

# RiboPure™-Bacteria Kit

(Part Number AM1925)

## *Protocol*

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**P/N 1925M Revision C**

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# I. Introduction

## A. Background

RiboPure™-Bacteria is a rapid RNA isolation kit which combines disruption of bacterial cell walls with Zirconia Beads, phenol extraction of the lysate, and glass-fiber filter purification of the RNA. It can be used to isolate total RNA from a variety of gram-negative and gram-positive bacteria. RiboPure-Bacteria was extensively tested with *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Campylobacter fetus*, and *Rhodobacter sphaeroides*, however, the kit will work with almost any gram-negative or gram-positive bacterial species.

The RiboPure-Bacteria method disrupts bacterial cell walls by beating cells mixed with RNAwiz and 0.1 mm Zirconia Beads on a vortex adapter (e.g. P/N AM10024) for 10 min. The lysate is then mixed with chloroform and centrifuged to form three distinct phases. The upper aqueous phase contains RNA, the semi-solid interphase contains DNA, and the lower organic phase contains mostly proteins, polysaccharides, fatty acids and other cellular debris. The RNA is then diluted with ethanol and bound to a silica filter. The RNA bound to the filter is washed to remove contaminants, and eluted in a low ionic strength solution. The kit also includes Ambion DNA-free™ reagents for the optional post-elution removal of contaminating genomic DNA, and for the subsequent removal of the DNase I and divalent cations in the buffer using Ambion's exclusive DNase Inactivation Reagent.

The entire RNA isolation procedure requires approximately 1 hr, starting with fresh, snap-frozen, or RNAlater® treated cells. The optional DNA removal step requires ~30 min of additional time. The resulting RNA is of superb quality, free of DNA and proteins, and suitable for use in Northern blotting, RT-PCR, mRNA enrichment, and microarray analysis. As with all glass fiber filter purification methods, 5S ribosomal RNAs and tRNAs are not quantitatively recovered using the RiboPure-Bacteria Kit.

## B. Reagents Provided with the Kit and Storage

The RiboPure-Bacteria Kit contains reagents for 50 RNA isolations. Note that the kit is shipped at room temperature, which will not affect its stability.

Amount	Component	Storage
30 g	Zirconia Beads	-20°C
200 µL	DNase I	-20°C
1 mL	10X DNase I Buffer	-20°C
2 mL	DNase Inactivation Reagent	-20°C
25 mL	RNawiz*	4°C
35 mL	Wash Solution 1†	4°C
50 mL	Wash Solution 2/3 Concentrate Add 40 mL 100% ethanol before use	4°C
5 mL	Elution Solution	any temp‡
50	0.5 mL Screw Cap Tubes	room temp
50	Filter Cartridges	room temp
100	Collection Tubes (2 mL)	room temp
100	1.5 mL Tubes (RNase-free)	room temp

\* This reagent contains phenol, which is a poison and an irritant. Contact with RNawiz may cause burns. Use gloves and other personal protection equipment when working with this reagent.

† This reagent contains guanidinium thiocyanate, a potentially hazardous substance; use it with appropriate caution.

If a precipitate develops in Wash Solution 1 during storage, warm it to 37°C before use to dissolve the precipitate.

‡ Store Elution Solution at -20°C, 4°C, or room temp.

## C. Required Materials Not Provided with the Kit

### Reagents

- 100% ethanol: ACS grade or better
  - for preparation of Wash Solution 2/3
  - to bind the RNA to the glass fiber filter
- Chloroform (*without* isoamyl alcohol): ACS grade or better

### Cell disruption

Vortex adapter (e.g. Ambion P/N AM10024) to hold microfuge tubes for cell disruption, and a vortex mixer (on which the vortex adapter fits).

### Microcentrifuge

Some centrifugations in the procedure must be done at 4°C (section [II.C](#) steps [6](#) and [8](#)).

### (optional)

To use vacuum pressure to pull solutions through the Filter Cartridges the following supplies and equipment are needed:

- Vacuum manifold attached to a vacuum pump
- 50 sterile 5 mL syringe barrels to support the Filter Cartridges

## D. Related Products Available from Applied Biosystems

<p>Vortex Adapter P/N AM10024</p>	<p>The Vortex Adapter attaches to Vortex-Genie<sup>®</sup> 2 (Scientific Industries) vortex mixers to hold microcentrifuge tube for hands-free agitation.</p>
<p>Electrophoresis Reagents See web or print catalog for P/Ns</p>	<p>Ambion offers gel loading solutions, agaroses, acrylamide solutions, powdered gel buffer mixes, nuclease-free water, and RNA and DNA molecular weight markers for electrophoresis. Please see our catalog or our website (<a href="http://www.ambion.com">www.ambion.com</a>) for a complete listing as this product line is always growing.</p>
<p>MICROBEnrich™ Kit P/N AM1901</p>	<p>The MICROBEnrich Kit employs a novel technology to remove over 90% of mammalian RNA from complex mixtures of host-bacterial RNA samples. If desired the enriched bacterial RNA obtained can be enriched for bacterial mRNA using the MICROBExpress Kit</p>
<p>MICROBExpress™ Kit P/N AM1905</p>	<p>The MICROBExpress Kit employs a novel technology to remove &gt;95% of the 16S and 23S rRNA from total RNA of a broad spectrum of gram-positive and gram-negative bacteria.</p>
<p>RNAlater® Solution P/N AM7020, AM7021</p>	<p>RNAlater Tissue Collection: RNA Stabilization Solution is an aqueous sample collection solution that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples. RNAlater Solution eliminates the need to immediately process samples or to freeze samples in liquid nitrogen. Samples can be submerged in RNAlater Solution for storage at RT, 4°C, or -20°C without jeopardizing the quality or quantity of RNA that can be obtained.</p>
<p>RNaseZap® Solution P/N AM9780, AM9782, AM9784</p>	<p>RNaseZap RNase Decontamination Solution is simply sprayed, poured, or wiped onto surfaces to instantly inactivate RNases. Rinsing twice with distilled water will eliminate all traces of RNase and RNaseZap Solution.</p>

## **II. RiboPure-Bacteria Procedure**

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### **A. Buffer and Equipment Preparation**

#### **Before Using the Kit for the First Time**

#### **Prepare Wash Solution 2/3**

- a. Add 40 mL ACS grade 100% ethanol to the bottle labeled Wash 2/3 Concentrate. Mix well.
- b. Place a check mark in the empty box on the label to indicate that the ethanol has been added.

#### **Open the container of Zirconia Beads**

The Zirconia Beads are supplied in a squeeze bottle. To open the container, remove the small red cap and cut the dispensing tip ~3–4 mm from the end with an RNase-free razor blade or scissors. Replace the red cap over the cut end and return the Zirconia Beads to a –20°C freezer. Storing the beads at –20°C keeps them cold in preparation for use in RNA isolation, and also keeps the beads dry.

#### **Equipment Preparation**

#### **Lab bench and pipettors**

Before working with RNA, it is always a good idea to clean the lab bench and pipettors with an RNase decontamination solution (e.g. Ambion RNaseZap Solution).

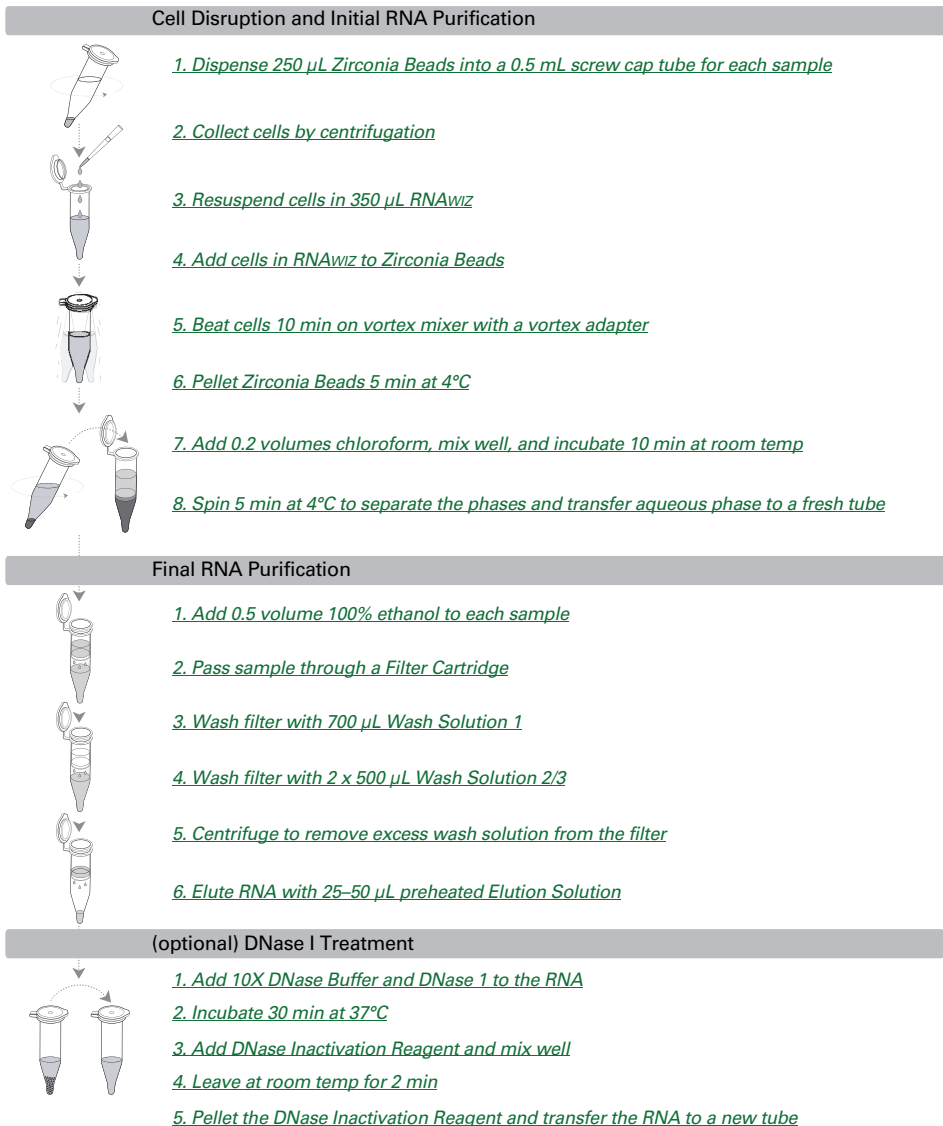
#### **Gloves and RNase-free technique**

- Wear laboratory gloves at all times during this procedure and change them frequently. They will protect you from the reagents, and they will protect the RNA from nucleases that are present on skin.
- Use RNase-free tips to handle the wash solutions and the Elution Solution, and avoid putting used tips into the kit reagents.

#### **Microfuge tubes**

Use the tubes supplied with the kit; they have been tested for RNase contamination and are certified RNase-free.

Figure 1. Procedure Overview



## B. Amount of Starting Material

The RiboPure-Bacteria procedure is designed for small scale RNA isolation from bacterial cells. The chart below lists the maximum recommended number of cells of various species to use in the procedure. Typically these are the number of cells present in 1–10 mL of logarithmic phase cultures in a rich medium such as LB or BHI.

For organisms not on the list start with  $0.5\text{--}1 \times 10^9$  cells.

	Maximum amount starting material	Expected RNA yield
<i>Escherichia coli</i>	$1 \times 10^9$ cells	90 µg
<i>Bacillus subtilis</i>	$5 \times 10^8$ cells	60 µg
<i>Pseudomonas aeruginosa</i>	$1 \times 10^{10}$ cells	90 µg
<i>Staphylococcus aureus</i>	$1 \times 10^9$ cells	40 µg

## C. Cell Disruption and Initial RNA Purification



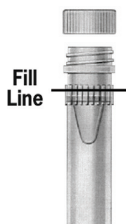
### NOTE

All centrifugation steps should be done at an RCF of  $\sim 13,000\text{--}16,000 \times g$ . This is typically near the maximum speed setting on a microfuge.

### 1. Dispense 250 µL Zirconia Beads into a 0.5 mL screw cap tube for each sample

For each sample, pour  $\sim 250$  µL of ice-cold Zirconia Beads into a 0.5 mL screw cap tube (supplied with the kit) using the picture below to estimate the volume of beads.

Figure 2. Estimating Volume of Zirconia Beads.



### 2. Collect cells by centrifugation

- Collect the cells from a bacterial culture or from an *RNAlater* suspension by centrifugation for 30–60 sec.
- Thoroughly remove the supernatant from the pellet, and discard the supernatant.

### 3. Resuspend cells in 350 µL RNAwiz

Add 350 µL RNAwiz to the cell pellet and resuspend by vortexing vigorously for 10–15 sec.

- 4. Add cells in RNeasy to Zirconia Beads** Transfer the cells in RNeasy from step [C.3](#) on page 6 to a tube containing 250  $\mu$ L Zirconia Beads. Securely fasten the lid.
- 5. Beat cells 10 min on vortex mixer with a vortex adapter** Position the sample tubes horizontally on the vortex adapter with the tube caps towards the center. Turn the vortex mixer on at maximum speed, and beat 10 min.
- The bacterial cells should be lysed after this treatment.
- 6. Pellet Zirconia Beads 5 min at 4°C**
- Centrifuge for 5 min at 4°C.
  - Transfer the bacterial lysate to a fresh 1.5 mL Tube (supplied with the kit) and discard the Zirconia Beads. Estimate the lysate volume while transferring the lysate; typically 200–250  $\mu$ L of lysate is recovered at this step.
- 7. Add 0.2 volumes chloroform, mix well, and incubate 10 min at room temp** Add 0.2 volumes chloroform to the lysate from step [6.b](#) above. Shake vigorously for 30 sec, then incubate 10 min at room temp.
- Adding the chloroform and incubating at room temp will allow the aqueous and organic phases to be separated by centrifugation.
- 8. Spin 5 min at 4°C to separate the phases and transfer aqueous phase to a fresh tube**
- Centrifuge for 5 min at 4°C.
  - Transfer the aqueous phase (top), containing the partially purified RNA, to a fresh 1.5 mL Tube. Estimate the lysate volume while transferring the lysate; typically 200–250  $\mu$ L of aqueous phase is recovered at this step.

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## D. Final RNA Purification



### NOTE

Perform all centrifugation steps at  $\sim 13,000$ – $16,000 \times g$ . Alternatively, for steps [2–4](#) below, the solutions can be drawn through the Filter Cartridges with vacuum pressure if desired. Simply place the Filter Cartridges into sterile 5 mL syringe barrels mounted on a vacuum manifold.

- Inspect Wash Solution 1 to see if a precipitate formed after storage at 4°C. If so, warm the solution to 37°C to dissolve the precipitate.
- Place 50–100  $\mu$ L Elution Solution per sample into an RNase-free tube and preheat it in a heat block set to 95–100°C.

- 1. Add 0.5 volume 100% ethanol to each sample** Add 0.5 volumes of 100% ethanol to the aqueous phase recovered in step [C.8.b](#) on page 7 and mix thoroughly.

**2. Pass sample through a Filter Cartridge**

- a. Place a Filter Cartridge into a 2 mL Collection Tube for each sample.

**IMPORTANT**

*Briefly inspect the Filter Cartridges before use. Occasionally, the glass fiber filters may become dislodged during shipping. If this is the case, gently push the filter down to the bottom of the cartridge using the wide end of a RNase-free pipette tip.*

- b. Transfer the sample to the Filter Cartridge, close the lid, and centrifuge for ~1 min or until all the liquid is through the filter.
- c. Discard the flow-through and return the Filter Cartridge to the same Collection Tube.

**NOTE**

*The RNA is now bound to the filter in the Filter Cartridge.*

**3. Wash filter with 700  $\mu$ L Wash Solution 1**

- a. Wash the filter by adding 700  $\mu$ L Wash Solution 1 to the Filter Cartridge, and centrifuge for ~1 min or until all of the liquid is through the filter.
- b. Discard the flow-through and return the Filter Cartridge to the same Collection Tube.

**4. Wash filter with 2 x 500  $\mu$ L Wash Solution 2/3**

- a. Wash the filter by adding 500  $\mu$ L Wash Solution 2/3 to the Filter Cartridge, and centrifuge for ~1 min or until all of the liquid is through the filter.
- b. Discard the flow-through and return the Filter Cartridge to the same Collection Tube.
- c. Repeat with a second 500  $\mu$ L aliquot of Wash Solution 2/3.

**5. Centrifuge to remove excess wash solution from the filter**

- a. Spin the Filter Cartridge for 1 min to remove excess wash. (This step should be done using a centrifuge, not vacuum pressure.)
- b. Transfer the Filter Cartridge to a fresh 2 mL Collection Tube.

**6. Elute RNA with 25–50  $\mu$ L preheated Elution Solution**

- a. Elute RNA by applying 25–50  $\mu$ L Elution Solution, preheated to 95–100°C, to the center of the filter.
- b. Centrifuge for 1 min.
- c. Repeating the elution step with a second 25–50  $\mu$ L aliquot of preheated Elution Solution will maximize total RNA yields.

## E. (optional) DNase I Treatment

The DNase I treatment describe below will remove trace amounts of genomic DNA from the eluted RNA.

### 1. Add 10X DNase Buffer and DNase 1 to the RNA

Add the following to the RNA and mix gently but thoroughly:

Amount	Component
1/9th volume	10X DNase Buffer
4 $\mu$ L	DNase 1 (2 U/ $\mu$ L)

### 2. Incubate 30 min at 37°C

Incubate 30 min at 37°C so that the DNase 1 can digest the genomic DNA.

### 3. Add DNase Inactivation Reagent and mix well



#### NOTE

*The DNase Inactivation Reagent may become difficult to pipette after multiple uses. If this happens, add Elution Solution equal to ~10–20% of the bed volume of the remaining reagent and vortex thoroughly to recreate a pipettable slurry.*

a. Add a volume of DNase Inactivation Reagent equal to 20% of the volume of RNA treated to each sample.

For example if 100  $\mu$ L of RNA is treated with DNase, add 20  $\mu$ L of DNase Inactivation Reagent.

Vortex the DNase Inactivation Reagent tube vigorously to ensure it is completely resuspended. To pipette the reagent, insert the pipet tip well below the surface and observe the material in the tip to ensure that it is mostly white, without a significant amount of clear fluid. If treating multiple samples, re-vortex the reagent as needed.

b. Vortex the tube of RNA after adding the DNase Inactivation Reagent to mix well.

### 4. Leave at room temp for 2 min

Store at room temp for 2 min flicking the tube once or twice during this period to resuspend the DNase Inactivation Reagent.

### 5. Pellet the DNase Inactivation Reagent and transfer the RNA to a new tube

Centrifuge the sample for ~1 min at maximum speed to pellet the DNase Inactivation Reagent, then transfer the RNA solution to a new RNase-free tube (not supplied with the kit).

### III. Assessing RNA Yield and Quality

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#### A. UV Absorbance

The concentration and purity of RNA can be determined by diluting an aliquot of the preparation (usually a 1:100 to 1:200 dilution) in TE (10 mM Tris-HCl pH 8, 1 mM EDTA) and reading the absorbance in a spectrophotometer at 260 nm and 280 nm. Be sure to zero the spectrophotometer with the TE used for sample dilution.

##### Concentration

An  $A_{260}$  of 1 is equivalent to 40  $\mu\text{g}$  RNA/mL.

The concentration ( $\mu\text{g}/\text{mL}$ ) of RNA is therefore calculated as follows:

$$A_{260} \times \text{dilution factor} \times 40 \mu\text{g}/\text{mL}$$

Following is a typical example:

RNA is eluted in 40  $\mu\text{L}$   
6  $\mu\text{L}$  of the prep is diluted 1:50 into 294  $\mu\text{L}$  of TE  
 $A_{260}=0.42$   
RNA concentration =  $0.42 \times 50 \times 40 \mu\text{g}/\text{mL} = 840 \mu\text{g}/\text{mL}$  or 0.84  $\mu\text{g}/\mu\text{L}$

Since there are 34  $\mu\text{L}$  of the prep left after using 6  $\mu\text{L}$  to measure the concentration, the total amount of remaining RNA is:

$$34 \mu\text{L} \times 0.84 \mu\text{g}/\mu\text{L} = 28.56 \mu\text{g}$$

Be aware that any contaminating DNA in the RNA prep will lead to an overestimation of yield, since all nucleic acids absorb at 260 nm.

##### Purity

The ratio of  $A_{260}$  to  $A_{280}$  values is a measure of RNA purity, and it should fall in the range of 1.8 to 2.1. Even if an RNA prep has an  $A_{260}:A_{280}$  ratio outside this range, it may function well in common applications such as Northern blotting, RT-PCR, and RNase protection assays.

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#### B. Denaturing Agarose Gel Electrophoresis

Ambion NorthernMax® reagents for Northern blotting include everything needed for denaturing agarose gel electrophoresis. These products are optimized for ease of use, safety, and low background, and they include detailed instructions for use.

An alternative to using the NorthernMax reagents is to use the procedure described below for electrophoresis in a formaldehyde denaturing agarose gel. This procedure is modified from “Current Protocols in Molecular Biology”, Section 4.9 (Ausubel et al., eds.). It is more difficult and time-consuming than the NorthernMax method, but it gives similar results.

## 1. Prepare the gel



### CAUTION

Formaldehyde is toxic through skin contact and inhalation of vapors. Manipulations involving formaldehyde should be done in a chemical fume hood.

- a. For 100 mL of gel solution, dissolve 1 g agarose in 72 mL water and cool to 60°C.
- b. Add 10 mL 10X MOPS running buffer, and 18 mL of 37% formaldehyde (12.3 M).

10X MOPS running buffer	
Concentration	Component
400 mM	MOPS, pH 7.0
100 mM	sodium acetate
10 mM	EDTA

- c. Pour the gel and allow it to set. The wells should be large enough to accommodate at least 60  $\mu$ L. Remove the comb, and place the gel in the gel tank. Cover with a few millimeters of 1X MOPS running buffer.

## 2. Prepare the RNA samples

- a. Plan to run 1  $\mu$ g of each RNA sample on the gel. Add nuclease-free water to bring the sample volumes to 11  $\mu$ L.
- b. Add the following to each RNA sample

Amount	Component
5 $\mu$ L	10X MOPS running buffer
9 $\mu$ L	12.3 M formaldehyde
25 $\mu$ L	formamide

- c. Heat samples at 55°C for 15 min.
- d. Add 10  $\mu$ L formaldehyde loading dye

Formaldehyde loading dye	
Amount	Component
1 mM	EDTA
0.25%	bromophenol blue
0.25%	xylene cyanol
50%	glycerol
60 $\mu$ g/mL	(optional) ethidium bromide

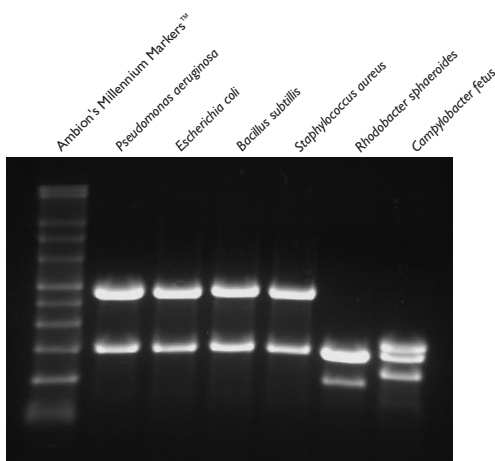
## 3. Electrophoresis

- a. Load the samples, and run the gel at 5 V/cm until the bromophenol blue (the faster-migrating dye) has migrated one-half to two-thirds of the length of the gel.

- b. Visualize the gel on a UV transilluminator. (If ethidium bromide was not added to the formaldehyde loading dye, post-stain the gel for ~20 min in 1X MOPS running buffer with 0.5 µg/mL ethidium bromide, and destain with two 10 min incubations in water.)

#### 4. Results

Figure 2 shows a typical denaturing agarose gel containing RNAs isolated from several different bacteria with the RiboPure-Bacteria Kit. The 16S and 23S ribosomal RNA (rRNA) bands should be fairly sharp, intense bands (size is dependent on the organism from which the RNA was obtained). The intensity of the upper band should be about twice that of the lower band. Smaller, more diffuse bands representing low molecular weight RNAs (tRNA and 5S ribosomal RNA) may be present, however, these RNAs are not quantitatively recovered using this kit. DNA contamination of the RNA preparation (if present) will be evident as a high molecular weight smear or as band migrating more slowly than the 23S rRNA band. Degradation of the RNA will be apparent as a smearing of ribosomal RNA bands and high background fluorescence in the lane.



**Figure 3. Formaldehyde Gel Analysis of Total RNA Isolated Using the RiboPure™-Bacteria Kit**

Total RNA (2 µg) isolated using the RiboPure-Bacteria Kit was separated on a 1% agarose gel in formaldehyde/MOPS buffer. The source organisms are indicated. Note that the larger rRNA band in each lane is nearly twice as intense as the smaller rRNA band. This is an indication that the RNA is intact (rRNA size is dependant on the organism). The slight smearing behind the rRNA bands represents mRNA. No genomic DNA contamination is visible on this gel.

## C. Agilent Bioanalyzer Analysis of Purified Total RNA

### 1. Evaluating total RNA with a bioanalyzer

The Agilent bioanalyzer with an associated LabChip® Kit provides a particularly effective method for evaluating total RNA integrity. Follow the instructions for RNA analysis provided with the RNA LabChip Kit. If necessary dilute the total RNA samples to the recommended concentration before analysis on the bioanalyzer.

### 2. Expected Results

Total RNA purified using glass fiber-based isolation methods contains <30% of the 5S rRNA and tRNAs present in total RNA purified using one step reagents and classic guanidinium/phenol procedures. The ratio of 23S:16S rRNAs areas is a measure of RNA purity, and it should fall in the range of 1.5–2.0. The ratios may differ for bacterial species that process the 23S rRNA (e.g. *R. sphaeroides*, *Campylobacter fetus*). Figure 4 shows typical Agilent 2100 Bioanalyzer electropherograms of total RNA from various bacterial species. Degradation of RNA will be evidenced by breakdown products causing the baseline to increase between the 16S and 23S rRNA peaks.

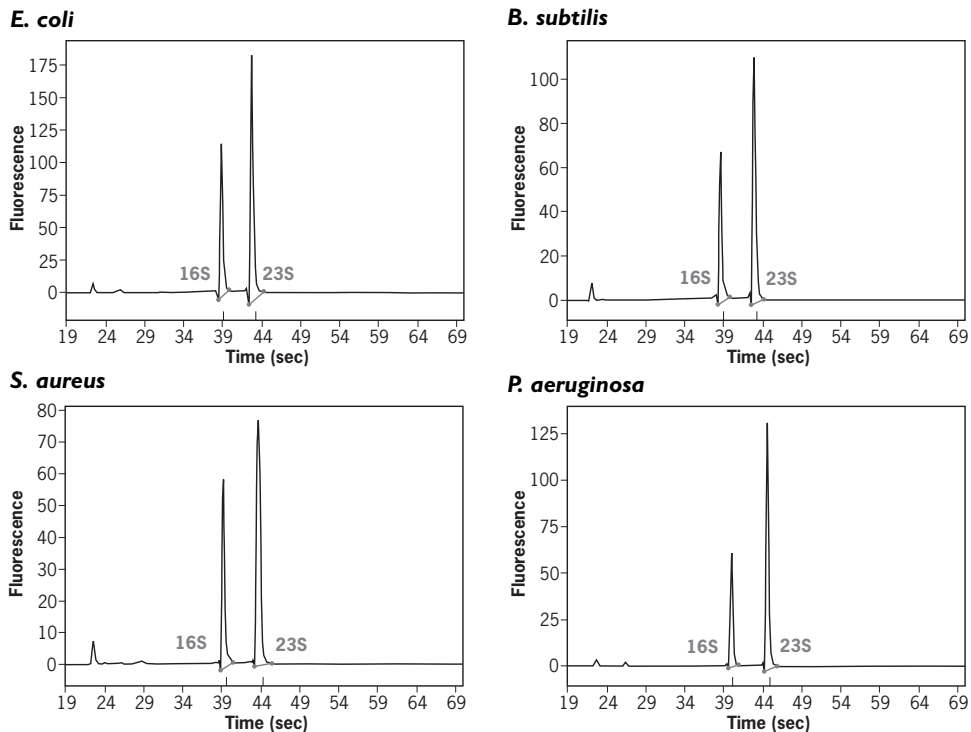


Figure 4. Agilent bioanalyzer electropherograms of total RNA from various bacterial species.

Total RNA was isolated using the RiboPure-Bacteria Kit and analyzed on an Agilent 2100 bioanalyzer using the RNA 6000 LabChip Kit. The 23S:16S rRNA ratios are as follows: *E. coli*: 1.84, *S. aureus*: 1.70, *B. subtilis*: 1.69, *P. aeruginosa*: 2.00.

## IV. Troubleshooting

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### A. RNA Looks Degraded

#### Problems during electrophoresis

##### **Ribosomal RNA (rRNA) is overloaded**

Running more than about 5 µg of RNA in a single lane may cause smearing and/or smearing of the rRNA bands. Remember, rRNA comprises about 80% of total RNA, so if 5 µg of total RNA is loaded in a gel lane, there will be about 1.3 µg and 2.6 µg of RNA in the 16S and 23S rRNA bands respectively.

##### **Samples are incompletely denatured**

To completely denature RNA, the sample should be incubated with the appropriate amount of denaturing loading dye for an adequate period of time. Reagents and protocols developed for Northern blotting should completely denature the RNA.

##### **Gel was run too fast**

Smearing may occur if gels are run at more than 5 volts/cm as measured between the electrodes. For example, if the distance between the electrode wires in the electrophoresis chamber measures 15 cm, the gel should be run at a constant 75 volts. For long runs (>3 hr) the buffer should be circulated to avoid the formation of pH gradients in the gel. This can be accomplished in various ways: manual circulation of the buffer every 15–30 min throughout the run (be sure samples have migrated into the gel first), continuous circulation of the buffer from one chamber to the other with a pump, or continuous circulation of the buffer using magnetic stir bars placed in both chambers.

#### RNA is degraded

##### **Improper handling of cells**

It is extremely important to immediately inactivate RNases released by cell disruption either by flash freezing at –80°C or colder (e.g. in liquid nitrogen or in a dry ice/ethanol bath), by resuspending cells in RNA*later*, or by disrupting cells in RNAWIZ. Some cell disruption will occur during harvesting of bacterial cultures by centrifugation; therefore, bacterial pellets should be processed to inactivate RNases immediately after harvesting.

##### **Exogenous RNase contamination**

Once the lysate is bound to the filter cartridge and the washing steps remove the RNase inactivating reagents present in the RNAWIZ, all the typical precautions against RNase contamination should be observed. Gloves should be worn at all times, and changed frequently to avoid the introduction of “finger RNases”. The bags containing the Collection and 1.5 mL Tubes, and the solution bottles should be kept closed when

they are not in use to avoid contamination with dust. Any tubes or solutions not supplied with the kit, which will contact the RNA, should be bought or prepared so that they are free from RNases.

For more information see Ambion Technical Bulletins #159 and 180 (available on our web site [www.ambion.com](http://www.ambion.com)) for more information on avoiding RNase contamination.

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## B. RNA Yield and Quality is Lower than Expected or Inconsistent

### Poor cell disruption

To efficiently lyse bacterial cells with Zirconia Beads, the cells must be vortexed for at least 10 min at maximum speed using the vortex adapter. Reducing the disruption time or speed will result in lower RNA yield due to insufficient cell lysis. This is commonly seen with gram-positive species (e.g. *Staphylococcus aureus*, *Bacillus subtilis*).

### Too many cells per RNA isolation reaction

Purification of high quality total RNA is limited by the number of cells which can be efficiently disrupted. The use of too many cells will result in lower yields as well as decreased quality. If too many cells are used the 23S:16S ratio which is an indicator of mRNA quality will decline dramatically. It is important to use the guidelines for cell number provided in section [II](#) on page 4 to ensure the highest possible yield and quality.

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## C. DNA and Ethanol Contamination

### DNA contamination

Ambion DNA-free, provided with the RiboPure-Bacteria Kit as an optional procedure, is an ideal method for removing contaminating genomic DNA. While the RiboPure-Bacteria Kit has been optimized to produce extremely pure RNA, for sensitive downstream applications like RT-PCR and microarray expression analysis, it is recommended that the DNase step be added to the RNA isolation procedure. With DNA-free, DNase is inactivated quickly and easily without jeopardizing your RNA in a heat treatment, and without organic solvent extractions and alcohol precipitations.

### Ethanol contamination

Following the washes with Wash Solution 2/3 (step [II.D.4](#) on page 8) the filter must be sufficiently dried by centrifugation at 16,000 × g for 1 min (do not use a vacuum manifold for this step). After centrifugation, remove the Filter Cartridge from the Collection Tube carefully to avoid contact with the flow-through as this will result in carryover of ethanol. If the filter is not dried, ethanol will be present in the eluted RNA and may cause inhibitory effects in subsequent enzymatic reactions.

## V. Appendix

### A. Safety Information

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The MSDS for any chemical supplied by Applied Biosystems or Ambion is available to you free 24 hours a day.

**IMPORTANT**

*For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.*

#### To obtain Material Safety Data Sheets

- Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: [www.ambion.com/techlib/msds](http://www.ambion.com/techlib/msds)
- Alternatively, e-mail your request to: [MSDS\\_Inquiry\\_CCRM@appliedbiosystems.com](mailto:MSDS_Inquiry_CCRM@appliedbiosystems.com). Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery.
- For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

#### Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

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## B. Quality Control

### Functional Testing

*Staphylococcus aureus* total RNA is prepared according to the kit instructions. The RNA is checked for integrity and minimum yield requirements.

### Nuclease testing

Relevant kit components are tested in the following nuclease assays:

#### **RNase activity**

Meets or exceeds specification when a sample is incubated with labeled RNA and analyzed by PAGE.

#### **Nonspecific endonuclease activity**

Meets or exceeds specification when a sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

#### **Exonuclease activity**

Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.