PROTOCOL

ambion® | RNA
by life technologies™

RiboPure™-Yeast Kit

life technologies™

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Part Number 1926M Rev. E
02/2011

RiboPure™-Yeast Kit

(Part Number AM1926)

Protocol

I. Introduction

IMPORTANT

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

A. Background

RiboPure™-Yeast is a rapid RNA isolation kit that combines disruption of yeast cells 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 yeast species. RiboPure-Yeast was extensively tested with *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris,* and *Candida albicans.*

The RiboPure-Yeast method disrupts yeast cell walls by beating cells mixed with an aqueous lysis buffer, SDS, phenol and 0.5 mm Zirconia Beads on a vortex adaptor (e.g. P/N AM10024) for 10 min. Up to 3×10^8 cells can be processed at a time. The lysate is then centrifuged to separate the aqueous phase, which contains the RNA, from the lower organic phase which contains proteins, polysaccharides, and other cellular debris. The aqueous lysate is diluted with Binding Buffer and ethanol, and is drawn through a glass-fiber filter which immobilizes the RNA. Contaminants are washed from the filter, and finally the RNA is eluted in a low ionic strength solution. Residual DNA is removed by treating the RNA with Ambion DNA-*free*™ reagents, included in the kit. As with all glass-fiber filter purification methods, 5S ribosomal RNAs and tRNAs are not quantitatively recovered using the RiboPure-Yeast Kit. The entire RNA isolation procedure and DNase treatment require approximately 1.5 hr, starting with fresh, snap-frozen, or RNA*later*® treated cultured yeast cells. The resulting RNA is of superb quality, free of DNA and proteins, and suitable for use in virtually any downstream application.

B. Reagents Provided with the Kit and Storage

The RiboPure-Yeast Kit contains reagents for 50 RNA isolations.

Amount	Component	Storage
50	1.5 mL Screw Cap Tubes	room temp
50	Filter Cartridges	room temp
100	Collection Tubes (2 mL)	room temp
24 mL	Lysis Buffer	4°C
2.4 mL	10% SDS*	4°C
24 mL	Phenol:Chloroform:IAA	4°C
95 mL	Binding Buffer	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	4°C
80 g	Zirconia Beads	–20°C
550 µL	10X DNase 1 Buffer	–20°C
200 µL	DNase 1	–20°C
550 µL	DNase Inactivation Reagent	–20°C

* If a precipitate develops during storage, dissolve the precipitate before use by warming the solution to 37°C.

C. Required Materials Not Provided with the Kit.

100% ethanol

Use ACS grade 100% ethanol or better. You will need 40 mL to add to the Wash Solution 2/3 Concentrate, and 1.25 mL per sample for use in step II.D.3 on page 7.

Vortex mixer and adapter

A laboratory vortex mixer fitted with a vortex adapter (e.g. P/N AM10024) is used to hold microfuge tubes in a horizontal position for cell disruption.

Tubes with lids

Sterile 4–15 mL capacity tubes with tight fitting lids are needed to collect samples in step II.C.6 on page 6, and they must be large enough to accommodate the Binding Buffer and ethanol that will be added in steps II.D. and 3 on page 7.

Microcentrifuge

A typical laboratory microcentrifuge (capable of >10,000 x g) at room temperature is needed for several steps in the procedure.

(optional) Vacuum manifold and supplies

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

Heat block or other incubator at 37°C and 95°C

- If precipitate forms in the reagent bottle(s), the 10% SDS and Wash Solution 1 will require incubation at 37°C to help redissolve the solution(s).
- The Elution Solution must be heated to 95–100°C in preparation for eluting RNA from the Filter Cartridge (step II.D.8 on page 7).
- The DNase reaction is incubated at 37°C (step II.E.2 on page 8).

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.
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.
RNase-free Tubes & Tips See our product catalog	Ambion RNase-free tubes and tips are available in most commonly used sizes and styles. They are guaranteed RNase- and DNase-free. For more information, see our product catalog at www.invitrogen.com/ambion.

II. RiboPure™-Yeast Procedure

A. Buffer and Equipment Preparation

Before using the kit for the first time

Add 40 mL 100% ethanol to the Wash Solution 2/3

Add 40 mL ACS grade 100% ethanol to the bottle of Wash 2/3 Concentrate. Mix well, and place a check mark in the empty box on the label to indicate that the ethanol was 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.

B. Amount of Starting Material and Expected RNA Yield

Use up to 3 x 10^8 yeast cells

The RiboPure-Yeast procedure is designed for small scale RNA isolation from up to 3 x 10^8 yeast cells (per Filter Cartridge).

Storing yeast cultures for RNA isolation

If a yeast culture won't be processed immediately, it can be stored by flash freezing at –80°C or colder (e.g., in liquid nitrogen or a dry ice/ethanol bath), or by resuspending cells in RNA*later* Solution.

RNA yield from flash frozen yeast cells, and cells stored in RNA*later* Solution will be slightly lower than from freshly harvested non-frozen cells.

Expected RNA yield

The chart below shows the expected RNA yield from different amounts of *S. cerevisiae* cells grown in YPD medium for 18–20 hr at 30°C in a shaking incubator (300 rpm). Other yeast species (*Schizosaccharomyces pombe, Pichia pastoris*, and *Candida albicans*) grown in those conditions gave similar RNA yields. RNA yield from cells grown to a later stage of growth was lower than the values shown in the table.

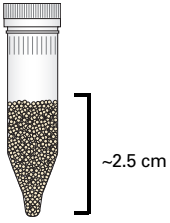
Table 1. Expected RNA yield using the RiboPure-Yeast Kit

Sample size	Expected yield
1 x 10^7 cells	5–10 µg
1 x 10^8 cells	80–100 µg
3 x 10^8 cells	250–300 µg

C. Cell Disruption and Initial RNA Purification

1. Dispense 750 µL Zirconia Beads into a 1.5 mL screw cap tube for each sample.

For each sample, pour 750 µL ice-cold Zirconia Beads into a 1.5 mL Screw Cap Tube supplied with the kit. Estimate the volume of beads by pouring them to a height of ~2.5 cm (1 inch) from the bottom of the tube—see the picture below as a guide.



2. Collect up to 3 x 10^8 cells by centrifugation

a. Collect up to 3 x 10^8 cells from a yeast culture by centrifugation for 2 min at >12,000 x g.
To collect cells from an RNA*later* suspension, centrifuge for at least 5 min, because cells in RNA*later* solution are more difficult to pellet than cells in culture media.

b. Thoroughly remove and discard the supernatant from the pellet.

3. Resuspend cells in lysis reagents

Add the following to each yeast cell pellet ***in the order shown***, and resuspend by vortexing vigorously for 10–15 seconds:

Amount	Component
480 µL	Lysis Buffer
48 µL	10% SDS
480 µL	Phenol:Chloroform:IAA

4. Add mixture to Zirconia Beads

Transfer the mixture of cells and lysis reagents to one of the prepared tubes containing 750 µL cold Zirconia Beads, and securely fasten the lid.

5. Beat cells for 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 for 10 min to lyse the yeast cells.

6. Centrifuge for 5 min at room temp, then transfer the aqueous phase to a fresh 4–15 mL tube

a. Centrifuge for 5 min at 16,100 x g at room temp to separate the aqueous phase, containing the RNA, from the organic phase.

b. Transfer the aqueous phase (top), containing the partially purified RNA, to a fresh 4–15 mL capacity tube with a tight fitting lid. (The tube should be large enough to hold ~3.7 mL.)
Typically, the recovered volume will be approximately 530 µL.

D. Final RNA Purification

1. Before you start:

Check Wash Solution 1 and the 10% SDS for precipitation, and redissolve by heating to 37°C if necessary

A precipitate may form in the Wash Solution 1 and/or in the 10% SDS during storage. Examine the bottles carefully before use, and if a precipitate is visible, redissolve it by heating the solution(s) to 37°C, and agitating the bottle occasionally.

Preheat Elution Solution to ~95°C

Place 50–100 µL Elution Solution per sample into an RNase- free tube and place it in a heat block set to 95–100°C. The preheated Elution Solution will be used in step D.8.

Inspect the Filter Cartridges

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.


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.


NOTE

For steps 4–6 below, the solutions can be drawn through the Filter Cartridges with vacuum pressure (instead of centrifugation) if desired. Simply place the Filter Cartridges into sterile 5 mL syringe barrels mounted on a vacuum manifold attached to a vacuum source, and apply the vacuum before beginning step 4; leave the vacuum on until you remove the Filter Cartridge from the syringe barrel after completing step 6.

2. Add 1.9 mL Binding Buffer to each sample

Add 1.9 mL Binding Buffer to aqueous phase recovered in step C.6 and mix thoroughly. (This corresponds to 350 µL Binding Buffer for each 100 µL of partially purified RNA.)

3. Add 1.25 mL 100% ethanol to each sample

Add 1.25 mL 100% ethanol (ACS grade or better) and mix thoroughly. (This corresponds to 235 µL ethanol for each 100 µL of partially purified RNA.)

4. Draw sample through a Filter Cartridge

a. Apply 700 µL of the sample mixture to a Filter Cartridge assembled in either a Collection Tube (supplied) or a 5 mL syringe barrel on a vacuum manifold.
The total volume of the lysate/ethanol mixture is ~3.7 mL, since the Filter Cartridge can only accommodate 700 µL, you will need to draw the lysate mixture through the Filter Cartridge in several applications of 700 µL at a time.

b. Centrifuge for ~0.5–1 min or until the lysate/ethanol mixture is through the filter.
If you are using a vacuum manifold, turn on the vacuum to draw the lysate through the filter. Leave the vacuum on to pull the washes through in subsequent steps.

c. Discard the flow-through and return the Filter Cartridge to the Collection Tube.

d. Repeat as necessary to pass the entire sample through the Filter Cartridge.


NOTE

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

5. Wash filter with 700 µL Wash Solution 1

a. Wash the filter by adding 700 µL Wash Solution 1 to the Filter Cartridge, and centrifuging for ~1 min or until all of the liquid is through the filter.
If you are using a vacuum manifold, just apply the wash solution, and the vacuum pressure will draw it through the filter.

b. Discard the flow-through and return the Filter Cartridge to the same Collection Tube.

6. 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. Draw it through the filter as in the previous step.

b. Repeat with a second 500 µL aliquot of Wash Solution 2/3.

7. Centrifuge for 1 min to remove excess wash solution from the filter

a. Centrifuge 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.

8. Elute RNA in 2 x 50 µL preheated Elution Solution

The RNA yield values shown in Table 1 on page 5 were obtained using two sequential elutions with 50 µL of Elution Solution each. We recommend using this volume of Elution Solution for yeast samples at or near

the maximum recommended size (3 x 10^8 cells). With significantly smaller samples, however, or to recover more highly concentrated RNA, as little as 2 x 25 µL of Elution Solution can be used for this step.

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. Repeat the elution step with a second 25–50 µL aliquot of preheated Elution Solution into the same Collection Tube.

E. DNase I Treatment

RNA isolated using RiboPure-Yeast will contain contaminating chromosomal DNA. To remove it, we recommend including this DNase I treatment. The DNase Treatment and Removal Reagents provided with the RiboPure-Yeast Kit include ultrapure DNase I and reaction buffer that are rigorously tested to be sure that no RNase is present. This procedure makes use of a simple process to inactivate the DNase without the risk incurred by heating the RNA, or the hassles of adding EDTA or extracting with phenol/chloroform.

1. Assemble the DNase digestion reaction

Assemble the DNase digestion reaction at room temp.

Amount	Component
50–100 µL	RNA sample (from step II.D.8 on page 7)
1/10th vol	10X DNase 1 Buffer (i.e. 5–10 µL)
4 µL	DNase I (8 U)

2. Incubate 30 min at 37°C

Incubate the DNase digestion reaction 30 min at 37°C.

3. Treat with 0.1 volume DNase Inactivation Reagent

a. Resuspend the DNase Inactivation Reagent by flicking or vortexing the tube, then add 0.1 volume DNase Inactivation Reagent to each sample.
For a typical 114 µL DNase digestion reaction, use 11 µL DNase Inactivation Reagent.

b. Mix by vortexing and allow reactions to stand 5 min at room temp.

c. Centrifuge 2–3 min at top speed in a microfuge (≥10,000 x g) at room temp to pellet the DNase Inactivation Reagent. Transfer the RNA (supernatant) to a fresh tube.

F. RNA Storage

Store RNA samples at –20°C for up to 1–2 months, or at –80°C for longer than 2 months.

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 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.

RNA 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

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.

RNA 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

The overall quality of RNA can be assessed by electrophoresis in a denaturing agarose gel system; this will also give some information about RNA yield. A denaturing gel system is suggested because most RNA forms extensive secondary structure via intramolecular base pairing, which prevents it from migrating strictly according to its size. Either formaldehyde- or glyoxal-based denaturing gel systems can be used to evaluate your RNA, We offer the NorthernMax® line of reagents for agarose gel analysis of RNA.

Be sure to include a positive control RNA on the gel so that unusual results can be attributed to a problem with the gel or a problem with the RNA under analysis. RNA molecular weight markers (such as Ambion Millennium™ Markers P/N AM7150, AM7151), an RNA sample known to be intact, or both, can be used for this purpose.

Expected appearance of denatured RNA on agarose gels

Figure 1 shows a typical denaturing agarose gel containing RNA samples isolated from several different species of yeast with the RiboPure-Yeast Kit. 18S and 28S ribosomal RNA (rRNA) from high quality *S. cerevisiae* RNA samples migrate as fairly sharp, intense bands at 1800 nt and 3000 nt. The intensity of the 28S rRNA (slower band) should be about twice that of the 18S band. Smaller, more diffuse bands representing low molecular weight RNAs (tRNA and 5S rRNA) may

also be present, however, these are not quantitatively recovered using this kit. If there is any genomic DNA contamination of the RNA preparation, it will be evident as a high molecular weight smear or as a band migrating slower than the 28S rRNA band. Degraded RNA will be apparent as a smearing of rRNA bands and high background fluorescence in the lane.

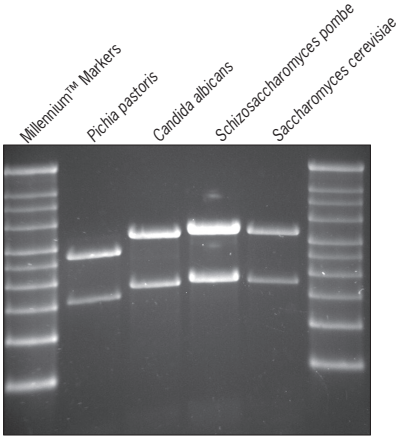


Figure 1. Yeast RNA Isolated with the RiboPure™-Yeast Kit

RNA was isolated from the indicated yeast species using the RiboPure™-Yeast Kit. Samples were run on a denaturing agarose gel stained with ethidium bromide. The tight migration of the rRNA bands, and the relative intensity of the 28S to 18S bands on this gel indicate that the RNA is high quality.

C. Agilent 2100 Bioanalyzer Analysis

The Agilent 2100 bioanalyzer with the RNA 6000 Nano LabChip® Kit provides a particularly effective method for evaluating total RNA integrity. Follow the instructions for RNA analysis provided with the RNA 6000 Nano LabChip Kit. We find that this system performs best by loading 1 µL of a 50–250 ng/µL RNA solution. If necessary, dilute RNA samples to this concentration for analysis on the bioanalyzer.

Expected bioanalyzer results

Total RNA purified using glass fiber-based isolation methods contain <30% of the 5S rRNA and tRNA present in total RNA purified using one step reagents and classic guanidinium/phenol procedures. The ratio of 28S:18S rRNA areas is a measure of RNA purity and should fall in the range of 1.5–2.0. If any degraded RNA is present in the prep, the breakdown products will cause the baseline between the 18S and 28S rRNA peaks to increase.

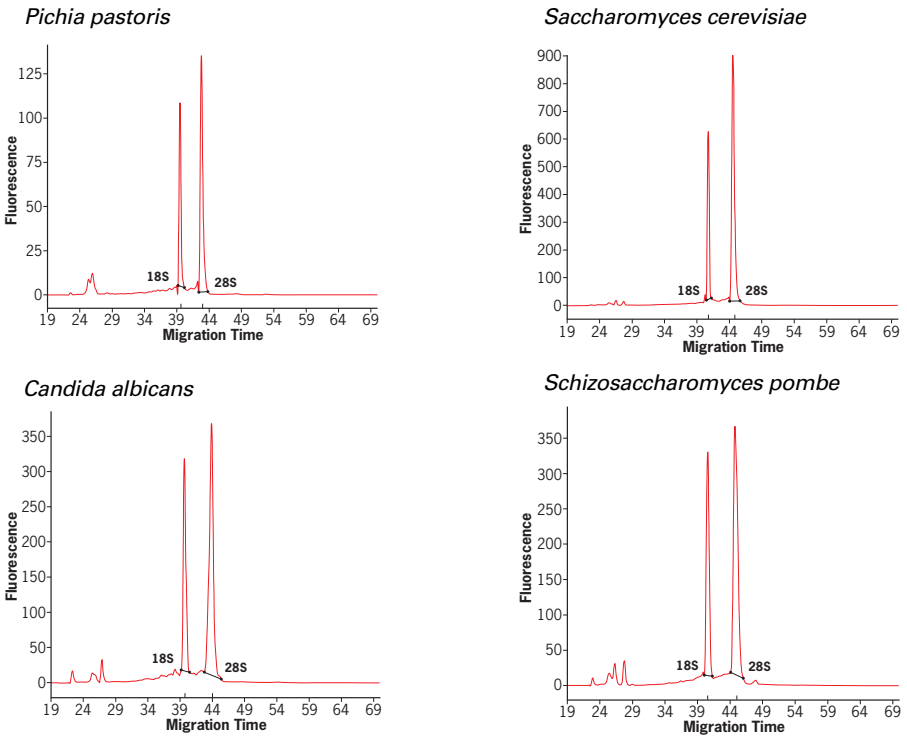


Figure 2. Electropherograms of Yeast RNA Isolated with the RiboPure™-Yeast Kit.

IV. Troubleshooting

A. RNA Looks Degraded

1. Improper handling of cells

Some cell disruption may occur during centrifugation to harvest yeast cultures; therefore, yeast pellets should be processed immediately after harvesting to inactivate RNases. RNases can be inactivated by starting the RiboPure-Yeast procedure, by treating cells with RNA*later* Solution, or by flash freezing in liquid nitrogen.

2. Exogenous RNase contamination

Once the RNA is bound to the Filter Cartridge and the washing steps are complete, the RNase inactivating reagents present in the Lysis and Binding Buffers will be gone, and 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 Tubes, and all 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.

3. Problems during agarose gel electrophoresis

a. 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. 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 18S and 28S rRNA bands, respectively.

b. 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.

c. 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.

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

1. Poor cell disruption

To efficiently lyse yeast cells with Zirconia Beads, the cells must be vortexed for at least 10 min at maximum speed using a vortex adapter. Reducing the disruption time or vortex speed will result in lower RNA yield due to insufficient cell lysis.

2. Cells were grown for too long

Yeast cultures that have been grown past the exponential growth phase yield less RNA than cells harvested during exponential growth. This is believed to be related to changes in yeast cell wall characteristics that occur with time in culture.

3. 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 maximum number of cells that should be used in this procedure is 3×10^8. Using more cells will result in lower RNA yield.

4. Freezing yeast cells before RNA isolation decreases yield

RNA yield from flash frozen yeast cells will be slightly lower than from freshly harvested, non-frozen cells. In our experiments, however, RNA ***quality*** was not affected by freezing cells prior to RNA isolation.

5. Ethanol contamination

Ethanol carryover in RNA may cause inhibitory effects in subsequent enzymatic reactions. Consider the following recommendations to avoid ethanol contamination:

- Be sure to dry the filter as indicated in step II.D.7 on page 7. Do not use a vacuum manifold for this step because it will not dry the filter as effectively as centrifugation.
- Also, after drying the filter, remove the Filter Cartridge from the Collection Tube ***carefully*** to avoid contact with the flow-through as this will result in carryover of ethanol.

V. Appendix

A. Quality Control

Functional Testing

Following the protocol, obtain ≥80 µg of Total RNA from 10^8 cells. DNA contamination should be less than 0.05%. The rRNA ratio on the Agilent Bioanalyzer should be >1.3.

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:

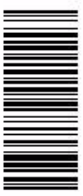
www.invitrogen.com

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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