Phenol-Free Total RNA Purification Kit

Code	Description	Size
N788-KIT	**Phenol-Free Total RNA Purification Kit**	50 preparations
N788-KIT-SAMPLE	**Phenol-Free Total RNA Purification Kit**	5 preparations

General Information

VWR Life Science AMRESCO's Phenol-Free Total RNA Purification Kit provides a rapid, spin-column chromatography method for the isolation and purification of total RNA. RNA from cultured animal cells, tissue samples, blood, bacteria, yeast, fungi, plants and viruses is preferentially purified from other cellular components using a proprietary resin as the separation matrix and without the use of phenol or chloroform. 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.

- Purifies all sizes of RNA from rRNA down to miRNA
- Procedure is phenol- and chloroform-free
- Purified RNA is suitable for all downstream applications

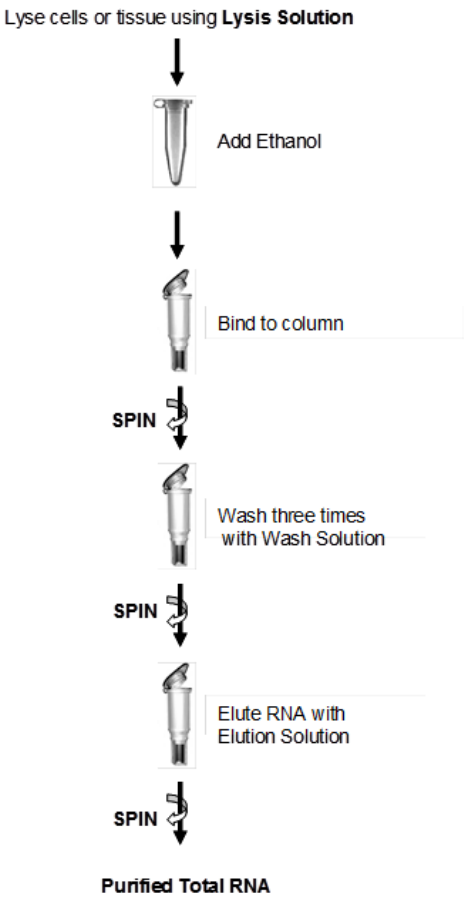
Storage/Stability

Store at room temperature (18 – 26°C). Stable for one year in unopened containers.

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Directions for 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	$3x10^6$ Cells
Animal Tissues	10 mg, unless specified
Brain Tissue	25 mg
Heart Tissue	5 mg
Blood	100 µL
Bacteria	$1x10^9$ Cells
Yeast	$1x10^8$ Cells
Fungi	50 mg
Plant Tissues	50 mg
Plasma/Serum (non-heparin treated)	200 µL
Time to Complete 10 Purifications	20 minutes
Average Yields:	
HeLa Cells ($1x10^6$ Cells)	15 µg
E. Coli ($1x10^9$ Cells)	5 µg

Purification Procedure Summary

Cells are first lysed in Lysis Solution and ethanol is added to the lysate, which is then loaded onto the spin-column. RNA binds to the resin in a manner that is dependent on the ionic concentration of the buffer, while contaminating proteins are removed in the flow-through or retained on top of the resin. The bound RNA is then washed with Wash Buffer in order to remove and remaining impurities. Purified total RNA is eluted in Elution Buffer and may be used in standard downstream applications or stored frozen for future use.

Materials Supplied

Component	N788-KIT	N788-KIT-SAMPLE
Phenol-Free RNA Lysis Solution	N785-40ML	N785-3.5ML
Phenol-Free RNA Wash Buffer	N786-22ML	N786-2ML
Phenol-Free RNA Elution Buffer	N787-6ML	N787-0.5ML
RiboZol™ Plus Spin Columns	ZZ-F1MLRNACOLUMN (50)	ZZ-F1MLRNACOLUMN (5)
RiboZol™ Plus Collection Tubes	ZZ-F1MLRNATUBE (50)	ZZ-F1MLRNATUBE (5)

Required Materials Not Supplied

Nuclease-free water
RNase decontaminating solution, such as Nucleaseliminator™ (E891)
Bench top microcentrifuge
Elution tubes (RNase-free)
95 – 100% ethanol
β-mercaptoethanol (optional)
For Animal Cell Protocol: PBS – RNase-free
For Nasal or Throat Swabs: Sterinle, single-use cotton swabs
For Fungi, Plant, or Animal Tissue Protocol: Liquid nitrogen, mortar and pestle and 70% ethanol
For Bacterial Protocol: Lysozyme-containing TE buffer
For Gram-negative bacteria – 1 mg/mL lysozyme in TE buffer
For Gram-positive bacteria – 3 mg/mL lysozyme in TE buffer
For Yeast Protocol: Resuspension buffer with lyticase: 50 mM Tris pH 7.5, 10 mM EDTA , 1 M Sorbitol, 1 unit/µL lyticase
For Plasma/Serum Protocol: MS2 RNA, 0.8 µg/µL (optional)
For On-Column DNA Removal Protocol: DNase I (optional)

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 a defense against RNases.

- The RNA work area should be located away from microbiological work stations.
- Clean, disposable gloves should be worn at all times when handling reagents, samples, tubes, pipettes, etc. Change gloves frequently to avoid contamination.
- Equipment/reagents should be designated for RNA work only.
- All solutions and water for RNA work must be nuclease-free.
- Clean all surfaces with RNase decontamination solutions.
- Keep all purified RNA samples on ice during downstream applications.

Protocol/Procedure

Notes:

- Precautions: Blood of human and animal subjects is considered potentially infectious, universal precautions should be followed when working with these types of samples. All necessary precautions as recommended by the specific region should be taken.
- It is important to work quickly during this procedure to minimize sample degradation.
- The steps for preparing the lysate (Step 1) are different depending on the starting material, all subsequent steps (Steps 2-5) are identical in all cases.
- Centrifugation is performed at room temperature.
- Ensure all solutions are at room temperature prior to use.

Preparing Wash Buffer

N788-KIT: Add 50 mL of 95 – 100% ethanol to the supplied bottle of Wash Buffer to yield a final volume of 72 mL.

N788-KIT-SAMPLE: Add 4.6 mL of 95 – 100% ethanol to the Wash Buffer to yield a final volume of 6.6 mL.

Preparing Lysis Solution

If the use of β-mercaptoethanol is not desired, use Lysis Solution as provided.

Optional: The use of β-mercaptoethanol in lysis is highly recommended for most animal tissues (particularly those known to have a high RNase content, such as pancrease), plant tissues, and

nasal or throat swabs. It is recommended for use with RNA that is intended to be used in sensitive downstream applications.

Add 10 µL of β-mercaptoethanol to each 1 mL of Lysis Solution required.

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

Starting Material	Section
Cell Culture-Monolayers (Adherent Cells), Integrated Viral RNA from cell Culture Monolayers	1A(i)
Cell Culture-Suspension, Lifted Cells, Integrated Viral RNA from Cell Culture Suspension	1A(ii)
Animal Tissue, Integrated Viral RNA from Tissues	1B
Blood, Heparin Treated Serum/Plasma, Integrated Virlal RNA from Blood	1C
Nasal or Throat Swabs, Integrated Viral RNA from Swabs	1D
Bacteria	1E
Yeast	1F
Fungi	1G
Plant	1H
Free Viral Particals	1I
Serum or Plasma (using EDTA or sodium citrate as the anti-coagulant)	1J

1A. Lysate Preparation from Cultured Animal Cells

Notes:

- The maximum recommended input of cells is $3x10^6$. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain 10^6 cells.
- Cell pellets can be stored at -70°C for later use or used directly in the procedure. Determine the number of cells present before freezing.
- Frozen pellets should be stored no longer than 2 weeks to ensure RNA integrity.
- Frozen pellets should not be thawed prior to beginning the protocol. Add the Lysis Solution directly to the frozen cell pellets (Step 1A(ii) c).

1A(i). Lysate Preparation from Adherent Cells (monolayer) or Integrated Viral RNA from 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 cell culture plate.
c. Lyse cells by gently tapping culture dish and swirlying buffer around the plate surface for five minutes.
d. Transfer lysate to a microcentrifuge tube.
e. Add 200 µL of 95 – 100% ethanol to the lysate.
f. 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 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 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 microliters of media may be left behind with the pellet in order to ensure 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 dislodged before proceeding to the next step.
d. Add 200 µL of 95 – 100% ethanol to the lysate.
e. 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 5 – 10 times at this point, to shear the genomic DNA prior to column loading.

Proceed to Step 2

1B. Lysate Preparation from Animal Tissues or Integrated Viral RNA from Tissues
Notes:

- RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. It is important to work quickly.

- Fresh or frozen tissues may be used. Tissues should be flash frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long term storage of several months. Do not thaw prior to weighing or grinding.
- Tissues stored in RNA stabilization reagents are compatible with this procedure. Prior to isolation, carefully remove the tissue and dry excessive liquid.

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.
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 to shear the genomic DNA prior to column loading.
g. Using a pipette, transfer the lysate into and RNase-free tube.
h. Spin the lysate for 2 minutes at 14,000 x *g* to pellet any cell debris. Transfer the supernatant to another RNase-free tube. Note the volume of the supernatant/lysate.
i. Add an equal volume of 70% ethanol to the lysate volume collected. Vortex to mix.

Proceed to Step 2.

1C. Lysate Preparation from Blood or Integrated Viral RNA from Blood
Notes:

- To prevent clogging of the column apply no more than 100 µL of blood per column.
- It is recommended to use non-coagulating fresh blood with EDTA as the anticoagulant.

a. Transfer up to 100 µL of non-coagulating blood to an RNase-free tube.
b. Add 350 µL of Lysis Solution to the blood. Lyse cells by vortexing for 15 seconds. Ensure that the mixture becomes transparent before proceeding to the next step.
c. Add 200 µL of 95 – 100% ethanol to the lysate.
d. Mix by vortexing for 10 seconds.

Proceed to Step 2

1D. Lysate Preparation from Nasal or Throat Swabs or Integrated Viral RNA from Swabs

a. Add 600 µLof Lysis solution to an RNase-free tube.

b. Gently brush a sterile, single use cotton swab inside the nose or mouth of the subject.
c. Using sterile techniques, cut the cotton swab 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 five minutes at room temperature.
d. Using a pipette, transfer the lysate into another RNase-free tube. Note the volume.
e. Add an equal volume of 70% ethanol to the 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 and kept on ice until needed.
- Bacterial pellets can be stored at -70°C for later use, or used directly in this procedure.
- Frozen pellets should not be thawed prior to beginning the protocol. Add lysozyme-containing TE buffer directly to the frozen pellet (Step 1Ec).

a. Pellet bacteria by centrifuging at 14,000 x *g* for 1 minute.
b. Decant the supernatant and carefully remove any remaining media by aspiration.
c. Re-suspend the bacteria thoroughly in 100 µL of the appropriate lysozyme-containing TE buffer (see table below) by vortexing.
d. Incubate at room temperature for the time indicated in the table below.

Bacteria Type	Lysozyme-Containing TE Buffer	Incubation TIme
Gram-Negative	1 mg/mL	5 minutes
Gram-Positive	3 mg/mL	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 they lysate.
g. 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 this protocol. Add the Lyticase-containing Resuspension Buffer directly to the frozen yeast pellet (Step 1Fc)

a. Prepare sufficient Lyticase-containing Resuspension Buffer according to the chart below. 100 µL is required for each sample. This solution should be prepared with sterile RNase-free reagents and kept on ice until ready for use.

Lyticase-containing Resuspension Buffer

Reagent	Concentration
Tris, pH 7.5	50 mM
EDTA	10 mM
Sorbitol	1 M
β-mercaptoethanol	0.1%
Lyticase	1 unit/µL

b. Pellet yeast by centrifuging at 14,000 x *g* for 1 minute.
c. Decant supernatant and carefully remove any remaining media by aspiration.
d. Re-suspend 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.
g. Mix by vortexing for 10 seconds.

Proceed to Step 2

1G. Lysate Preparation from Fungi
Notes:

- Fresh or frozen fungi may be used. Fungal tissues should be flash-frozen with 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 fungi tissue to thaw prior to beginning procedure.

- No more than 50 mg of fungi should be used in order to prevent clogging of the column.

  a. Weigh out no more than 50 mg of fungi.
  b. Transfer the fungus into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the fungus thoroughly using the pestle.
     Note: Ground fungus can be stored at -70°C and RNA purification performed at a later time.
  c. Allow the liquid nitrogen to evaporate without allowing the fungus to thaw.
  d. Add 600 µL of Lysis Solution to the fungi sample and continue to grind until the sample has been homogenized.
  e. Using a pipette, transfer the lysate into an RNase-free tube.
  f. Sping the lysate for 2 minutes at 14,000 x *g* to pellet any cell debris. Transfer the supernatant/lysate to another RNase-free tube, and note the volume.
  g. Add an equal volume of 95 – 100% ethanol that is equivalent to the lysate volume collected. Vortex to mix.

Proceed to Step 2

1H. Lysate Preparation from Plants
Notes:

- The maximum recommended input of plant tissue is 50 mg or $5x10^6$ plant cells.
- Fresh or frozen plant tissue may be used. Plant tissues should be flash-frozen with liquid nitrogen and transferred immediately to a -70°C freezer for long term storage.
- Do not allow frozen plant tissue to thaw prior to beginning procedure.

  a. Transfer a maximum of 50 mg plant tissue or $5x10^6$ plant cells into a mortar filled with an appropriate amount of liquid nitrogen to cover the sample. Grind the sample into a fine powder.
  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 tube.
  e. Spin the lysate at 14,000 x *g* for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free tube, note the volume.
  f. Add an equal volume of 95 – 100% ethanol to the lysate. Vortex to mix.

1I. Lysate Preparation from Free Viral Particles
Note: To prevent clogging no more than 100 µL of viral suspension should be used.

a. Transfer up to 100 µL of viral suspension to an RNase-free tube.
b. Add 350 µL of Lysis Solution. Lyse viral cells by vortexing for 15 seconds. Ensure that the mixture is transparent before proceeding.
c. Add 200 µL of 95 – 100% ethanol to the lysate.
d. Mix by vortexing for 10 seconds.

Proceed to Step 2

1J. Lysate Preparation from Plasma or Serum

Note:

- It is recommended to use this kit to isolate RNA from plasma or serum prepared by standard protocol from non-coagulating fresh blood using EDTA or sodium citrate as the anticoagulant. For plasma prepared from fresh blood using heparin as an anticoagulant refer to the protocol **1C Lysate Preparation from Blood**.
- It is recommended that no more than 200 µL of plasma or serum be used in order to prevent clogging of the column.
- Avoid multiple freeze-thaw cycles, aliquot the appropriate volume for usage prior to freezing.
- The yield of RNA from plasma and serum is highly variable. In general the expected yield could vary from 1 – 100 ng per 100 µL plasma or serum. In addition the expected A260:A280 ratio as well as the A260:A230 ratio will be lower (< 1.80) than the normal acceptable range from other cells or tissues. These isolated RNAs can still be used effectively in downstream applications.

a. Transfer up to 200 µL of plasma or serum to an RNase-free tube.
b. Add 300 µL of Lysis Solution to every 100 µL of plasma/serum.
c. Mix by vortexing for 10 seconds.

Optional Step: Add 0.7 µL of 0.8 µg/mL MS2 RNA per sample. MS2 RNA could increase the consistency of downstream applications such as RT-PCR. However, the use of MS2 RNA is not recommended for applications involving the global gene expression analysis such as microarrays or sequencing.

d. Add 400 µL of 95 – 100% ethanol to every 400 µL of the lysate.
e. Mix by vortexing for 10 seconds.

Proceed to Step 2

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

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 at 14,000 x *g* **(≥ 3,500 rpm for Serum/Plasma Lysates).** If any lysate remains in the column, centrifuge for an additional minute, until all lysate has passed through the column.
c. Discard the flow through tube and place the column into a collection tube.
d. If the lysate volume exceeds 600 µL, repeat steps 2b and 2c as necessary.

Optional Step: Phenol-free Total RNA Purification Kit isolates RNA with minimal amounts of genomic DNA present. At this point, complete the optional **On-Column DNA Removal Protocol**, available in Appendix A, to remove residual DNA that may affect sensitive downstream applications.

3. Column Wash

a. Apply 400 µL of Wash Buffer to the column and centrifuge for 1 minute at 14,000 x *g*. Centrifuge for an additional minute if lysate remains in the column.
b. Discard the flow through and place into a new collection tube.
c. Repeat steps 2a and 2b for a total of three washes.
d. Discard the flow through tube and place the column into a collection tube.
e. Spin for 2 minutes at 14,000 x *g* in order to dry the resin. Discard the collection tube.

4. RNA Elution

a. Place the column into a new RNase-free elution tube.
b. Add 50 µL of Elution Solution to the column.
c. Centrifuge for 2 minutes at **200 x *g*** followed by **14,000 x *g*** (if the entire 50 µL has not been eluted spin the column for an additional minute at 14,000 x *g*).

Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate RNase-free 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.

Troubleshooting Guide

Problem	Cause	Solution
Poor RNA Recovery	Incomplete lysis of cells or tissues	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 Buffer supplied with the kit be used for maximum 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 – 100% ethanol is added to the supplied Wash Buffer prior to use.
	Low RNA content in cells or tissue	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.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature through out the procedure. Temperatures below 15°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 "*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 Peform 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.

Appendix A

Protocol For Optional On-Column DNA Removal

Phenol-free Total RNA Purification Kit isolates RNA with minimal amounts of genomic DNA present. 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/ul RNase-free DNase I (0649) in 20 mM Tris, pH 8.3, 2 mM $MgCl_2$. A 100 ul aliquot is required for each column to be treated.
2. Perform the appropriate total RNA Isolation Procedure depending on the starting material, up to and including Step 2 Binding to Column (Steps 1 and 2 of all protocols).
3. Apply 400 ul of Wash Solution to the column and centrifuge for 2 minutes at 14,000xg. Discard the flow through tube and place the column into a collection tube.
4. Apply 100 ul of the RNase-free DNase I solution prepared in step 1 and centrifuge at 14,000xg for 1 minute. Spin for an additional minute if solution still remains in the column.
5. Pipette the eluate that is present in the collection tube back onto the same column (this will ensure maximum DNase I activity and maximum yields of RNA, particularly for small RNA species.)
6. Incubate the column assembly at 25-30°C for 15 minutes.
7. Proceed directly to the second wash step in the Column Wash section (Step 3c).

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Directions for Use

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