Poly(A)Purist™ Kit

(Part Number AM1916)

Protocol

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I. Introduction

A. Overview

Eukaryotic mRNAs contain a stretch of "A" residues at their 3' ends. The Poly(A)Purist™ Kit uses this characteristic to select mRNA from total RNA preparations. The total RNA used as starting material in this procedure can be prepared from any eukaryotic tissue or cultured cell source using any method, for example Ambion® TōTALLY RNA™, TRI Reagent®, or RNAqueous® Kits. ***This kit is optimized to select poly(A) RNA from total RNA in a very low ionic-strength buffer and it cannot be used with crude cell or tissue lysates.*** The procedure is similar to published methods for oligo(dT) selection of poly(A) RNA, but the binding and wash solutions are novel. These optimized reagents greatly increase the specificity of poly(A) selection and shorten the procedure. Total RNA in dilute aqueous solution (e.g. water, TE, or THE RNA Storage Solution) is combined with the proprietary Binding Solution, and a pre-measured aliquot of Oligo(dT) Cellulose is added to it. The mixture is incubated with continual rocking or shaking, allowing hybridization between the poly(A) sequences found on most mRNAs and the Oligo(dT) Cellulose. The Oligo(dT) Cellulose is then transferred to a Spin Column and washed to remove nonspecifically bound material and ribosomal RNA. Finally, the poly(A) RNA is eluted using pre-warmed THE RNA Storage Solution.

The poly(A) RNA can be used immediately after elution from the Oligo(dT) Cellulose, or it can be concentrated by ethanol precipitation. After just a single round of oligo(dT) selection, the poly(A) RNA will be essentially free of DNA and protein and sufficiently pure for virtually all uses, such as Northern blotting, RT-PCR, microinjection, cDNA library construction, S1 and RNase protection assays, in vitro translation, subtractive cDNA cloning and reverse transcription for creating labeled cDNA for gene arrays. The poly(A) RNA can be subjected to a second oligo(dT) selection to eliminate traces of ribosomal RNA, however, this is rarely required.

B. Reagents Provided with the Kit and Storage

The Poly(A)Purist Kit includes reagents for 6 isolations of poly(A) RNA from 0.2–2mg of total RNA.

Amount	Component	Storage
5 mL	Nuclease-free Water	any temp*
6 each	Microfuge Tubes	room temp
6 each	Microfuge tubes with Spin Columns	room temp
8 mL	2X Binding Solution	4°C
20 mL	Wash Solution 1	4°C
20 mL	Wash Solution 2	4°C
6 each	Oligo(dT) Cellulose (100 mg aliquots)	4°C
8 mL	THE RNA Storage Solution	–20°C
1 mL	5 M Ammonium Acetate	–20°C
100 µL	Glycogen (5 mg/mL)	–20°C

* Store Nuclease-free Water at –20°C, 4°C, or room temp.

Note that the entire kit is shipped at room temperature which will not affect its stability.

C. Required Materials Not Provided with the Kit

- 100% Ethanol (analytical reagent grade)
- Microcentrifuge capable of RCF 12,000 x g
- ***(optional)*** Materials and equipment for RNA analysis
  - Spectrophotometer
  - Reagents and apparatus for preparation and electrophoresis of agarose gels
  - RiboGreen® RNA Quantitation Assay and Kit (Molecular Probes Inc.)

D. Related Products Available from Applied Biosystems

RNA Isolation Kits See web or print catalog for P/Ns	Family of kits for isolation of total or poly(A) RNA. Included in the product line are kits using classical GITC and acidic phenol, one-step disruption/denaturation, phenol-free glass fiber filter or magnetic bead binding, and combination kits.
Electrophoresis Reagents See web or print catalog for P/Ns	Ambion offers gel loading solutions, agaroses, acrylamide solutions, powdered gel buffer mixes, nuclease-free water, and RNA and DNA molecular weight markers for electrophoresis. Please see our catalog or our website (www.ambion.com) for a complete listing as this product line is always growing.
Millennium™ Markers and BrightStar® Biotinylated Millennium™ Markers P/N AM7150 and AM7170	Ambion's Millennium™ Markers are designed to provide very accurate size determination of single-stranded RNA transcripts from 0.5 to 9 kb and can be used in any Northern protocol. They are a mixture of 10 easy-to-remember sizes of in vitro transcripts: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6 and 9 kb.
RNase-free Tubes & Tips see our web or print catalog	Ambion RNase-free tubes and tips are available in most commonly used sizes and styles. They are guaranteed RNase- and DNase-free. See our latest catalog or our website (www.ambion.com/prod/tubes) for specific information.
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. Poly(A)Purist Procedure

CAUTION

Spin columns containing Oligo(dT) Cellulose should not be centrifuged at over 5000 X g.

The microfuge tubes supplied with the Poly(A)Purist Kit may not fit in some microcentrifuges, for example some models of microcentrifuges manufactured by Heraeus. We recommend that 1 or 2 tubes be test-spun to make sure that your microcentrifuge is deep enough to accommodate them.

Start with total RNA

The Poly(A)Purist Kit is optimized for isolation of high quality poly(A) RNA from total RNA in a low ionic-strength environment, and cannot be used with cell or tissue lysates, which routinely contain high levels of interfering ions.

Precipitate RNA to remove salt if necessary

Total RNA prepared from a solid-phase extraction method such as RNAqueous can be used immediately after elution because such samples are unlikely to have high levels of salt. On the other hand, RNA isolated by methods that include organic extractions for example using the products TRI Reagent® or TōTALLY RNA, may have a substantial amount of residual salt. If RNA from these types of procedures has been precipitated only a single time, we recommend doing a second alcohol precipitation to remove residual salt before starting the Poly(A)Purist procedure (this is described in step 1 below).

Save an aliquot of your total RNA

It is also a good idea to retain a small aliquot (~1–2 µg if possible) of the total RNA used in this procedure to check on a gel after the poly(A) RNA isolation is finished.

A. Preparation of Total RNA

1. (optional) Alcohol precipitate total RNA to remove residual salt

RNA with minimal salt left over from the isolation procedure will work best in Poly(A)Purist. If total RNA was isolated using a one-step reagent, or a multi-step organic procedure, and it was precipitated only once as part of the procedure, precipitate the RNA again to remove excess salt.

Add the following to the RNA:

- 0.1 volume 5 M Ammonium Acetate or 3 M sodium acetate

NOTE

There may not be enough 5 M Ammonium Acetate supplied with the kit to precipitate large volumes of total RNA. Using the suggested volumes, 250 µL is needed for the final precipitation (step 2 on page 8).

- 1 µL Glycogen
  The glycogen acts as a carrier to increase precipitation efficiency from dilute RNA solutions; it is unnecessary for solutions with ≥200 µg RNA/mL)
- 2.5 volumes 100% ethanol
- Mix thoroughly by vortexing.

a. Precipitate at –20°C overnight, or quick freeze it in either ethanol and dry ice, or in a –70°C freezer for 30 min.

b. Recover the RNA by centrifugation at ≥12,000 x g for 20–30 min at 4°C.

c. Carefully remove and discard the supernatant. The RNA pellet may not adhere tightly to the walls of the tubes, so we suggest removing the supernatant by gentle aspiration with a fine-tipped pipette.

d. Centrifuge the tube briefly a second time, and aspirate away any additional fluid that collects with a fine-tipped pipette.

e. Add 1 mL 70% ethanol, and vortex the tube a few times. Repellet the RNA by microcentrifuging, for 10 min at 4°C. Remove supernatant carefully as in steps c and d above.

2. Start with 0.2–2 mg total RNA

Follow the instructions below for either RNA pellets (2a) or for RNA in solution (2b).

2a. RNA pellets: resuspend in water and adjust to 1X Binding Solution in a final volume of 0.5–1.5 mL

- Resuspend 0.2–2 mg RNA in 0.25–0.75 mL Nuclease-free Water (included with the kit). Vortex vigorously to completely resuspend the pellet.
  At this step, the exact RNA concentration is not critical. In general, use ~0.25 mL Nuclease-free Water to resuspend relatively small amounts of RNA (i.e. ~0.2 mg), and use closer to 0.75 mL water for RNA pellets that are closer to 2 mg.
- Add an equal volume 2X Binding Solution and mix thoroughly.

2b. RNA solutions: adjust to 1X Binding Solution in a final volume of 0.5–1.5 mL

- Starting with 0.2–2 mg RNA in water, TE, or THE RNA Storage Solution, add Nuclease-free Water to bring the sample volume to 0.25–0.75 mL.
  At this step, the exact RNA concentration is not critical. In general, bring the volume to ~0.25 mL for relatively small amounts of RNA (i.e. ~0.2 mg), and bring the sample volume closer to 0.75 mL for RNA samples that are closer to 2 mg.
- Add an equal volume 2X Binding Solution and mix thoroughly.

B. Bind to Oligo(dT) Cellulose

1. Add each RNA sample to 1 tube Oligo(dT) Cellulose, mix well

Mix by inversion to thoroughly resuspend the resin. If necessary, clumps can be broken up by pipetting up and down.

2. Heat the mixture for 5 min at 65–75°C

Incubating the RNA/oligo(dT) mixture at 65–75°C for 5 min denatures secondary structure and maximizes hybridization between the poly(A) sequences found on most mRNAs, and the poly(T) sequences on the Oligo(dT) Cellulose.

3. Rock the tube gently for 30–60 min at room temp

Incubate for 30–60 min at room temperature (RT) with gentle agitation. Typically 90% of the possible poly(A) binding will occur in first 30 min. If the incubation time is extended to 60 min an additional 5% will occur. Constant rocking or agitation will increase the efficiency of poly(A) RNA binding to the Oligo(dT) Cellulose.

4. Pellet the Oligo(dT) Cellulose

a. Centrifuge at 2,000 to 4,000 x g for 3 min at RT.

b. Remove the supernatant by aspiration and save it on ice until the recovery of poly(A) RNA has been verified.

5. Start preheating the THE RNA Storage Solution to 60–80°C

Preheated THE RNA Storage Solution will be used to elute the poly(A) RNA from the Oligo(dT) Cellulose near the end of the procedure (step D.1 on page 7)

C. Wash the Oligo(dT) Cellulose

1. Wash the Oligo(dT) Cellulose twice with 0.5 mL Wash Solution 1 each time

These washes remove nonspecifically bound material and ribosomal RNA. Do them as follows:

a. Add 0.5 mL Wash Solution 1 to the Oligo(dT) Cellulose pellet, and vortex briefly to mix well.

b. Place a spin column for each RNA prep into a 2 mL microfuge tube, and transfer the Oligo(dT) Cellulose suspension to the spin column. Check that your microcentrifuge is deep enough for the 2 mL tubes before attempting to spin the sample(s).

c. Pass the Wash Solution 1 through the Oligo(dT) Cellulose by centrifuging at 2,000–4,000 x g for 3 min at RT. Discard the filtrate from the microfuge tube, and put the Spin Column back in the tube.

d. Add 0.5 mL Wash Solution 1 to the Oligo(dT) Cellulose, close the tube, and vortex briefly to thoroughly mix the wash solution with the cellulose.

e. Spin the Wash Solution 1 through the Oligo(dT) Cellulose at 2,000–4,000 x g for 3 min at RT. Discard the filtrate from the microfuge tube, and put the Spin Column back in the tube.

2. Wash the Oligo(dT) Cellulose3 times with 0.5 mL Wash Solution 2 each time

a. Add 0.5 mL Wash Solution 2 to the Oligo(dT) Cellulose, close the tube, and vortex briefly to thoroughly mix.

b. Spin the Wash Solution 2 through the Oligo(dT) Cellulose at 2,000–4,000 x g for 3 min at RT. Discard the filtrate from the microfuge tube, and put the Spin Column back in the tube.

c. Repeat steps a and b (above), this is the second wash.

d. Repeat steps a and b again, this is the third wash

D. Recover the Poly(A) RNA

IMPORTANT

All centrifugations in this section should be at ~5,000 X g at RT.

1. Elute the poly(A) RNA twice with 200 µL aliquots of warm THE RNA Storage Solution

a. Place the Spin Column into a new microfuge tube (use one that was provided with the kit).

b. Add 200 µL of warm (60–80°C) THE RNA Storage Solution to the Oligo(dT) Cellulose. Close the tube and vortex briefly to thoroughly mix.

c. Immediately centrifuge at ~5000 x g for 2 min to force it through the column.
THE RNA Storage Solution strips the poly(A) RNA from the Oligo(dT) Cellulose. Most of the poly(A) RNA is now at the bottom of the microfuge tube.

d. Leave the Spin Column in the tube, and repeat steps b and c above with a second 200 µL aliquot of warm THE RNA Storage Solution. This second elution strips any remaining poly(A) RNA from the Oligo(dT) Cellulose into THE RNA Storage Solution.

e. Discard the spin column. [If you intend to do a second round of oligo(dT) selection without first checking the results from a single round, reserve the The Oligo(dT) Cellulose for the second round and repeat the procedure starting at step II.A.2b on page 5.]

2. Precipitate RNA with:
0.1 volume 5 M NH_4 Ac
1 µL Glycogen
2.5 volumes ethanol

a. Add the following to the eluted poly(A) RNA:

Amount	Component
40 µL	5 M Ammonium Acetate (NH_4Ac)
1 µL	Glycogen*
1.1 mL	100% ethanol

* The glycogen acts as a carrier which increases the efficiency of precipitation; it will not interfere with quantitation by UV light absorbance.

b. Leave the precipitation mixture at –20°C overnight, or quick freeze it in either ethanol and dry ice, or in a –70°C freezer for 30 min.


NOTE

At this point the RNA can be stored at –70°C if desired.

c. Recover the RNA by centrifugation at ≥12,000 x g for 20–30 min at 4°C.

d. Carefully remove and discard the supernatant. The RNA pellet may not adhere tightly to the walls of the tubes, so we suggest removing the supernatant by gentle aspiration with a fine-tipped pipette.

e. Centrifuge the tube briefly a second time, and aspirate away any additional fluid that collects with a fine-tipped pipette.

3. (optional) Wash the pellet with 70% ethanol

Add 1 mL 70% ethanol, and vortex the tube a few times. Repellet the RNA by microcentrifuging, for 10 min at 4°C. Remove supernatant carefully as described in steps d and e above.

4. Resuspend the poly(A) RNA in THE RNA Storage Solution

Dissolve the poly(A) RNA pellet in 20–50 µL THE RNA Storage Solution (provided with the kit). If necessary, heat the mixture to 60–80°C to get the RNA into solution.

We recommend storing the RNA at –70°C.

5. (optional) Second round of oligo(dT) selection

A second round of oligo(dT) selection is typically not necessary to obtain poly(A) RNA that is suitable for most molecular biology applications. If desired, however, you can add a second round of oligo(dT) selection by simply repeating the procedure starting at step II.A.2b on page 5.

III. Assessing Yield and Quality of Poly(A) RNA

A. Quantitation of RNA

1. UV absorbance

The concentration and purity of RNA can be determined by diluting an aliquot of the preparation (usually a 1:50 to 1:100 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. 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. Be sure to zero the spectrophotometer with the TE used for sample dilution.

a. 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 resuspended in 40 µL DEPC Water/EDTA
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 only 34 µL of the prep left after sacrificing 6 µL to measure the concentration, the total amount of remaining RNA is:
34 µL X 0.84 µg/µL = 28.56 µg

b. 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 of this range, it may function well in common applications such as Northern blotting, RT-PCR, and RNase protection assays.

2. Ethidium bromide spot assay

Another technique that can be used to quantitate dilute samples of RNA is an ethidium bromide spot assay. Make a standard curve with several 2-fold dilutions of an RNA solution of known concentration. Using 2 µg/mL ethidium bromide as the diluent, start at about 80 ng/µL RNA, and make several 2-fold dilutions, ending about 1.25 ng/µL RNA. Make a few dilutions of the unknown RNA as well. The final concentration of ethidium bromide in all the samples should be 1 µg/mL. Spot 2 µL of the RNA standards and the unknown RNA dilutions onto plastic wrap placed on a UV transilluminator. Compare the fluorescence of the RNAs to estimate the concentration of the unknown RNA sample. Make sure that the unknown sample dilutions are in the linear range of ethidium bromide fluorescence. This assay will detect as little as 5 ng of RNA with an error of about two-fold.

3. Fluorescent dye

If you have a fluorometer, or a fluorescence microplate reader, Molecular Probes' RiboGreen® fluorescence-based assay for RNA quantitation is a convenient and sensitive way to measure RNA concentration.

B. Denaturing Agarose Gel Electrophoresis

Most poly(A) RNA forms extensive secondary structure via intramolecular base pairing. Because of this, it is best to use a denaturing gel system to size-fractionate RNA. Be sure to include a positive control on the gel so that any 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.

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. Expected results

The 28S and 18S ribosomal RNA (rRNA) bands are typically visible in poly(A) RNA; the bands should be sharp and discrete (size is dependent on the organism from which the RNA was obtained). It is difficult to assess the quality of poly(A) RNA from an agarose gel; it should look like a diffuse smear from about 500 bases to about 7 kb, with the majority of the material running at about 2 kb.

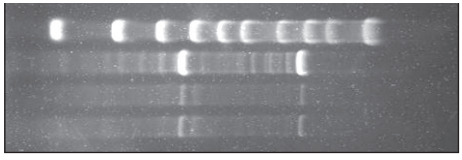

Figure 1. Total RNA and poly(A) RNA

Poly(A) RNA was isolated from 100 µg aliquots of total RNA from mouse liver using the Poly(A)Purist Kit. One-quarter of the poly(A) RNA obtained (~0.3 µg), and 1 µg of the input total RNA were fractionated on a 1% agarose denaturing (glyoxal) gel. The samples were pre-stained with ethidium bromide. Note the sharpness of the bands from remaining ribosomal RNA and the background smear of fluorescence from the poly(A) RNA.

IV. Troubleshooting

A. Low Yield

If the yield of RNA is lower than expected, consider the following explanations and remedies:

1. Poly(A) RNA is scarce in the source tissue

The actual amount of poly(A) RNA depends on cell type and physiological state. Only 1 to 5% of total cellular RNA is poly(A) RNA. Expected yields of poly(A) RNA vary widely among tissues. If you are accustomed to working with RNA from tissues such as liver or kidney which have a relatively high proportion of poly(A) RNA, you may have unrealistically high expectations of poly(A) RNA yields from tissues such as muscle or brain.

2. The RNA is degraded

The total RNA input may have been degraded. Check some of the input total RNA on a denaturing gel. Also, see below.

B. Degraded RNA

1. Rule out gel problems

If the RNA looks degraded as assessed on a denaturing agarose gel, there could be a problem with the gel, or the RNA could have been exposed to RNase at some point in the procedure. Since high quality poly(A) RNA looks like a smear when run on a gel, degraded poly(A) RNA is difficult to identify by looking at a gel. That's why it is important to run an intact control RNA on the gel for comparison.

If the test RNA looks degraded, but the control RNA produces sharp bands, then the test RNA is probably degraded. There are troubleshooting suggestions on the next few pages for avoiding RNase at each step in the RNA isolation procedure.

If both the control RNA(s) and the test RNA look smeared, try using fresh reagents for the gel, the running buffer, and the gel loading solution. It is not uncommon for these reagents to go bad after time and use, and this can cause smeary gels.

2. Avoiding RNA degradation during sample collection and storage

Sample collection

To minimize the degradation of poly(A) RNA during sample collection, the tissue should be dissected immediately after sacrificing the source organism, and rapidly extracted or placed in one of the following until it can be extracted:

- Ambion® RNA*later*® tissue storage and RNA stabilization solution
- cold phosphate-buffered saline (PBS) on ice
- liquid nitrogen

Samples to be stored in RNA*later* can be a maximum of 0.5 cm in one dimension, therefore, many tissue samples must be divided into pieces to allow good penetration of the RNA*later*. Smaller pieces freeze faster, and may be easier to manipulate later. Try to remove as much extraneous material as possible from samples that will be frozen or processed fresh, for example remove adipose tissue from heart, and remove gall bladder from liver. Extraneous material can be removed from tissue stored in RNA*later* at any time. Finally, some tissues benefit from perfusion with cold PBS to eliminate some of the red blood cells.

Sample storage

Instructions for storage of cell and tissue samples in Ambion RNA*later* can be found in the RNA*later* protocol.

Cells can be stored in the Lysis Solution at –70°C if desired. They should not be stored as cell pellets because it is difficult to effectively lyse frozen cell pellets.

Tissue samples can also be snap-frozen by immersion in liquid nitrogen, then transferred to a –70°C freezer for long-term storage. RNA processing will be easier and there will be less opportunity for RNA degradation in the sample if the pieces are weighed before snap freezing (especially small pieces such as mouse organs), to minimize post-freezing manipulation.

3. Avoiding degradation of RNA during storage

Poly(A) RNA can be damaged by repeated cycles of freeze-thawing (RNA Methodologies, a Laboratory Guide, 1992). To avoid repeated freeze-thawing, poly(A) RNA samples should be stored in small aliquots at –70°C or –80°C in THE RNA Storage Solution provided with the kit.

If degradation problems are encountered after prolonged storage, it may be desirable to store the RNA as an ethanol precipitate (i.e., add 2 volumes of ethanol to the prep in aqueous solution). The RNA can be recovered by centrifugation, after adjusting the salt concentration to 0.25 M with potassium acetate.

Alternatively, RNA can be stored in formamide at –20°C; RNase A activity is greatly reduced by storing the RNA in formamide (Chomczynski, 1992).

C. Impure RNA

1. Residual salt

Salt contamination can inhibit enzymatic reactions, in this procedure, salt can be carried over from the Ammonium Acetate precipitation. Try to avoid this by removing all of the supernatant after the precipitation with the double centrifugation described in steps 2.d and e on page 8. Any remaining salt can be removed by washing the RNA pellet with 70% EtOH as described in step 3 on page 8.

2. A_{260}:A_{280} ratio below 1.7

If protein contamination is suspected to be a problem due to a low A_{260}:A_{280} ratio, organic extraction(s) with an equal volume of phenol/$CHCl_3$ or $CHCl_3$/isoamyl alcohol (49:1 or 24:1 mixture) may be beneficial. Chloroform extraction also removes residual phenol. Despite these efforts, the A_{260}:A_{280} ratio may sometimes remain below 1.8, especially for RNA isolated from tissues such as liver and kidney. For most applications, a low A_{260}:A_{280} ratio will probably not affect the results. We have used poly(A) RNA with A_{260}:A_{280} ratios ranging from 1.4 to 1.8 with good results in RNase Protection Assays, Northern blots, in vitro translation experiments, and RT-PCR.

3. Ribosomal RNA contamination

Since ribosomal RNA (rRNA) makes up about 80% of total RNA, it is very difficult to recover RNA that does not have some rRNA. Typically a single oligo(dT) selection using Poly(A)Purist reduces rRNA to levels acceptable for virtually all molecular biology procedures. To use the RNA in procedures that cannot tolerate even trace amounts of ribosomal RNA, it may be desirable to add a second round of oligo(dT) selection. To do this, simply re-start the procedure at step II.A.2b on page 5.

V. Appendix

A. References

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B. Quality Control

Functional Testing

All components are tested in a functional RNA Isolation procedure as described in the protocol.

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.

C. Safety Information

Chemical safety guidelines

To minimize the hazards of chemicals:

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

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining the MSDS

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At **www.appliedbiosystems.com**, select **Support**, then **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At **www.ambion.com**, go to the web catalog page for the product of interest. Click **MSDS**, then right-click to print or download.
- E-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com) or telephone (650-554-2756; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1–2 weeks for processing.

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