USER GUIDE

ambion®
by life technologies™

RNAqueous®-96 Automated Kit

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RNAqueous®-96 Automated Kit

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

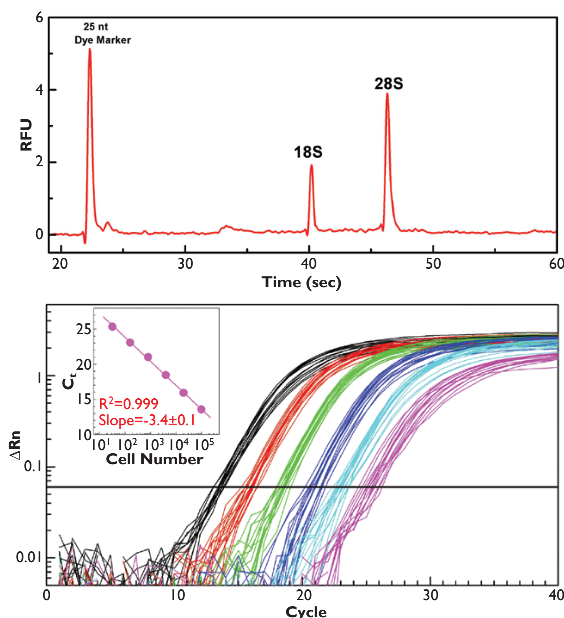
Introduction

Overview of the procedure

The RNAqueous®-96 Automated Kit is designed for rapid high throughput purification of total RNA from cells or tissue with a multichannel pipettor or an automated liquid handling system. The method is based on disrupting samples in a Lysis Solution containing guanidinium thiocyanate and other ingredients for rapid solubilization of cellular membranes and simultaneous inactivation of cellular nucleases (Chirgwin et al. 1979; Chomczynski and Sacchi 1987). After lysis, the sample is mixed with an ethanol solution, and is passed over a glass fiber filter contained in the wells of a 96-well plate. The RNA in the sample binds to the filter, and it may be treated with DNase while bound to the filter if desired. The filters are then washed to remove proteins and other contaminants, and the RNA is eluted in a small volume of Nuclease-free Water, or in Elution Solution. Elution Solution is 0.1 mM EDTA, which will chelate trace amounts of divalent cations. Small cellular RNAs such as tRNA and 5S ribosomal RNA are not quantitatively recovered with this kit.

These procedures were developed by Life Technologies' automation specialists and R&D scientists on our in-house robotic workstations. Every step in the procedures is fine-tuned to yield a user-friendly, highly effective method for high throughput RNA purification. Users of other liquid handling systems can program their equipment using the detailed instructions in this booklet.

Figure 1 Yield and Quality of Total RNA Isolated using the RNAqueous®-96 Automated Kit. RNA was extracted from the following quantities of K562 suspension cells: 32, 160, 800, 4,000, 20,000 and 100,000 cells using the RNAqueous®-96 Automated Kit. The standard procedure was used with a MultiPROBE® II HT EX liquid handling system (Perkin Elmer). Total RNA yield was ~18 pg/cell. The Agilent 2100 Bioanalyzer™ trace shows the quality of the total RNA obtained; notice the 2:1 28S/18S rRNA ratio and the lack of DNA contamination (top). Also, the amount of total RNA recovered was linearly proportional to cell input as quantitated by real-time RT-PCR (5% of the total RNA was used in a 25 µL RT-PCR) using human GAPDH primer/probe set on an ABI 7700 (bottom).



Kit components and storage conditions

Amount	Component	Storage
4	Filter Plates	room temp
4	Collection Plates with lids	room temp
4	Culture Plates	room temp
1	Heat Plate with lid	room temp
100 mL	Nuclease-free Water	any temp†
100 mL	Elution Solution	any temp†
100 mL	Lysis/Binding Solution	4°C
100 mL	Rebinding Mix (Add 35 mL 100% ethanol before use)	4°C
2 x 175 mL	Wash Solution Concentrate (Add 140 mL 100% ethanol to each bottle before use)	4°C
1.25 mL	DNase 1 (20 U/µL)	–20°C
12 mL	DNase 1 Buffer	–20°C

† Store components at –20°C, 4°C, or room temp.

Required materials not provided with the kit

- 100% ethanol, ACS grade or better
- For completely automated RNA isolation with the RNAqueous®-96 Automated Kit, the robotic liquid handler must have the following features:
  - 200 µL pipetting tool
  - Gripper tool
  - Heating device to heat eluent to 65–80°C, this can be a jacket to heat the eluent reservoir directly, or a heating tile capable of heating water in a 96-well flat bottom plate to at least 65°C, which can be reached by the gripper and pipetting tools.
  - Vacuum manifold and detachable filter collar that can be controlled by the workstation operation software.
  - Vacuum supply that can be set to 4–7" Hg (580–650 torr).
  - 5 reservoirs with reservoir holder(s): Refer to the deck layout diagram in section "Deck layout" on page 8 for required minimum reservoir capacities.

RNAqueous®-96 Automated procedure

Preparation of equipment and reagents

Gloves and RNase-free technique

Wear laboratory gloves while preparing samples and reagents, and while setting up the workstation. They will protect you from the reagents, and they will protect the RNA from nucleases that are present on skin. Use RNase-free pipettes to handle the kit reagents, and avoid putting used pipettes into the reagent containers.

Vacuum manifold set-up

Seat the Filter Plate on the vacuum manifold, and set the bleed gauge so that the vacuum pressure will be 4–7" Hg (580–650 torr).

Heating device setup

There may be as much as a 10–20°C difference between the temperature setting of the heating device and the temperature of solutions in the reservoir or in the wells of the Heat Plate. Set the heating device so that the contents will be 65–80°C before they are transferred to the Filter Plate for RNA elution in step 6. on page 14.

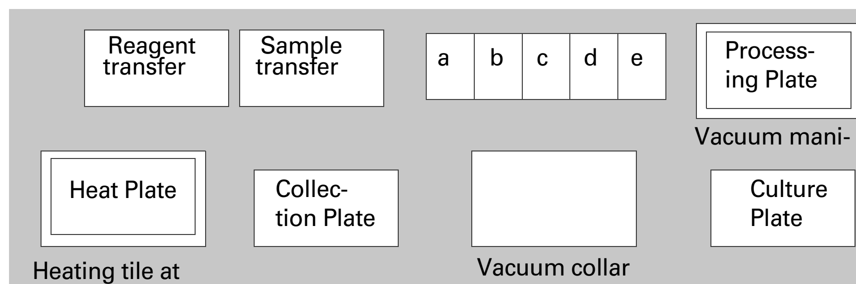
Liquid handling

The shape of the disposable pipet tips will affect the optimum dispense and aspiration speeds; use the recommendations in section "RNA isolation procedure and automation guidelines" on page 10 as a starting point for optimization.

To avoid cross contamination and to minimize tip usage, dispense all reagents 1–2 mm above the solution already in the well. If the system doesn't have a liquid tracking feature, be sure to account for the liquid level at the end of the dispensing event when setting the dispense height.

Deck layout

The suggested labware orientation shown below is designed for fast liquid handling and to minimize the possibility of contamination.



Reservoir	per plate	4 plates w/o refilling
a. Elution Solution or Nuclease-free Water	25 mL	100 mL
b. Wash Solution	90 mL	350 mL
c. Rebinding Mix	25 mL	100 mL
d. DNase 1 in DNase Buffer	2.5 mL	10 mL
e. Lysis/Binding Solution	25 mL	100 mL

Choice of eluent

Total RNA can be eluted from the Filter Plate at the end of the procedure with either Nuclease-free Water or Elution Solution (both are provided with the kit). Ambion recommends using Elution Solution (0.1 mM EDTA) for long term RNA storage, and Nuclease-free Water when the RNA will be used soon after isolation and/or in downstream applications that cannot tolerate 0.1 mM EDTA.

Add 35 mL 100% ethanol to the Rebinding Mix

Add 35 mL 100% ethanol (ACS grade or better) to the bottle labeled Rebinding Mix. Mix well. Place a check mark on the label to indicate that the ethanol has been added.

Add 140 mL 100% ethanol to each bottle of Wash Solution

Add 140 mL of 100% ethanol (ACS grade or better) to each bottle labeled Wash Solution Concentrate. Mix well. Place a check mark on the label to indicate that the ethanol has been added.

Prepare DNase 1 solution

Prepare the DNase 1 solution just before starting the RNA isolation procedure.

Component	Per plate	For 4 plates
DNase 1 Buffer	2.2 mL	8.8 mL
DNase 1 (20 U/µL)	300 µL	1.2 mL

Sample collection and preparation

Sample size

In general the RNAqueous®-96 Automated Kit is designed for the following sample sizes:

- Mammalian cells in culture: 100–500,000 cells
  (≤100,000 cells per well works best)
- Tissue samples: 0.1–1.0 mg tissue

Using larger samples increases RNA yield, but the increase may not scale linearly with the amount of sample used. At some point, RNA yield may be compromised by using too much sample because of interference with RNA binding to the filter by other cellular components. The best approach to optimize sample size for a particular type of material may be to do a pilot experiment using increasing sample amounts, in order to determine the point at which filtration times become too long, and the yield and/or purity of RNA is diminished.

The sample type influences the amount of lysate that can be processed without clogging the RNAqueous®-96 Automated filters. In general, the lysate from cultured cells is less likely to clog the filters than lysate from solid tissue, even when close to 10^6 cells are used. Also, some types of tissue are more problematic than others, for example brain and liver lysates rarely clog the Filter Plate whereas kidney, skeletal muscle, and spleen more often cause filter clogging.

1. Cells in culture

   It is generally not necessary to wash cells with phosphate buffered saline (PBS) before lysis unless filter clogging has been a problem. If this is the case, wash cells by resuspending in cold PBS and collect them by centrifugation before lysis to help alleviate filter clogging.

   Adherent cells: remove the culture medium with the liquid handling system or remove it manually

   - To use the liquid handling system, start at step i. on page 11.
   - To start the procedure manually: Thoroughly remove the culture medium and continue with step ii. on page 11.

   Cells grown in suspension:

   Pellet cells by low speed centrifugation, thoroughly remove the culture medium, and continue with step ii. on page 11.

2. Solid tissue samples:

   Sample collection

   If the source organism will be sacrificed, tissue should be harvested as quickly as possible after sacrifice. Immediately after obtaining and weighing the tissue, inactivate RNases by one of the following methods:

   - Immediately disrupt the tissue in Lysis/Binding Solution.
   - Snap freeze the tissue in liquid nitrogen, and store at –70°C or colder.
   - Place samples in Ambion® RNA*later*® Solution (pieces must be <0.5 cm^2 in at least one dimension), and store up to 1 week at 25°C, up to 1 month at 4°C, or indefinitely at –20°C.

   We recommend RNA*later* solution for both short- and long-term storage of tissue samples because, unlike tissue that is stored by freezing in liquid nitrogen, tissue stored in RNA*later* usually does not need to be ground to a powder in liquid nitrogen for RNA isolation.

Sample disruption

Thoroughly disrupt 0.1–1 mg tissue samples in 200 µL Lysis/Binding Solution. Because the sample size is so small, many sample types can probably be disrupted by dropping them into a homogenization vessel containing the Lysis/Binding Solution, and processing with a rotor-stator homogenizer, a sonicator, or with a manual tissue homogenizer. Extremely hard tissues, and/or tissues that are very high in RNase content may require freezing the tissue in liquid nitrogen and grinding in a mortar and pestle, and then homogenizing as described for thorough disruption of the tissue.

RNA isolation procedure and automation guidelines

IMPORTANT! The vacuum pressure for this procedure should be 4–7" Hg (580–650 torr). If centrifugation is used to pass liquids through the Filter Plate, spin for 1–2 min at approximately 1900 x g. In general, RNA yield is more consistent when centrifugation is used to pass liquids through the filters than when vacuum pressure is used.

You may, however, need to fine-tune labware positions, aspiration and dispense heights. The dimensions of the plates supplied with the kit are listed in , "RNAqueous®-96 Automated plate specifications" on page 19.

1. Lyse cells and bind RNA to the Filter Plate

   Instructions are provided for different sample types below. Follow the instructions for cultured cells (a. below), or tissue sample lysates (b. on page 12).

a. Cultured cells

i. Remove culture medium from the wells of the Culture Plate.

Tip change	Aspiration	Dispense	Blowout
Yes†	100 µL/sec	300 µL/sec	no delay 20 µL

† Return to tip box for reuse at lysate loading step if desired.

ii. Add 200 µL Lysis/Binding Solution to each well of the Culture Plate.

Tip change	Aspiration	Dispense	Blowout
no tip change	100 µL/sec	300 µL/sec	no delay 20 µL

iii. Gently shake for 5 min to lyse the cells.

With the DPC MicroMix 5 mixer, we use Form 14 (orbital speed 12.5/ 17.5 Hz) and Amplitude 6.

iv. Add 100 µL 100% ethanol to each well of the Culture Plate.

Tip change	Aspiration	Dispense	Blowout
no tip change	200 µL/sec	200 µL/sec	1 sec delay 20 µL

v. Mix by pipetting up and down, and transfer the mixture to the wells of the Filter Plate.

Tip change	Aspiration	Dispense	Blowout
yes, change tip for each sample	200 µL/sec	300 µL/sec Use tip-touch†	1 sec delay 20 µL

† Include a tip touch when using tips with relatively large openings, such as those used on Beckman robots; a tip touch is not necessary with tips that have smaller openings such as those used for Tecan and Perkin Elmer robots. Tip touch moves tips towards the wall of the well after dispensing to remove any droplets adhering to the outside of the tips; this increases accuracy and decreases cross contamination. If your system does not have tip touch capability, an alternative solution is to adjust the dispense height, so that the tip opening is 0.5–1 mm below the final liquid surface level. Capillary force will minimize the occurrence of hanging droplets.

The total volume per well at this step is slightly less than 300 µL due to volume shrinkage when ethanol mixes with aqueous solution. Using 200 µL tips, each sample will require two pipetting events to transfer all of the lysate/ethanol mixture to the Filter Plate. Program 200 µL for the first

transfer, and 100 µL for the second transfer; this minimizes sample loss from the viscous solution clinging to the wall of the Culture Plate well. Also, we recommend transferring all lysate/ethanol mixture from the first column of wells first before going on to the next column of wells.

vi. Apply the vacuum until the lysate is pulled through the Filter Plate.

The time required varies with the number and type of cells. Normally 3 min is adequate for up to 500,000 cells per well.

b. Tissue samples

i. Disrupt tissue in 200 µL Lysis/Binding Solution and transfer to a Culture Plate. Place it in the Culture Plate position on the deck layout diagram.

ii. Add 100 µL 100% ethanol to each well of the Culture Plate.

Tip change	Aspiration	Dispense	Blowout
no tip change	200 µL/sec	200 µL/sec	1 sec delay 20 µL

iii. Mix by pipetting up and down, and transfer the mixture to the wells of the Filter Plate.

Tip change	Aspiration	Dispense	Blowout
yes, change tip for each sample	200 µL/sec	300 µL/sec	1 sec delay 20 µL

iv. Apply the vacuum until the lysate is pulled through the Filter Plate.

The time required varies with the size and type of tissue sample. Normally 3 min is adequate for ≤1 mg tissue samples.

2. (optional) DNase 1 digestion

This DNase treatment removes contaminating DNA from RNA preparations. This treatment is optional, and can be omitted if DNA contamination is not a concern, for example when using the RNA for Northern blots, or when the RNA will be used in RT-PCR with primers designed to span an intron so that genomic DNA will not be amplified.

a. Wash Filter Plate with 300 µL Wash Solution

Add 300 µL Wash Solution to each well of the Filter Plate, and apply the vacuum for 2 min or until the Wash Solution is through the filter.

Tip change	Aspiration	Dispense	Blowout
no tip change	200 µL/sec	300 µL/sec	1 sec delay 20 µL

b. Add 20 µL DNase 1 (diluted with DNase 1 Buffer) to each well of the Filter Plate and incubate 15 min at room temp.

Tip change	Aspiration	Dispense	Blowout
no tip change	200 µL/sec Include 20 µL waste volume for multiple dispense	300 µL/sec	no delay 20 µL

Note: Do not apply the vacuum in this step.

3. Preheat Elution Solution (or Nuclease- free Water) for RNA elution

   Total RNA can be eluted with either Nuclease-free Water or Elution Solution.

   If using the Heat Plate to preheat the RNA eluent, distribute 200 µL Elution Solution (or Nuclease-free Water) per well to the Heat Plate (which is sitting on the heating tile) and cover with the Lid. This step can be scheduled to run during the DNase 1 digestion.

Tip change	Aspiration	Dispense	Blowout
no tip change	200 µL/sec	400 µL/sec	no delay 20 µL

4. Wash Filter Plate with 200 µL Rebinding Mix

   a. Add 200 µL Rebinding Mix to each well of the Filter Plate.

Tip change	Aspiration	Dispense	Blowout
no tip change	200 µL/sec	300 µL/sec	1 sec delay 20 µL

   b. Wait 1 min, then apply the vacuum for 1 min or until the Rebinding Mix is through the filter.

5. Wash Filter Plate with 2 x 200 µL Wash Solution, and dry the filter for 5 min

   a. Add 200 µL Wash Solution to each well of the Filter Plate.

Tip change	Aspiration	Dispense	Blowout
no tip change	<200 µL/sec	<300 µL/sec	1 sec delay 20 µL

   b. Apply the vacuum for 1 min or until the Wash Solution is through the filter.

   c. Add a second 200 µL Wash Solution to each filter well of the filter plate.

Tip change	Aspiration	Dispense	Blowout
no tip change	200 µL/sec	200 µL/sec	1 sec delay 20 µL

   d. Apply the vacuum for 5 min to draw the Wash Solution through the filter, and to dry the filter plate.

6. Elute the purified total RNA with 55 µL preheated Elution Solution or Nuclease-free Water
   a. Remove the Filter Plate together with the filter collar from the vacuum manifold.
   b. Open the lid of the Collection Plate.
   c. Place the Collection Plate on the vacuum manifold.
   d. Return the Filter Plate/filter collar back to the top of the vacuum manifold.
      Make sure that the ends of filter drip directors sit on, or slightly above (+2 or –1 mm), the tops of the Collection Plate wells. If the vacuum manifold is too deep, add a spacer to lift the Collection Plate to the proper height.
   e. (If using Heat Plate to preheat the eluent: open the lid of the Heat Plate.) Transfer 55 µL preheated Elution Solution (or Nuclease-free Water) to the Filter Plate.

Tip change	Pre-wet tip	Aspiration	Dispense	Blowout
no tip change	yes, prewet before first transfer	100 µL/sec	300 µL/sec	20 µL

   f. Apply the vacuum for 1 min or until all of the RNA-containing eluent is through the filter.
7. Elute the RNA a second time with 50 µL preheated Elution Solution or Nuclease-free Water
   a. Transfer 50 µL preheated Elution Solution (or Nuclease-free Water) to the Filter Plate.

Tip change	Pre-wet tip	Aspiration	Dispense	Blowout
no tip change	yes, prewet before first transfer	100 µL/sec	300 µL/sec	20 µL

   b. Apply the vacuum for 1 min or until all of the eluent is through the filter.
   c. Close the lid of the Heat Plate.
   d. Remove the Filter Plate/filter collar from the Collection Plate.
   e. Remove the Collection Plate from the vacuum manifold.
   f. Close the lid of the Collection Plate.
      The purified total RNA is now in the Collection Plate.
8. Change the Filter, Collection, and Culture Plates to prepare for the next run
   a. Return the Filter Plate/filter collar back to the top of vacuum manifold.
   b. Place fresh Filter Plate and Collection Plates in position, and refill the reservoirs (if necessary) for the next run.
   c. Load the Culture Plate for the next run.

Troubleshooting

Filter plate clogs

Sample amount

Loading more than the recommended amount of lysate, or loading a lysate made from more than the recommended amount of sample material can cause RNAqueous®-96 Automated Filter Plates to clog.

Problems with vacuum pressure

A common cause of low vacuum pressure is worn-out or dirty gaskets. Check the vacuum pressure on the Filter Plate by simply lifting it while the vacuum is applied. If the vacuum pressure is built up properly, the filter, collar and vacuum manifold should be stacked together tightly by the negative pressure. If you can easily lift the Filter Plate off the collar or vacuum manifold, try cleaning or replacing the gaskets. Although increasing the vacuum setting may give better sealing, it may also cause the Elution Solution to splash, thus we don't recommend vacuum pressure higher than 7" Hg.

The lysate is too viscous

If the vacuum pressure builds up properly but the liquid does not pass through the Filter Plate, reduce the viscosity of the lysate by using more Lysis/Binding Solution to homogenize the sample, and/or using smaller samples (fewer cells or smaller piece of tissue). Viscosity can also be reduced by shearing the DNA. This can be done by passing the sample through a syringe needle (20–25 gauge) several times, or by sonicating the lysate.

DNA contamination remains after DNase treatment

Residual Wash Solution on the filter

Be sure to completely remove the Wash Solution before the DNase treatment. If needed, extend vacuum time at step 2. on page 12. The presence of residual wash solution may decrease the enzyme's activity.

Prewarm the DNase solution, and increase the digestion time

Prewarm the diluted DNase solution to 37°C before putting it into the reservoir, and increase the DNase incubation to 30 minutes (step b. on page 13) to get a more thorough DNA digestion.

Design PCR primers to span one or more intron/exon boundaries

If contaminating DNA is suspected because PCR product is made from the minus-RT control in RT-PCR, using PCR primers that span an intron/exon boundary will minimize the effect of the contaminating DNA. Primers that span multiple introns or long introns, will typically span a region of genomic DNA that would be too large for amplification under the conditions used to amplify the desired shorter RT-PCR product. Primers spanning short introns generally yield a product from contaminating DNA that is distinguishable by size from the RT-PCR product amplified from RNA.

Include a post-elution DNase treatment

The DNase and DNase buffer provided with the RNAqueous®-96 Automated kit are optimized for on-the-filter digestion, and may not be ideal for treatment of RNA in solution. A better option for post-elution DNase treatment is to use the Ambion® DNA-*free*™ Kit (Part no. AM1906). It contains high quality RNase-free, DNase 1 and reaction buffer for degrading DNA. A novel reagent for removing the DNase without the hassles or hazards of phenol extraction or alcohol precipitation is also included.

Lower than expected yield of RNA

Tissue has low RNA content

Be sure the expectations for RNA yield are realistic for the type of sample used: some tissues, for example brain and skeletal muscle, have relatively low RNA content.

Sample size was too large

Using too much starting sample may reduce the RNA binding efficiency of the RNAqueous®-96 Automated filters. The maximum amount of starting material that can be used without compromising RNA yield and quality depends on the type of sample (cultured cells vs. tissue samples, and tissue source), and may need to be determined experimentally for particular samples. See section “Sample size” on page 9 for more information on sample size.

RNA is degraded

Run a positive control on gels to rule out gel artifacts

When assessing RNA yield and quality by electrophoresis on denaturing agarose gels, always run a positive control RNA sample known to be intact as a control for electrophoresis conditions and for degradation introduced during gel analysis.

Improper handling of samples before RNA isolation

Most problems with RNA degradation are caused by improper storage or handling of samples before lysis/disruption. Solid tissue must be harvested quickly, and either homogenized in Lysis/Binding Solution, placed into RNA*later* storage solution, or snap-frozen in liquid nitrogen to prevent degradation of the RNA by intracellular RNases.

Contaminated RNAqueous®-96 Automated Kit components or pipette tips

A less likely cause of RNA degradation is due to contaminating RNases introduced by pipette tips or mishandling of the RNAqueous®-96 Automated Kit reagents. To avoid contaminating the kit reagents, take precautionary measures such as using RNase-free pipette tips, wearing gloves when opening tubes and bottles (especially the Elution Solution and Nuclease-free Water), and keeping the vessels closed when not in use. Ambion RNase*Zap*® Solution (Part nos. AM9780–AM9784) can be used to eliminate RNases from the lab bench and from other equipment such as pipettors.

Low yield of cDNA and/or RT-PCR product

Residual salt or ethanol in the RNA sample

Problems with using the RNA as template for reverse transcription (cDNA synthesis) may be due to inhibition of the reverse transcriptase by residual salt or ethanol in the RNA sample.

- Thorough removal of Wash Solution (step 5. d. on page 13) is critical when the RNA will be used for reverse transcription.

- If the RNA was concentrated by ethanol precipitation after the RNAqueous®-96 Automated procedure, be sure to remove all traces of the ethanol supernatant.

Mixing experiment

To determine whether low yield of RT-PCR product is due to inhibitors in the RNA sample, consider doing a mixing experiment using RNA known to support RT-PCR, with and without addition of the questionable RNA. Reduced yields of amplified product in the mixed sample indicates the presence of inhibitors in the suspect RNA.

Variation in RNA yield between wells

Uneven vacuum pressure

Uneven vacuum pressure during the filtration and elution steps can cause variation in RNA yield between wells of the RNAqueous®-96 Automated Filter Plate. At each step of the procedure that requires the vacuum to be applied, make sure that the liquid is thoroughly drawn through all the wells of the Filter Plate.

Other suggestions

- Centrifugation usually results in more consistent RNA yield. If uniform RNA yield is your top priority, we recommend using centrifugation, instead of vacuum pressure, to pull solutions through the filter.
- Well to well reproducibility requires accurate delivery of uniform volumes of RNA Elution Solution to each well.
- Be sure to elute RNA in at least 50 µL of Elution Solution, and apply it directly to the center of the filters for the best results.
- Before quantifying RNA yield, mix the eluted RNA in the Collection Plate. The RNA solutions from the two consecutive elutions may take a long time to mix by diffusion alone.

Supplemental Information

RNAqueous®-96 Automated plate specifications

Filter Plate (800 µL Glass Fiber Filter Plate)		
well diameter	6.91 mm	(0.272")
well depth	28.00 mm	(1.102")
well-center to well-center	9.00 mm	(0.354")
plate length	123.26 mm	(4.852")
plate width	81.16 mm	(3.194")
plate height	30.73 mm	(1.209")
drip director length	4.76 mm	(0.187")

Culture Plate (Flat Bottom plate)		
Well diameter	6.93 mm	(0.273")
Well depth	11.25 mm	(0.442")
Well-center to well-center	9.00 mm	(0.354")
Plate length	124.42 mm	(4.889")
Plate width	81.87 mm	(3.223")
Plate Height	14.31 mm	(0.563")

Culture Plate Lid		
Lid length	127.16 mm	(5.006")
Lid width	84.73 mm	(0.336")
Lid Height	9.06 mm	(0.356")

Collection Plate (U bottom plate)		
Well diameter	6.71 mm	(0.264")
Well depth	10.60 mm	(0.417")
Well-center to well-center	9.00 mm	(0.354")
Plate length	123.72 mm	(4.871")
Plate width	81.52 mm	(3.208")
Plate Height	14.31 mm	(0.563")

Plate Lid		
Lid length	127.00 mm	(5.000”)
Lid width	84.91 mm	(3.343”)
Lid Height	14.31 mm	(0.563”)

Heat Plate (Flat Bottom plate)		
Well diameter	6.76 mm	(0.267”)
Well depth	10.72 mm	(0.422”)
Well-center to well-center	9.00 mm	(0.354”)
Plate length	123.80 mm	(4.874”)
Plate width	81.42 mm	(3.206”)
Plate Height	14.31 mm	(0.563”)

Additional procedures

Quantitation and assessment of RNA purity by UV absorbance

The concentration and purity of RNA can be determined by reading the absorbance of an aliquot of the preparation in a spectrophotometer at 260 nm and 280 nm. If you dilute the sample (usually a 1:10 to 1:100 dilution) in TE (10 mM Tris-HCl pH 8, 1 mM EDTA) to read the absorbance, be sure to zero the spectrophotometer with the TE used for sample dilution. The buffer used for dilution need not be RNase-free (unless you want to recover the RNA), since slight degradation of the RNA will not significantly affect its absorbance.

1. Concentration

   An A_{260} of 1 is equivalent to 40 µg RNA/mL.

   The concentration (µg/mL) of RNA is therefore calculated by multiplying the A_{260} X dilution factor X 40 µg/mL.

   Following is a typical example:

   RNA is eluted in 100 µL Elution Solution
   5 µL of the prep is diluted 1:20 into 100 µL of TE
   A_{260} = 0.10
   RNA concentration = 0.10 X 20 X 40 µg/mL = 80 µg/mL or 0.08 µg/µL
   Since there are 95 µL of the prep left after using 5 µL to measure the concentration, the total amount of remaining RNA is:
   95 µL X 0.08 µg/µL = 7.6 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.

2. Purity

   The ratio of A260 to A280 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 of this range, it may function well in common applications such as RT-PCR, Northern blotting, and RNase protection assays.

Quantitation of RNA by Ribogreen®

Ribogreen® reagent from Molecular Probes can be used as a sensitive method for quantitating RNA in solution. Follow the manufacturer's instructions for use.

Assessing RNA on denaturing agarose gels

The overall quality of an RNA preparation may be assessed by electrophoresis on a denaturing agarose gel; 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, and this prevents it from migrating strictly according to its size. 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, an RNA sample known to be intact, or both, can be used for this purpose.

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.

Related products available from Life Technologies

Magnetic Stand-96 Part no. AM10027	The Ambion Magnetic Stand-96 has powerful magnets positioned to capture beads to one side of the well. This capture pattern makes it very easy to remove supernatants manually without disturbing the beads, and therefore may be preferred by beginning users. In some applications, however, pellets formed with the Magnetic Stand-96 may be difficult to resuspend. If this occurs, we recommend the 96-well Magnetic-Ring Stand (Part no. AM10050).
MessageSensor™ RT Kit Part no. AM1745	The MessageSensor RT Kit for one-step qRT-PCR includes an optimized set of reagents for exceptionally sensitive reverse transcription. The kit is designed to be used for single-tube amplification of mRNA using either real-time or end-point amplification strategies.
RNA*later*® Solution Part nos. 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 Part nos. 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.

B Safety

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.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this 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/**

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Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**

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

Obtaining support

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

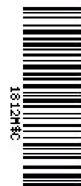
www.lifetechnologies.com

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.



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