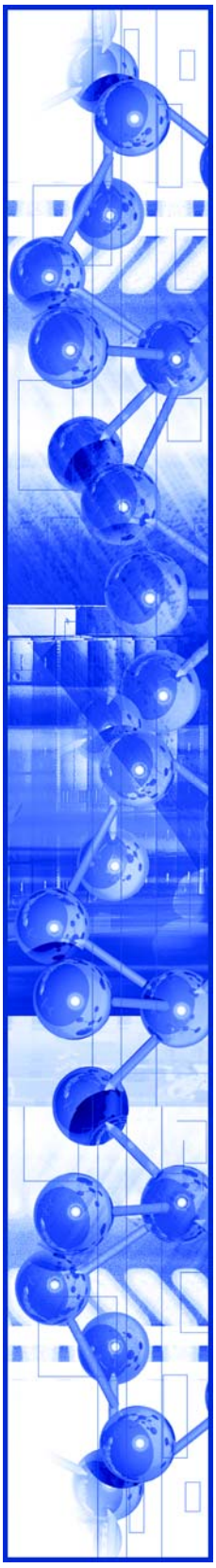


Phenol-free Total RNA Purification Kit

Code	Description	Size
N788-Kit	Phenol-free Total RNA Purification Kit *Includes*: Lysis Solution, 40 ml Wash Solution, 22ml Elution Solution, 6 ml Mini Spin Columns, 50 Collection Tubes, 50 *Sufficient material for 50 preparations*	1 Kit

General Information

AMRESCO's Total RNA Purification Kit provides a rapid method for the isolation and purification of total RNA from cultured animal cells, tissue samples, blood, bacteria, yeast, fungi, plants and viruses. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. 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.

Purification Technology

Purification is based on spin column chromatography using a proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves lysing the cells or tissue of interest with the provided Lysis Solution (please see the flow chart on page 2). Ethanol is then added to the lysate, and the solution is loaded onto the spin-column. The resin binds RNA in a manner that depends on ionic concentration of the buffer. Only the RNA will bind to the column, while the contaminating proteins will be removed in the flow-through or retained on the top of the resin. The bound RNA is then washed with the provided Wash Solution in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Solution. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Storage/Stability:

All 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.

Application Disclaimer

For Research Use Only.
Not for Therapeutic or Diagnostic Use.

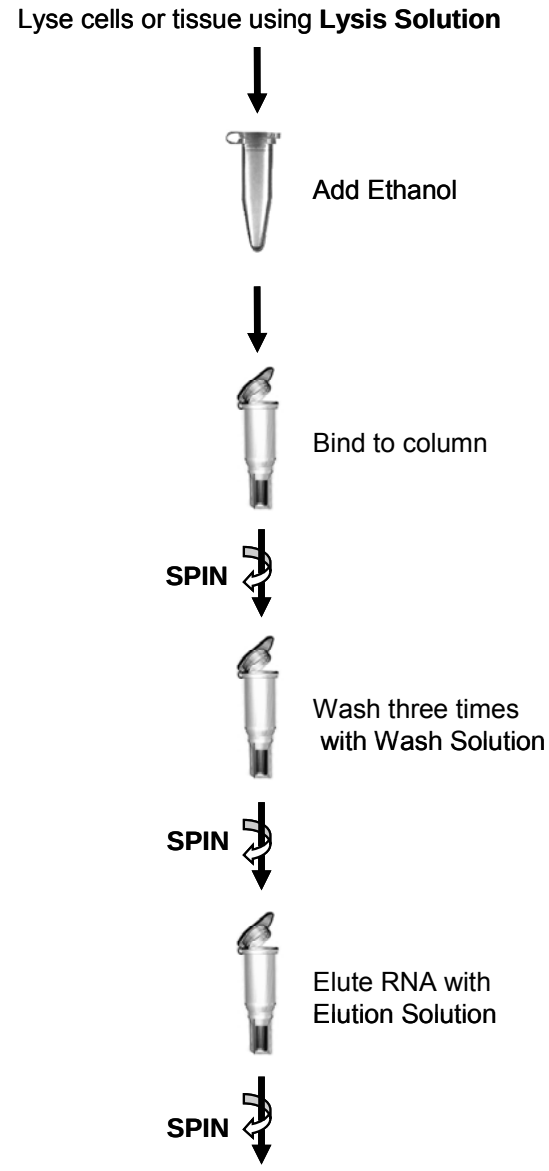
Kit Specifications	
Maximum Column Binding Capacity	50 µg
Maximum Column Loading Volume	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Animal Cells Animal Tissues Blood Bacteria Yeast Fungi Plant Tissues	 3×10^6 cells 10 mg (for most tissues) 100 µL 1×10^9 cells 1×10^8 cells 50 mg 50 mg
Time to Complete 10 Purifications	20 minutes
Average Yields HeLa Cells (1×10^6 cells) *E. coli* (1×10^9cells)	 15 µg 50 µg

Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to destroy enzymatic activity. 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. Gloves should be changed frequently to avoid contamination*
- *Solutions, tips, tubes, lab coats, pipettes, etc. should be reserved for RNA procedures only*
- *All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water such as AMRESCO's Sterile, Nuclease Free Water (E476)*
- *Clean all surfaces with commercially available RNase decontamination solutions such as AMRESCO's NucleasEliminator (Code: E891)*
- *When working with purified RNA samples, ensure that they remain on ice during downstream applications*

Lysis Procedure



Procedure:

Customer Supplied Equipment:
For All Protocols
- Benchtop microcentrifuge
- Elution tubes (RNase-free)

Customer Supplied Reagents:
General
- 95 - 100% ethanol AMRESCO Code: E193
- β-mercaptoethanol (optional) AMRESCO Code: 0482

For Animal Cell Protocol
- PBS (RNase-free) AMRESCO Code: K812

For Animal Tissue Protocol
- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

For Nasal or Throat Swabs
- Sterile, single-use cotton swabs

For Bacterial Protocol
- Lysozyme-containing TE Buffer:
  - For Gram-negative bacteria, 1 mg/mL lysozyme in TE Buffer
  - For Gram-positive bacteria, 3 mg/mL lysozyme in TE Buffer

For Yeast Protocol
- Resuspension Buffer with Lyticase:
  - 50 mM Tris pH 7.5
  - 10 mM EDTA
  - 1 M Sorbital
  - 1 unit/μL Lyticase

For Fungi Protocol
- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

For Plant Protocol
- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

Precautions:

➥ ***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.***

1. Lysate Preparation from Various Cell Types

➥ *The steps for preparing the lysate are different depending on the starting material (Step 1). However, the subsequent steps are the same in all cases (Steps 2 – 5).*

➥ *It is important to work quickly during this procedure.*

Notes:
- *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.*

a. Prepare Wash Solution: Add 50 mL of 95% ethanol to the supplied bottle containing the concentrated **Wash Solution** to yield a final volume of 72 mL.

Optional: The use of β-mercaptoethanol in lysis is highly recommended for most animal tissues, particularly those known to have a high RNAse content (ex: pancreas), as well as for most plant tissues and nasal and throat swabs. It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 μL of β-mercaptoethanol 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.

Find the lysis procedure optimized for your starting material in the table below.

Optimized lysate procedures

Starting Material	Section
Cell Culture	
Monolayers (Adherent Cells)	**1A (i)**
Suspension & Lifted Cells	**1A (ii)**
Animal Tissue	**1B**
Blood	**1C**
Nasal or Throat Swabs	**1D**
Bacteria	**1E**
Yeast	**1F**
Fungi	**1G**
Plant	**1H**
Viruses	
Integrated Viral RNA from cell cultures	**1A**
Integrated Viral RNA from tissue	**1B**
Integrated Viral RNA from blood	**1C**
Integrated Viral RNA from nasal or throat swabs	**1D**
Free Viral Particles	**1I**

1A. Lysate Preparation from Cultured Animal Cells

Notes:

- *The maximum recommended input of cells is 3 x 10^6. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain 10^6 cells. A hemocytometer can be used in conjunction with a microscope to count the number of 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 no longer than 2 weeks to ensure RNA integrity.*
- *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) c).***

1A. (i) Lysate Preparation from Adherent (Monolayer) Cells

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. Add 200 µL of 95 – 100% ethanol to the lysate. Mix by vortexing for 10 seconds.

Note: *For input amounts greater than 10^6 cells, it is recommended that the lysate is passed through a 25 gauge needle attached to a syringe 5-10 times at this point, to shear the genomic DNA prior to column loading.*

Proceed to Step 2.

1A. (ii) Lysate Preparation from Suspension Cultures and Lifted Cells

a. Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than 200 x g (~2,000 RPM) for 10 minutes to pellet cells.

b. Carefully decant the supernatant. A few µL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.

c. 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.

d. Add 200 µL of 95 - 100% ethanol to the lysate. Mix by vortexing for 10 seconds.

Note: *For input amounts greater than 10^6 cells, it is recommended that the lysate is passed through a 25 gauge needle attached to a syringe 5-10 times at this point, to shear the genomic DNA prior to column loading.*

Proceed to Step 2.

1B. Lysate Preparation from Animal Tissue

Notes:

- *Phenol-free Total RNA Purification Kit is designed for isolating RNA from tissue samples up to 10 mg. Since RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized, it is important that the procedure be carried out as quickly as possible.*
- *Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months. When isolating total RNA from frozen tissues ensure that the tissue does not thaw during weighing or prior to grinding with the mortar and pestle.*
- *Tissues stored in RNA stabilization reagents are compatible with this isolation procedure. Prior to isolation, carefully remove the tissue from the storage reagent using forceps, and dry excessive liquid.*
- *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.*

Recommended Maximum Input Amounts of Animal Tissues

Tissue	Maximum Input Amount
Brain	25 mg
Heart	5 mg
Kidney	10 mg
Liver	10 mg
Lung	10 mg
Spleen	10 mg

a. Excise the tissue sample from the animal.

b. Determine the amount of tissue by weighing. Please refer to the table above 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.

e. Add 600 µL of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized.

f. Pass the homogenized lysate 5-10 times through a 25 gauge needle attached to a syringe.

g. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).

h. Spin the 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.

i. Add an equal volume of 70% ethanol to the lysate volume collected (Example: 100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

Proceed to Step 2.

1C. Lysate Preparation from Blood

Notes:

- *Blood from 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.*
- *To prevent clogging of the column, apply no more than 100 µL of blood per column.*
- *We recommend the use of this kit to isolate RNA from non-coagulating fresh blood using EDTA as the anti-coagulant.*
- *It is important to work quickly during this procedure.*

a. Transfer up to 100 µL of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).

b. Add 350 µL of **Lysis Solution** to the blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.

c. Add 200 µL of 95 – 100% ethanol to the lysate. Mix by vortexing for 10 seconds.

Proceed to Step 2.

1D. Lysate Preparation from Nasal or Throat Swabs

Notes:

- *Body fluid 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 these samples.*
- *It is important to work quickly during this procedure.*

a. Add 600 µL of **Lysis Solution** to an RNase-free microcentrifuge tube (not provided).

b. Gently brush a sterile, single-use cotton swab inside the nose or mouth of the subject.

c. Using sterile techniques, cut the cotton tip where the nasal or throat cells were collected and place into the microcentrifuge tube containing the **Lysis Solution**. Close the tube. Vortex gently and incubate for 5 minutes at room temperature.

d. Using a pipette, transfer the lysate into another RNase-free microcentrifuge tube. Note the volume of the lysate.

e. Add an equal volume of 70% ethanol (provided by the user) to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

Proceed to Step 2.

1E. Lysate Preparation from Bacteria

Notes:

- *Prepare the appropriate lysozyme-containing TE Buffer as indicated in the table below. This solution should be prepared with sterile, RNAse-free TE Buffer pH 8.0, (AMRESCO Code: E112) and kept on ice until needed. These reagents are to be provided by the user. AMRESCO Lysozyme (Code: 0663)*
- *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 x 10^9 cells/mL has an OD_{600} 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 1Ec)**.*

a. Pellet bacteria by centrifuging at 14,000 *x g* (~14,000 RPM) for 1 minute.

b. Decant supernatant, and carefully remove any remaining media by aspiration.

c. Resuspend the bacteria thoroughly in 100 µL of the appropriate lysozyme-containing TE buffer (see table below) by vortexing. Incubate at room temperature for the time indicated in the table below.

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

d. Add 300 µL of Lysis Solution and vortex vigorously for at least 10 seconds.

e. Add 200 µL of 95 – 100% ethanol to the lysate. Mix by vortexing for 10 seconds.

Proceed to Step 2.

1F. Lysate Preparation from Yeast

Notes:

- *It is recommended that no more than 10^8 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 1Fc)****.*

a. Prepare sufficient Lyticase-containing Resuspension Buffer, (50 mM Tris, pH 7.5, 10 mM EDTA, 1M Sorbitol, 0.1% β-mercaptoethanol and 1 unit/μL Lyticase). 100 μL of buffer is required for each sample. 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.

b. Pellet yeast by centrifuging at 14,000 *x g* (~14,000 RPM) for 1 minute.

c. Decant supernatant, and carefully remove any remaining media by aspiration.

d. Resuspend the yeast thoroughly in 100 μL of Lyticase-containing Resuspension Buffer by vortexing. Incubate at 37°C for 10 minutes.

e. Add 300 μL of Lysis Solution and vortex vigorously for at least 10 seconds.

f. Add 200 μL of 95 – 100% ethanol to the lysate. Mix by vortexing for 10 seconds.

Proceed to Step 2.

1G. Lysate Preparation from Fungi

Notes:

- *Fresh or frozen fungi may be used for this procedure. Fungal tissues 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.*
- *No more than 50 mg of fungi should be used for this procedure to prevent clogging of the column.*

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°C, such that the RNA purification can be performed at a later time.*

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

d. Add 600 μL of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized.

e. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).

f. Spin the 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. Add an equal volume of 70% ethanol that is equivalent to the lysate volume collected (100 μL of ethanol is added to every 100 μL of lysate). Vortex to mix.

Proceed to Step 2.

1H. Lysate Preparation from Plants

Notes:

- *The maximum recommended input of plant tissue is 50 mg or 5 x 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.*
- *It is important to work quickly during this procedure.*

a. Transfer a maximum of 50 mg of plant tissue or 5 x 10^6 plant cells into a mortar that contains sufficient liquid nitrogen to cover the sample. Grind the sample into a fine powder using a pestle in liquid nitrogen.

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

c. Add 600 µL of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized.

d. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).

e. Spin the 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.

f. Add an equal volume of 70% ethanol that is equivalent to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

Proceed to Step 2.

1I. Lysate Preparation from Viruses

Notes:

Refer to the table below to find the optimal procedure for viral RNA source material.

Starting Material	Section
Integrated Viral RNA from cell cultures	**1A**
Integrated Viral RNA from tissue	**1B**
Integrated Viral RNA from blood	**1C**
Integrated Viral RNA from nasal or throat swabs	**1D**
Free Viral Particles	**1I**

- *The procedure below (1I) is optimized for free viral particles only.*
- *To prevent clogging of the column, the volume of the viral suspension should be no greater than 100 µl.*
- *It is important to work quickly during this procedure.*

a. Transfer up to 100 µL of viral suspension to an RNase-free microcentrifuge tube (not provided).

b. Add 350 µL of **Lysis Solution**. Lyse viral cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.

c. Add 200 µL of 95 – 100% ethanol to the lysate. Mix by vortexing for 10 seconds.

Proceed to Step 2.

Note: From this point forward the remaining total RNA purification steps are identical for all lysates.

2. Binding RNA to Column

a. Assemble a column with one of the provided collection tubes.

b. Apply up to 600 µL of the lysate with the ethanol (from Step 1) onto the column and centrifuge for 1 minute.

Note: *Inspect the column to insure that the entire lysate volume has passed through into the collection tube. Spin for an additional minute if lysate remains in the column.*

c. Discard the flowthrough and reassemble the spin column with its collection tube.

d. If the lysate volume exceeds 600 µl, repeat steps 2b and 2c as necessary.

Optional Step:

Phenol-freeTotal RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol.

3. Column Wash

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

Note: *Inspect the column to insure that the entire lysate volume has passed through into the collection tube. Spin for an additional minute if lysate remains in the column.*

b. Discard the flowthrough and reassemble the spin column with its collection tube.

c. Repeat steps **3a** and **3b** to wash column a second time.

d. Wash column a third time by adding another 400 µL of **Wash Solution** and centrifuging for 1 minute.

e. Discard the flowthrough and reassemble the spin column with its collection tube.

f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. RNA Elution

a. Place the column into a fresh, RNase-free 1.7 mL elution tube.

b. Add 50 µL of **Elution Solution** to the column.

c. 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 50 µL 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 4b** and **4c**).*

5. Storage of RNA

The purified RNA sample may be stored at –20°C for a few days. Samples should be placed at –70°C for long term storage. RNA degradation can be minimized by storing purified RNA in buffers such as AMRESCO's Riboreserve™ RNA Storage Solution (Code: N633).

Appendix A

Protocol for Optional On-Column DNA Removal

Phenol-free Total RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications

1. Prepare a working stock of 0.25 Kunitz unit/µL RNase-free DNase I (such as AMRESCO's DNaseI , Code: 0649) in 20 mM Tris, pH 8.3, 2 mM $MgCl_2$. A 100 µL aliquot is required for each column to be treated.

2. Perform the appropriate Total RNA Isolation Procedure for your starting material up to and including "**Binding to Column**" (Steps 1 and 2 of all protocols)

3. Apply 400 µL of **Wash Solution** to the column and centrifuge for 2 minute. Discard the flowthrough. Reassemble the spin column with its collection tube.

4. Apply 100 µL of the RNase-free DNase I solution prepared in Step 1 to the column and centrifuge at 14, 000 x g (~14 000 RPM) for 1 minute.

   Note: *Ensure that the entire DNase I solution passes through the column. If needed, spin at 14, 000 x g (~14 000 RPM) for an additional minute.*

5. After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column.

   Note: *Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, particularly small RNA species.*

6. Incubate the column assembly at 25 - 30°C for 15 minutes.

7. Without any further centrifugation, proceed directly to the second wash step in the "**Column Wash**" section (Step 3c).

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 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 Wash Solution	Ensure that 50 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.
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
	High amounts of genomic DNA present in sample	The lysate may be passed through a 25 gauge needle attached to a syringe 5-10 times in order to shear the genomic DNA prior to loading onto the column.
Clogged Column	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°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 "*Working with RNA"* 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.
	Starting material may have a high RNase content	For starting materials with high RNAase content, it is recommended that β-mercaptoethanol be added to the Lysis Solution.
	Lysozyme or lyticase used may not be RNAse-free	Ensure that the lysozyme and lyticase being used with this kit is RNase-free, in order to prevent possible problems with RNA degradation.
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with 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 Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNase I digestion on the RNA sample after elution to remove genomic DNA contamination. See Appendix A.

Related Products

Code	Product
User Supplied Reagents	
E476	Water, Sterile, Nuclease-Free
E891	NucleasEliminator™
0482	β-Mercaptoethanol
E112	TE Buffer, 1X Sterile Solution
K812	PBS, 1X Sterile Solution
0633	Lysozyme, Egg white
0649	DNase I
N633	RiboReserve Storage Solution
0663	Lysozyme
0691	Sorbitol
RNA Electrophoresis	
N726	Formaldehyde-free RNA Gel Kit
0710-500G	Agarose I™, 500 g General Use (also available as tablets, K857-100TABS)
0658-4L	TBE Buffer, 10X Liquid Concentrate
0478-2PK	TBE Buffer, 10X Ready-Pack™
0796-1.6L	TAE Buffer, 25X Liquid Concentrate
N603	RiboReady 100B RNA Ladder
N604	RiboReady 1Kb RNA Ladder

Visit the AMRESCO website for additional related products
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Corporate Headquarters
AMRESCO Inc.
30175 Solon Industrial Pkwy.
Solon, OH 44139 USA
Tel: (440) 349-1199
Toll free: 1-800-829-2805 (US & Canada)
Fax: (440) 349-2441
e-mail: info@amresco-inc.com

www.interchim.com
interchim

211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com