solution** — To prevent RNase activity, tissues must be flash-frozen in liquid nitrogen, stored at –70 °C, and not allowed to thaw until they are disrupted in the Lysis Solution/2-ME Mixture. Alternatively, tissues may be stored in RNAlater, RNA Stabilization Solution for Tissue (**R0901**).
	Cause — Omitted column wash with Wash Solution 1 after On-Column DNase I Digestion. **Solution** — For future preparations, be sure to wash the column with 0.25 mL of Wash Solution 1 after DNase I digestion (Appendix I, step 7 on page 12). Use of Wash 1 before Wash 2 is essential for full recovery of RNA.
Unacceptable level of DNA contamination in purified RNA	**Cause** — Optional DNase I treatment was omitted. **Solution** — Treat samples with the optional on-column DNase I digestion using the On-Column DNase I Digestion Set (**DNASE10** and **DNASE70**), see Appendix I (page 12). Alternatively, after completing the GenElute Total RNA Purification procedure, treat the eluted RNA with a post-purification DNase I digestion using Amplification Grade DNase I (**AMPD1**).
	Cause — Binding column was overloaded. **Solution** — For future preparations, use fewer cells or smaller tissue samples.
Downstream applications are inhibited	**Cause** — Residual ethanol in eluate. **Solution** — Residual ethanol from Wash Solution 2 can inhibit enzymes such as reverse transcriptase used for RT-PCR. After the final wash of the binding column (Section D, step 4) do not allow the Wash Solution to contact the column. Re-spin the tubes if necessary.
	Cause — Residual salt in eluate. **Solution** — Residual guanidine thiocyanate will also inhibit enzymes. Transfer the binding column to a clean receiving tube before adding Wash Solution 2 (Section D, step 3). Wash twice with 500 µL of Wash Solution 2.

Appendix 1: On-Column DNase I Digestion

The vast majority of DNA is eliminated from RNA preparations by the purification procedures described in Section D in this technical bulletin. However, for very sensitive applications, such as quantitative RT-PCR, even minor DNA contamination can give false positive detection. Significantly lower levels of DNA contamination can be achieved by digesting with DNase I using the On-Column DNase I Digestion Set (**DNASE10** and **DNASE70**). For the most stringent removal of DNA, post-purification treatment with Amplification Grade DNase I (**AMPD1**) is recommended.

1. Prepare starting material as described in Sections A, B, or C.
2. Load lysate/ethanol mixture into a GenElute Binding Column as described in Section D, step 1.
3. Pipette 250 µL of Wash Solution 1 into the column and centrifuge at maximum speed for 15 seconds.
4. Mix 10 µL of DNase I (**D2816**) with 70 µL of DNase Digest Buffer (**D1566**) for each preparation. Mix by inversion. Do not vortex the DNase I or the DNase I/Digest Buffer mixture. DNase I is sensitive to physical denaturation.
   Note: The DNase I/Digest Buffer mixture may be prepared up to 2 hours in advance.
5. Add 80 µL of the DNase I/Digest Buffer mixture directly onto the filter in the Binding Column.
6. Incubate at room temperature for 15 minutes.
7. Pipette 250 µL of Wash Solution 1 into the column and centrifuge at maximum speed for 15 seconds. This is a critical step. Omission may result in 20–80% loss of RNA.
8. Transfer the binding column into a fresh 2.0 mL collection tube, and continue with Section D, step 3.

Appendix 2: RNA Clean-up Procedure

The GenElute Mammalian Total RNA Miniprep Kit is designed for isolating RNA from cells or tissue. However, the kit can also be used to clean up RNA previously isolated by different methods or remove proteins and unincorporated nucleotides from RNA after enzymatic reactions, such as labeling and DNase digestion. Up to 150 µg of RNA can be purified per binding column.

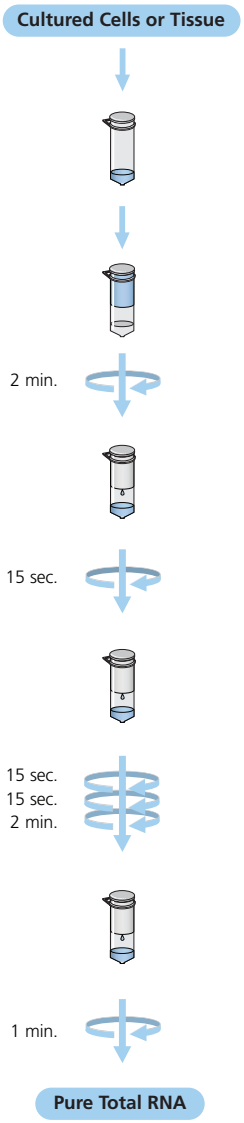
1. Adjust reaction volume containing RNA to 100 µL with Elution Solution. Add 350 µL of the Lysis Solution/2-ME Mixture and mix thoroughly.
2. Add 250 µL of 200 proof ethanol to the mixture from step 1 and mix thoroughly. Assemble a binding column (**CP9471**) with a 2 mL collection tube (**T5449**). Pipette the mixture onto the binding column. Centrifuge at maximum speed for 15 seconds.
3. Transfer the binding column to a new 2 mL collection tube. Wash with diluted Wash Solution 2 and elute RNA as in Section D, steps 3–5.

References

1. Ausubel, F. M.; et al. *Current Protocols in Molecular Biology*; John Wiley & Sons: NY, 1995; sections 4.1–4.10.
2. Farrell, Robert E., Jr.; *RNA Methodologies*; 2nd Edition; Academic Press: NY, 1998; pp. 37–53.
3. Sambrook, J.; et al. *Molecular Cloning: A Laboratory Manual*; 2nd Edition; Cold Spring Harbor Laboratory Press: Plainview, NY, 1989; pp. 7.3–7.5.

Experienced User Protocol

All spins at ; 14,000 × *g*.



1 Release RNA from Cells or Tissues

- ❑ Add 2-mercaptoethanol to lysis solution (10 µL 2-ME/1 mL lysis solution).
- ❑ Lyse cells/homogenize tissue in 250 or 500 µL of lysis solution/2-ME mixture.
- ❑ Transfer lysate to filtration column. *Spin 2 minutes*. Discard filtration column.

2 Bind RNA to Column

- ❑ Add equal volume of 70% ethanol to filtrate (250 or 500 µL). Mix thoroughly.
- ❑ Transfer up to 700 µL lysate/ethanol mixture to binding column. *Spin 15 seconds.*
- ❑ Discard flow-through & repeat if necessary.

3 Wash to Remove Contaminants

- ❑ Add 500 µL Wash Solution 1 to column. *Spin 15 seconds.*
- ❑ Transfer column to new collection tube.
- ❑ Add 500 µL Wash Solution 2 to column. **Note**: Ethanol must be added to Wash 2 concentrate before first use. *Spin 15 seconds*. Discard Wash Solution.
- ❑ Add second 500 µL Wash Solution 2 to column.
- ❑ *Spin 2 minutes* to remove ethanol.

4 Elute Purified RNA

- ❑ Transfer column to new collection tube.
- ❑ Add 50 µL elution solution to column. *Spin 1 minute* (repeat if > 100 µg RNA expected).

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052
Technical Service (800) 325-5832 • sigma-aldrich.com/techservice
Development/Custom Manufacturing Inquiries SAFC® (800) 244-1173
Safety-related Information sigma-aldrich.com/safetycenter

3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

SIGMA-ALDRICH®

2017 - 06567
11/2017