PROTOCOL

ambion® | RNA
by life technologies™

RiboPure™-Bacteria Kit

life technologies™

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RiboPure™-Bacteria Kit

(Part Number AM1925)

Protocol

I. Introduction

IMPORTANT

Before using this product, read and understand the "Safety Information" in the appendix in this document.

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 our exclusive DNase Inactivation Reagent.

The entire RNA isolation procedure requires approximately 1 hr, starting with fresh, snap-frozen, or RNA*later*® 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

* 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. 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

Vortex Adapter P/N AM10024	The Vortex Adapter attaches to Vortex-Genie® 2 (Scientific Industries) vortex mixers to hold microcentrifuge tube for hands-free agitation.
Electrophoresis Reagents See our product catalog	We offer gel loading solutions, agaroses, acrylamide solutions, powdered gel buffer mixes, nuclease-free water, and RNA and DNA molecular weight markers for electrophoresis. For available products, see our product catalog at www.invitrogen.com/ambion.
MICROB*Enrich*™ Kit P/N AM1901	The MICROB*Enrich* 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 enriched for bacterial mRNA using the MICROB*Express* Kit
MICROB*Express*™ Kit P/N AM1905	The MICROB*Express* Kit employs a novel technology to remove >95% of the 16S and 23S rRNA from total RNA of a broad spectrum of gram-positive and gram-negative bacteria.
RNA*later*® Solution P/N AM7020, AM7021	RNA*later* Tissue Collection: RNA Stabilization Solution is an aqueous sample collection solution that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples. RNA*later* Solution eliminates the need to immediately process samples or to freeze samples in liquid nitrogen. Samples can be submerged in RNA*later* Solution for storage at RT, 4°C, or –20°C without jeopardizing the quality or quantity of RNA that can be obtained.
RNase*Zap*® Solution P/N AM9780, AM9782, AM9784	RNase*Zap* 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 RNase*Zap* Solution.

II. RiboPure-Bacteria Procedure

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 RNase*Zap* Solution).

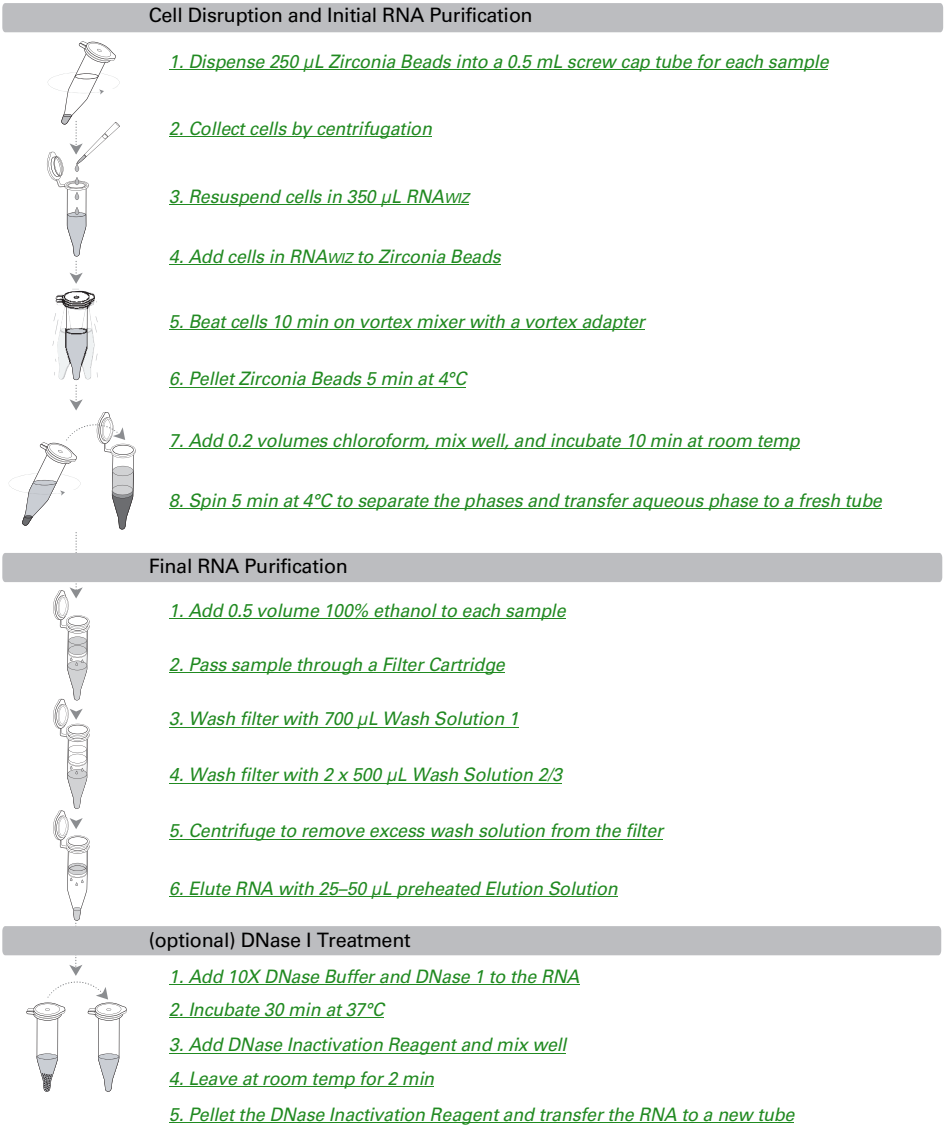
Gloves and RNase-free technique

- 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 0.5–1 x 10^9 cells.

	Maximum amount starting material	Expected RNA yield
Escherichia coli	1 x 10^9 cells	90 µg
Bacillus subtilis	5 x 10^8 cells	60 µg
Pseudomonas aeruginosa	1 x 10^{10} cells	90 µg
Staphylococcus aureus	1 x 10^9 cells	40 µg

C. Cell Disruption and Initial RNA Purification

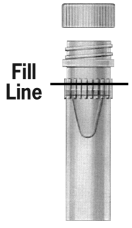
NOTE

All centrifugation steps should be done at an RCF of ~13,000–16,000 X 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 ~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

a. Collect the cells from a bacterial culture or from an RNA*later* suspension by centrifugation for 30–60 sec.

b. 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 RNAwiz to Zirconia Beads

Transfer the cells in RNAwiz from step C.3 on page 6 to a tube containing 250 µL 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

a. Centrifuge for 5 min at 4°C.

b. 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 µL 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

a. Centrifuge for 5 min at 4°C.

b. 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 µL of aqueous phase is recovered at this step.

D. Final RNA Purification

NOTE

Perform all centrifugation steps at ~13,000–16,000 X 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 µL 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 µL Wash Solution 1

a. Wash the filter by adding 700 µL 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 µL Wash Solution 2/3

a. Wash the filter by adding 500 µL 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 µL 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 µL preheated Elution Solution

a. Elute RNA by applying 25–50 µL 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 µL 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 µL	DNase 1 (2 U/µL)

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

a. Add a volume of DNase Inactivation Reagent equal to 20% of the volume of RNA treated to each sample.
For example if 100 µL of RNA is treated with DNase, add 20 µL of DNase Inactivation Reagent.

Vortex the DNase Inactivation Reagent tube vigorously to ensure it is completely resuspended. the 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.

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.

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

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 µg RNA/mL.

The concentration (µg/mL) of RNA is therefore calculated as follows:

A_{260} x dilution factor x 40 µg/mL

Following is a typical example:

RNA is eluted in 40 µL
6 µL of the prep is diluted 1:50 into 294 µL of TE
A_{260}=0.42
RNA concentration = 0.42 x 50 x 40 µg/mL = 840 µg/mL or 0.84 µg/µL

Since there are 34 µL of the prep left after using 6 µL to measure the concentration, the total amount of remaining RNA is:

34 µL x 0.84 µg/µL = 28.56 µg

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.

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 µL. 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 µg of each RNA sample on the gel. Add nuclease-free water to bring the sample volumes to 11 µL.

b. Add the following to each RNA sample

Amount	Component
5 µL	10X MOPS running buffer
9 µL	12.3 M formaldehyde
25 µL	formamide

c. Heat samples at 55°C for 15 min.

d. Add 10 µL formaldehyde loading dye

Formaldehyde loading dye	
Amount	Component
1 mM	EDTA
0.25%	bromophenol blue
0.25%	xylene cyanol
50%	glycerol
60 µg/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 3 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.

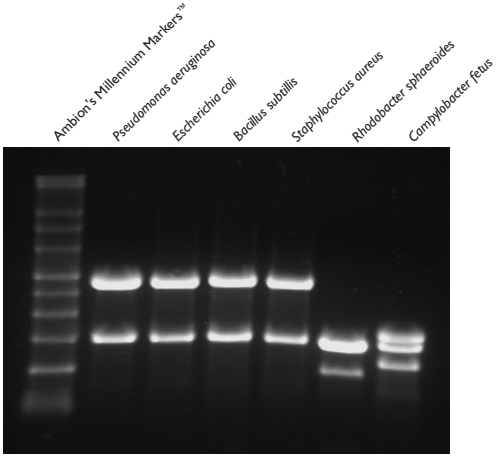


Figure 3. Formaldehyde Gel Analysis of Total RNA Isolated Using the RiboPure™-Bacteria Kit

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.

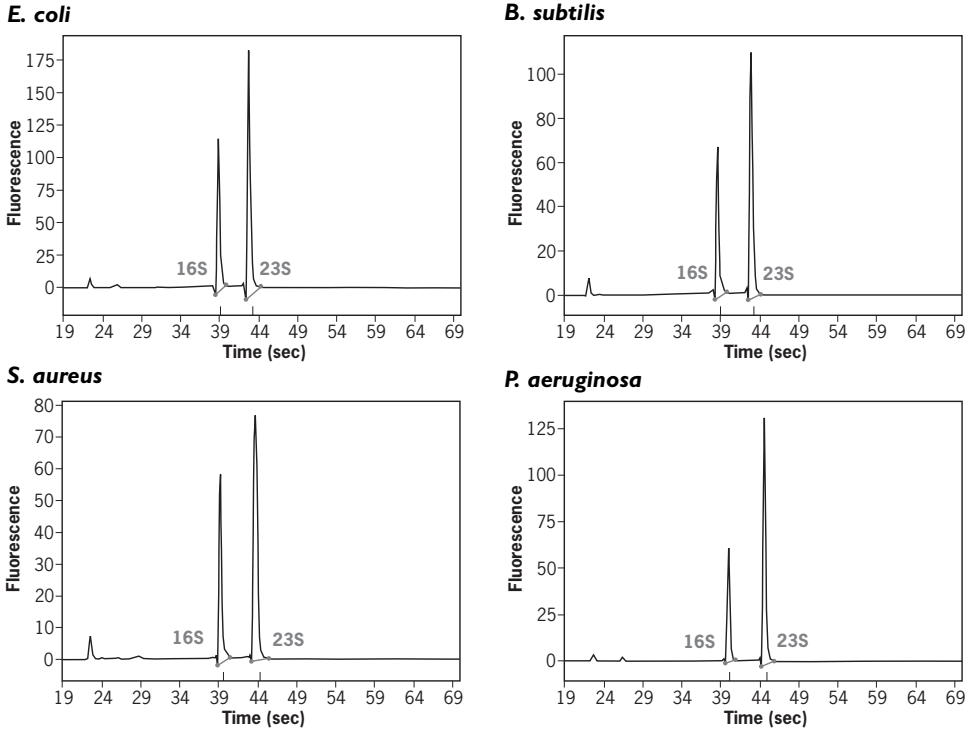


Figure 4. Agilent bioanalyzer electropherograms of total RNA from various bacterial species.

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

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 smiling 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, go to www.invitrogen.com/site/us/en/home/support/technical-support.html.

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.

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 x 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 in inhibitory effects in subsequent enzymatic reactions.

V. Appendix

A. 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.

B. Safety Information

WARNING

GENERAL SAFETY. *Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.*

- *Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.*
- *Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.*

1. Chemical safety

WARNING

GENERAL CHEMICAL HANDLING. *To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:*

- *Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.*

- *Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).*
- *Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).*
- *Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.*
- *Handle chemical wastes in a fume hood.*
- *Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)*
- *After emptying a waste container, seal it with the cap provided.*
- *Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.*
- *Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.*
- ***IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.*

2. Biological hazard safety

WARNING

***Potential Biohazard.** Depending on the samples used on the instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.*

WARNING

***BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:*

In the U.S.:

- *U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: www.cdc.gov/biosafety*
- *Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html*
- *Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.*

- *Additional information about biohazard guidelines is available at: www.cdc.gov*

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

VI. Documentation and Support

A. Obtaining SDSs

Safety Data Sheets (SDSs) are available from:

www.invitrogen.com/sds

or

www.appliedbiosystems.com/sds

Note: For the SDSs of chemicals not distributed by Life Technologies, **contact the chemical manufacturer.**

B. Obtaining support

For the latest services and support information for all locations, go to:

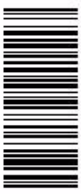
www.invitrogen.com

or

www.appliedbiosystems.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches



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