USER GUIDE

ambion®
by life technologies™

MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit

Compatible with PAXgene® Blood RNA Tubes

Catalog Number 4451894

Publication Part Number 4452006 Rev. B
Revision Date June 2011

life technologies™

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Information in this document is subject to change without notice.

APPLIED BIOSYSTEMS DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTENT ALLOWED BY LAW, IN NO EVENT SHALL APPLIED BIOSYSTEMS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT APPLIED BIOSYSTEMS IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

NOTICE TO PURCHASER: LIMITED LICENSE

This product or portions thereof is manufactured and sold under license from GE Healthcare under U.S. Patent Nos. 5,523,231 and 5,681,946 and other foreign patents. End users are specifically not authorized to and are forbidden from reselling, transferring or distributing any products either as a stand alone product or as a component of another product.

TRADEMARKS

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

Eppendorf and Thermomixer are registered trademarks of Eppendorf AG.

LabChip is a registered trademark of Caliper Life Sciences Inc.

NanoDrop is a registered trademark of NanoDrop Technologies LLC.

PAXgene is a registered trademark of PreAnalytiX GmbH. No sponsorship, endorsement, or affiliation is implied herein.

Safety-Lok and Vacutainer are registered trademarks of Becton, Dickinson and Company.

TaqMan is a registered trademark of Roche Molecular Systems, Inc.

VWR is a registered trademark of VWR International, LLC.

Part Number 4452006 Rev. B
June 2011

Contents

MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Compatible with PAXgene® Blood RNA Tubes)

Product information

Purpose of the product

The MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit is designed for purification of total RNA that includes microRNAs from 2.5 mL of human whole blood that has been collected and stabilized in a PAXgene® Blood RNA Tube. (PAXgene® tubes are available from PreAnalytiX; PreAnalytiX is a QIAGEN/BD Company.)

Procedure overview

Start with blood that has been drawn into PAXgene® Blood RNA Tubes (2.5 mL of blood per PAXgene® tube). The stabilized blood is pelleted and washed, subjected to a protease treatment, then transferred to a microcentrifuge tube or to a 96-well plate for RNA extraction. The 96-well plate format is amenable to robotic platforms.

After transferring to the tube or plate, the protease-treated lysate is extracted for RNA and treated with TURBO™ DNase, using streamlined MagMAX™ magnetic bead-based methodology. The magnetic beads can be fully dispersed in solution, allowing thorough nucleic acid binding, washing, and elution. The procedure consistently delivers maximum yields (approximately 3 to 18 µg) of high-quality RNA without the need to centrifuge spin columns.

Applications

You can use this MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit for:

- **Medium-throughput applications** – Perform the RNA isolations in the 96-well Processing Plate that is provided in the kit.
- **Low-throughput applications** – Perform the RNA isolations in 1.5-mL microfuge tubes (the tubes are not provided in the kit).

Note: You can also use the MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit to perform high-throughput applications on the MagMAX™ Express-96 Deep Well Magnetic Particle Processor. See "High-Throughput Applications" on page 21.

Kit contents and storage

Upon receipt, store each component as described in the following table.

Kit part number	Kit name	Kit contents			Storage conditions
		Box	Component	Quantity	
4451894	MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Compatible with PAXgene® Blood RNA Tubes)	1	Resuspension Solution (Compatible with PAXgene® Blood RNA Tubes)	18 mL	Room temperature
			Wash Solution 1 Concentrate†	16 mL	
			Wash Solution 2 Concentrate‡	2 × 30 mL	
			RNA Rebinding Buffer	10 mL	
			Elution Buffer	10 mL	
			Processing Plate (96-well)	1 plate	
			2.0 mL Tubes	100 tubes	
			RNA Binding Beads	2 mL	2 to 8°C Do not freeze.
		2	Proteinase (Compatible with PAXgene® Blood RNA Tubes)	0.6 mL	–15 to –25°C
			10X TURBO™ DNase Buffer	1 mL	
			TURBO™ DNase	1 mL	

† Before using the Wash Solution 1 Concentrate, add the correct volume of isopropanol (user-supplied), as indicated on the bottle.
‡ Before using the Wash Solution 2 Concentrate, add the correct volume of ethanol (user-supplied), as indicated on the bottle.

User-supplied materials

Unless other noted, all reagents and materials are available at **www.lifetechnologies.com** (Applied Biosystems).

IMPORTANT! For the Safety Data Sheet (SDS) of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Sample

Item	Source (Part no.)
PAXgene® Blood RNA Tubes (100 tubes)	PreAnalytiX GmbH (762165)
Blood samples, collected in PAXgene® Blood RNA Tubes	—

Reagents

Reagent	Source (Part no.)
100% Ethanol, ACS reagent grade or equivalent	MLS†
100% Isopropanol, ACS reagent grade or equivalent	MLS
Nuclease-free water	MLS
RNase*Zap*® surface decontamination solution	AM9780, AM9782, AM9784

† MLS: Major laboratory supplier

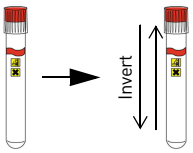
Laboratory consumables and equipment

Item	Source (Part no.)
Disposable gloves	MLS†
Pipette tips, aerosol-resistant, nuclease-free	MLS
Pipettes: • Disposable serological pipettes (5-mL), or equivalent • Pipetting device for serological pipettes • Single- and multichannel pipettes	MLS
Non-Stick RNase-free Microfuge Tubes (1.5-mL)	AM12450
Processing Plates (recommended if you process <96 samples at a time)	Fisher Scientific (AB-1127)
Liquid reservoirs (RNase-free)	MLS
Magnetic stand: • DynaMag™-2 magnet • Magnetic Stand-96 • 96 well Magnetic-Ring Stand	 • Life Technologies (Invitrogen; 123-21D) • AM10027 • AM10050
Microcentrifuge, with a 45-degree, fixed-angle rotor (capable of 16,000 × g)	MLS
MicroAmp® Clear Adhesive Film (for protecting unused wells of the 96-well Processing Plate)	4306311
Orbital shaker (for the 96-well Processing Plate)	MLS
Thermomixer (capable of 1000 rpm and 55°C)	MLS
Vortexer	MLS
Vortex Adapter-60 (for the 1.5-mL microfuge tubes)	AM10014
Centrifuge, with a swing-bucket rotor and blood-tube adapters (capable of 3000 × g)	MLS

† MLS: Major laboratory supplier

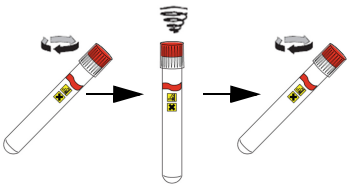
Workflow

Collect blood samples in the PAXgene® Blood RNA Tubes (page 11)

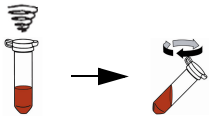


Prepare the PAXgene®-stabilized blood samples (page 11)

Centrifuge the samples and wash the crude RNA pellets

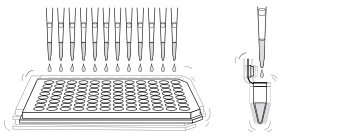


Resuspend the washed crude RNA pellets and digest with protease



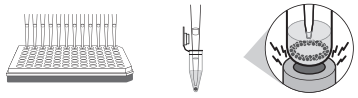
Purify the RNA (page 13)

Bind the RNA to the RNA Binding Beads, then magnetically capture the beads



Wash once with Wash Solution 1

Wash once with Wash Solution 2



Treat with TURBO™ DNase, then rebind the RNA

Wash twice with Wash Solution 2

Dry the beads

Elute the RNA

Estimated time required

If you use the 96-well Processing Plate, you can obtain 12 samples of purified RNA in ~1.7 hours. (The Processing Plate is provided in this kit.)

Note: This time estimate does not include the time needed for collecting the blood sample, incubating the sample in the collection tube, or thawing the sample.

Before you begin

Prepare the thermomixer

This procedure was developed using an Eppendorf® Thermomixer® R with a 2.0-mL thermoblock. To prepare your thermomixer:

- Set the speed to 1000 rpm
- Preheat to 55°C

Determine plate shaker compatibility and settings

This procedure was developed using a Thermo Scientific Barnstead Titer Plate Shaker 4625, which has a shaking speed range of 0 to 10 (40 to 110 rpm) and a shaker motion diameter of 0.120 inches (3 mm), with the following settings:

- Binding and wash steps – Moderate shaker speed, settings 6 to 7 (~600 to 750 rpm)
- DNase digestion, bead drying, and RNA elution steps – Vigorous shaker speed, settings 8 to 9 (~860 to 980 rpm)

If you are using a different plate shaker:

- Verify that the 96-well Processing Plate fits securely on your shaker.
- Determine the equivalent moderate and vigorous shaking speeds, as described above, for your plate shaker: Add water to a 96-well plate according to the table below, then determine the maximum setting that you can use on your shaker without any of the water spilling.

To determine the maximum speed for...	Add...	When to use
Small volumes	80 µL of water	Use this speed for the DNase digestion, bead drying, and RNA elution steps.
Large volumes	400 µL of water	Use this speed for the binding and wash steps.

Determine vortexer compatibility and settings

For the tube-based purification steps, use a vortexer that can accommodate the 1.5-mL microfuge tubes. This procedure was developed using a VWR® MiniVortexer with a Vortex Adapter-60 with the following settings (based on a range of 1 to 10):

- Binding and wash steps – Moderate vortex adaptor speed, settings 4 to 5
- DNase digestion and elution steps – Vigorous vortex adapter speed, settings 6 to 7

If you are using a different vortexer, determine your vortexer settings with the vortex adapter in place, as follows:

To determine a...	Perform these steps:	When to use
Moderate vortex adapter speed	1. Add 400 µL of water to each of two 1.5-mL microcentrifuge tubes. 2. Place the tubes securely in the vortex adapter, using one of the tubes as a counterbalance. 3. Set the vortexer to its lowest setting, then observe the motion of the water. 4. Gradually increase the speed to approximately half of the maximum setting to identify a gentle but thorough mixing speed.	Use this speed for the binding and wash steps
Vigorous vortex adapter speed	1. Add 80 µL of water to each of two 1.5-mL microcentrifuge tubes. 2. Place the tubes securely in the vortex adapter, using one of the tubes as a counterbalance. 3. Set the vortexer to just below its maximum setting, then observe the motion of the assembly. 4. Adjust the vortexer speed so that it agitates the tubes very rapidly, but does not cause the tube contents to turn to foam or the vortexer to become unstable.	Use this speed for the DNase digestion and the elution steps.

Follow these guidelines to prevent RNase contamination

- Wear laboratory gloves for this protocol. Gloves protect you from the reagents and protect the RNA from nucleases that are present on skin.
- Use RNase-free pipette tips to handle the reagents, and avoid putting used tips into the reagent containers.
- Clean lab benches and pipettes with an RNase decontamination solution, for example, RNase*Zap*® solution (Part no. AM9780).

Prepare the wash solutions

Prepare Wash Solution 1

Each RNA isolation requires 150 µL of prepared Wash Solution 1. To prepare Wash Solution 1:

1. To the bottle of Wash Solution 1 Concentrate, add 8 mL of 100% isopropanol.
2. Mix well by inverting 3 to 4 times.
3. Mark the bottle label to indicate that isopropanol was added.

   Note: The resulting mixture is referred to as *Wash Solution 1* in this protocol.

Store Wash Solution 1 at room temperature until needed.

Prepare Wash Solution 2

Each RNA isolation requires 450 µL of prepared Wash Solution 2. To prepare Wash Solution 2:

1. To each bottle of Wash Solution 2 Concentrate, add 24 mL of 100% ethanol.
2. Mix well by inverting 3 to 4 times.

3. Mark the bottle labels to indicate that ethanol was added.

   Note: The resulting mixture is referred to as *Wash Solution 2* in this protocol.

Store Wash Solution 2 at room temperature until needed.

Collect blood samples in the PAXgene® Blood RNA Tubes

This section provides brief procedures for collecting blood samples with PAXgene® Blood RNA Tubes. For additional information, refer to the current *PreAnalytiX® RNA Tube Product Circular* and to the product documentation for your blood collection set.

Note: If you are using the BD Vacutainer® Safety-Lok® Blood Collection Set, go to the Becton, Dickinson and Company Web site for additional information (**www.bd.com**).

Collect blood samples

WARNING! Biological samples have the potential to transmit infectious diseases. For safety and biohazard guidelines, see "Biological hazard safety" on page 26.

1. Draw 2.5 mL of blood directly into each PAXgene® Blood RNA Tube according to your laboratory's standard procedures.
2. Immediately after filling each PAXgene® tube, gently invert the tube 8 to 10 times to ensure that the RNA stabilizing additive makes uniform contact with the sample.

   IMPORTANT! Failure to mix the stabilizing additive with the blood leads to inadequate stabilization of the gene expression profile and can potentially compromise the RNA purification procedure.

3. Incubate the samples at room temperature (18 to 25°C) for 2 to 72 hours before processing.

Storage conditions

Refer to the current *PreAnalytiX® RNA Tube Product Circular* for recommendations on storing PAXgene® tubes that contain stabilized blood samples.

Prepare the PAXgene®-stabilized blood samples

Centrifuge the samples and wash the crude RNA pellets

1. If the blood samples are frozen:
   a. Thaw the samples in the PAXgene® tubes for at least 2 hours at room temperature (18 to 25°C).
   b. Gently invert the thawed blood samples 10 times.

   Note: For more information on thawing samples collected in PAXgene® tubes, refer to the current *PreAnalytiX® RNA Tube Product Circular*.
2. Centrifuge the PAXgene® tubes at 3000 × *g* for 10 minutes to pellet the crude RNA.
3. Carefully pour the supernatant from each tube.

4. To each tube, add 5 mL of nuclease-free water, then securely re-cap the tubes.
5. Vortex the tubes at vigorous speed until the crude RNA pellets are fully resuspended.
6. Centrifuge the tubes at 3000 × *g* for 10 minutes to re-pellet the crude RNA .
7. Carefully pour the supernatant from each tube.
8. Drain residual supernatant by inverting the tubes on absorbent paper for 2 minutes.
9. Using clean, absorbent paper, blot any remaining liquid from the tube rims.

Proceed immediately to the "Resuspend the washed crude RNA pellets and digest with protease" below.

Resuspend the washed crude RNA pellets and digest with protease

1. Just before use, prepare the resuspension mixture:
   a. Per the table below, calculate the total volume required for each component:
   volume per sample × the total number of samples

   Include 5% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Component	Volume per sample
Resuspension Solution†	174 µL
Proteinase†	6 µL
Total volume required for 1 sample	**180 µL**

† Compatible with PAXgene® Blood RNA Tubes, not compatible with Tempus® Blood RNA Tubes.

   b. Add the components to a microcentrifuge tube, then mix well by vortexing at moderate speed.
   c. Briefly (2 to 3 seconds) centrifuge to collect the resuspension mixture at the tube bottom.
   d. Place the resuspension mixture on ice until needed.
2. To each PAXgene® tube (from step 9 on page 12), add 180 µL of the prepared resuspension mixture, then securely re-cap the tubes.
3. Vortex the tubes at vigorous speed until the crude RNA pellets are fully resuspended.
4. For *each* PAXgene® tube, transfer the total volume of resuspended crude RNA pellet to one 2.0 mL Tube (provided with the kit), then securely cap and label each 2.0 mL Tube with the sample identification.
5. Place the 2.0 mL Tubes in the pre-heated thermomixer, then mix at 1000 rpm for 10 minutes at 55°C to digest.

IMPORTANT! It is critical that you perform the protease digestion at the specified temperature and mixing speed. Otherwise, RNA yields and quality may decrease significantly.

6. Centrifuge the 2.0 mL Tubes at 16,000 × *g* for 3 minutes to clarify the digested samples.
7. For *each* 2.0 mL Tube: Without disturbing the debris pellet, transfer the total volume (~180 to 200 µL) of RNA-rich supernatant to:
   - One 1.5-mL microfuge tube; securely cap and label each tube with the sample identification

     OR
   - One well of the 96-well Processing Plate

   Note: Small amounts of the debris pellet may be aspirated with the RNA-rich supernatant. This will not interfere with RNA purification.

STOPPING POINT You can store the RNA-rich supernatant at room temperature (18 to 25°C) for up to 4 hours. If you are using the 96-well Processing Plate, place a cover over all wells that contain the RNA-rich supernatant.

Purify the RNA

RNA purification procedure guidelines

- Perform the RNA purification procedure at room temperature (18 to 25°C), unless otherwise stated.
- When aspirating, be careful not to dislodge the RNA Binding Beads from the magnet.
- The capture time of the RNA Binding Beads depends on the magnetic stand that you use.
- When capturing the RNA Binding Beads on the magnetic stand, you can remove the supernatant after the solution becomes clear and the beads form a pellet at the magnet.
- When performing a tube-based purification, securely cap the tubes before shaking them on the vortex adaptor.
- When performing a tube-based purification, briefly (2 to 3 seconds) centrifuge the sample each time after shaking in the vortex adapter to collect the contents at the tube bottom.

Bind the RNA to the RNA Binding Beads, then magnetically capture the beads

1. Vortex the RNA Binding Beads at vigorous speed until fully resuspended.
2. To each microfuge tube or well (containing RNA-rich supernatant, from step 7 on page 13), add 20 µL of the vortexed RNA Binding Beads, then mix at moderate speed for 3 minutes:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).
3. To each microfuge tube or well, add 200 µL of 100% isopropanol, then mix at moderate speed for 5 minutes:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).

   Note: After you add the 100% isopropanol, the RNA Binding Beads may clump. This will not interfere with RNA purification.

4. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 3 minutes.
5. Carefully aspirate and discard all supernatant without disturbing the beads.

   Note: The supernatant may be dark and opaque during the first binding. When you remove the supernatant, note where the magnet contacts the wells of the tubes or plate so that you can angle the pipette tips away from the magnetic beads.
6. Remove the microfuge tubes or plate from the magnetic stand.

IMPORTANT! It is critical that you remove the tubes or plate from the magnetic stand before you perform the next step.

Wash once with Wash Solution 1

1. To each microfuge tube or well, add 150 µL of prepared Wash Solution 1, then mix at moderate speed for 1 minute:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).
2. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 2 minutes.
3. Carefully aspirate and discard all supernatant without disturbing the beads.
4. Remove the microfuge tubes or plate from the magnetic stand.

IMPORTANT! It is critical that you remove the tubes or plate from the magnetic stand before the next step.

Wash once with Wash Solution 2

1. To each microfuge tube or well, add 150 µL of prepared Wash Solution 2, then mix at moderate speed for 1 minute:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).
2. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 2 minutes.
3. Carefully aspirate and discard all supernatant without disturbing the beads.
4. Remove the microfuge tubes or plate from the magnetic stand.

IMPORTANT! It is critical that you remove the tubes or plate from the magnetic stand before the next step.

Treat with TURBO™ DNase, then rebind the RNA

1. Just before use, prepare the TURBO™ DNase mixture:
   a. Per the table below, calculate the total volume required for each component: *volume per sample × the total number of samples*

      Include 5% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Component	Volume per sample
Nuclease-free water	35 µL
10× TURBO™ DNase Buffer	5 µL
TURBO™ DNase	10 µL
Total volume required for 1 sample	**50 µL**

   b. Add the components to a microcentrifuge tube, then mix well by vortexing at gentle speed.
   c. Briefly (2 to 3 seconds) centrifuge to collect the TURBO™ DNase mixture at the tube bottom.
   d. Place the TURBO™ DNase mixture on ice until needed.
2. To each microfuge tube or well, add 50 µL of the prepared TURBO™ DNase mixture, then mix at vigorous speed for 10 minutes:
   - Shake the tubes on a vortex adaptor (settings 6 to 7).
   - Shake the plate on an orbital shaker (settings 8 to 9).
3. To each microfuge tube or well, add:
   - 50 µL of RNA Rebinding Buffer
   - 100 µL of 100% isopropanol

   Note: The RNA Binding Beads should not clump after you add the 100% isopropanol.
4. Mix at moderate speed for 3 minutes:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).
5. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 2 minutes.
6. Carefully aspirate and discard all supernatant without disturbing the beads.
7. Remove the microfuge tubes or plate from the magnetic stand.

IMPORTANT! It is critical that you remove the tubes or plate from the magnetic stand before the next step.

Wash twice with Wash Solution 2

1. To each microfuge tube or well, add 150 µL of prepared Wash Solution 2 (from page 10), then mix at moderate speed for 1 minute:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).
2. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 2 minutes.
3. Carefully aspirate and discard all supernatant without disturbing the beads.
4. Remove the microfuge tubes or plate from the magnetic stand.

   IMPORTANT! It is critical that you remove the tubes or plate from the magnetic stand before the next step.

5. Repeat steps 1 to 4 above once.

Dry the beads

To dry the beads in microfuge tubes:

1. Open the tubes, then invert the tubes on absorbent paper for 2 minutes at room temperature.
2. Inspect the tubes. If there is residual Wash Solution 2:
   a. Use a fine-tipped pipette to remove the supernatant, being careful not to disturb the beads. Discard the supernatant.
   b. Leaving the tubes open, invert for 1 minute more at room temperature.

To dry the beads in a 96-well Processing Plate:

1. Shake the plate on an orbital shaker at vigorous speed (settings 8 to 9) for 2 minutes at room temperature.
2. Inspect the plate. If there is residual Wash Solution 2, shake the plate at vigorous speed (settings 8 to 9) for 2 minutes more at room temperature.

Elute the RNA

1. To each microfuge tube or well, add 80 µL of Elution Buffer, then mix at vigorous speed for 4 minutes:
   - Shake the tubes on a vortex adaptor (settings 6 to 7).
   - Shake the plate on an orbital shaker (settings 8 to 9).

   Note: You can use 20 to 80 µL of Elution Buffer, depending on the final concentration you want to obtain.
2. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 3 minutes.
3. Being careful not to disturb the beads, transfer the supernatant to a nuclease-free container that is appropriate for your application. **Do not discard the supernatant; the purified RNA is in the supernatant.**

STOPPING POINT Store the purified RNA on ice for immediate use, at –20°C for up to 6 months, or at –80°C for long-term storage.

Assessing RNA yield and quality

RNA yield

Spectrophotometry

The concentration of an RNA solution can be determined by measuring its absorbance at 260 nm. We recommend using a Thermo Scientific NanoDrop® Spectrophotometer because it is extremely quick and easy to use: you can directly measure 0.5 to 2 µL of the RNA sample. For more information, go to **www.nanodrop.com**.

Alternatively, you can determine the RNA concentration by diluting an aliquot of the preparation in TE (10 mM Tris-HCl, pH 8, 1 mM EDTA) and reading the absorbance in a traditional spectrophotometer at 260 nm. An A_{260} of 1 is equivalent to an RNA concentration of 40 µg/mL, in a spectrophotometer with a 1-cm path length. Calculate the RNA concentration (µg/mL) as follows:

$$A_{260} \times dilution\ factor \times 40\ \mu g/mL = \mu g\ of\ RNA/mL$$

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

Fluorometry

If a fluorometer or a fluorescence microplate reader is available, Molecular Probes RiboGreen® fluorescence-based assay for RNA quantitation is a convenient and sensitive way to measure RNA concentration. Follow the manufacturer's instructions for using the RiboGreen assay.

RNA quality

Spectrophotometry

The A_{260}/A_{280} ratio of the RNA is an indication of its quality. The RNA isolated with this procedure should have an A_{260}/A_{280} ratio of 1.8 to 2.2. However, RNA with absorbance ratios outside of this range may still function well for qRT-PCR or other amplification-based downstream applications.

Agarose gel electrophoresis

You can also assess the quality of your RNA sample by agarose gel electrophoresis.

In higher-quality preparations, two broad bands representing 18S and 28S rRNA will be seen; only a smear will be visible in lower-quality preparations.

Microfluidic analysis

The Agilent 2100 Bioanalyzer used in conjunction with an RNA LabChip® Kit provides a powerful and sensitive method to assess RNA quality. To use this system, follow the instructions for RNA analysis provided with the RNA LabChip Kit.

The data mimics that seen on agarose gels. The 28S to 18S rRNA ratio is often used as an indicator of RNA quality. For total RNA isolated from human blood collected in a PAXgene® Blood RNA Tube using this kit, a 28S to 18S rRNA ratio of ≥1.0 is considered acceptable quality.

A more reliable method for evaluating RNA quality is to calculate the RNA Integrity Number (RIN) using a bioanalyzer. A metric developed by Agilent, the RIN analyzes information from both rRNA bands, as well as information contained outside the rRNA peaks (potential degradation products) to provide a fuller picture of RNA degradation states. For more information, search for **RIN** on the Agilent Web site (**www.chem.agilent.com**).

Troubleshooting

Observation	Possible cause	Recommended action
No RNA or low RNA yield	The drawn blood sample was <2.5 mL	During the phlebotomy procedure, make sure that the PAXgene® Blood RNA Tube is filled with 2.5 mL of blood.
	During the collection step, sample lysis was not complete	Immediately after collecting the blood sample, gently invert the PAXgene® tube 8 to 10 times to thoroughly mix the RNA stabilizing additive with the blood sample.
		After collecting the blood sample, incubate the sample for at least 2 hours at room temperature.
	The Proteinase was stored incorrectly	Store the Proteinase at 2 to 6°C for short-term storage, or at –15 to –25°C for long-term storage.
	During the protease digestion, the sample heating temperature and/or mixing speed were not sufficient	Always preheat the heat source before incubating the resuspended crude RNA pellet.
		Always heat the resuspended crude RNA pellet at 55°C.
		Always perform the incubation while mixing at 1000 rpm.
	The RNA Binding Beads were stored incorrectly	Store the RNA Binding Beads at room temperature for short-term storage, or at 2 to 6°C for long-term storage. **IMPORTANT!** Do not freeze the RNA Binding Beads.
	The mass of RNA Binding Beads added was not sufficient	Make sure that the RNA Binding Beads are fully resuspended before pipetting them into the 96-well Processing Plate or the 1.5-mL microfuge tubes.
	The RNA Binding Beads were added after the isopropanol	Add the RNA Binding Beads to the RNA-rich supernatant before adding the isopropanol.
	During the RNA binding steps, an incorrect volume of isopropanol was used	Use the volumes recommended in this user guide.
	During the RNA binding steps or washing steps, the RNA Binding Beads were lost	To prevent aspiration of the RNA Binding Beads in subsequent experiments, follow these guidelines: • Use sufficient magnetic capture time. • Aspirate the supernatant slowly. • Keep the pipette tip openings away from the captured RNA Binding Beads when aspirating the supernatant.

Observation	Possible cause	Recommended action
No RNA or low RNA yield *(Continued)*	After the TURBO™ DNase digestion, the user did not perform the RNA rebinding step	Always perform the RNA rebinding steps after the TURBO™ DNase digestion.
	During the RNA elution step, the RNA Binding Beads were not fully resuspended or dispersed	In general, the RNA Binding Beads will disperse more easily when the temperature of the mixture is >20°C.
		At the end of the elution step, make sure that the RNA Binding Beads are fully dispersed in Elution Buffer. If the RNA Binding Beads aggregate or fail to disperse during the final elution step, it may improve RNA yield to place the plate or tube in a 70°C incubator for 5 minutes, then repeat the 4-minute shaking incubation before capturing the beads. In subsequent experiments using sample types with bead-clumping problems, you can facilitate dispersion of the beads by preheating the Elution Buffer to 70 to 80°C before adding it to the samples.
		Do not over-dry the RNA Binding Beads before eluting. If the beads were over-dried, extend the shaking time to 10 minutes to rehydrate the beads.
Bead carryover	Loose beads were inadvertently transferred with the eluate	After transferring the eluate to the elution plate or tube, perform another collection on the magnetic stand, then transfer the purified RNA sample to a new plate or tube. **Note:** If the RNA Binding Beads are carried over into the eluate containing the RNA, they will cause the solution to be light brown in color. A small quantity of beads in the purified RNA sample does not inhibit RT reactions or RT-PCR.
Excess DNA content	During the TURBO™ DNase digestion, the ambient temperature was too cold	Because the TURBO™ DNA digestion is done at room temperature, it is important that the ambient temperature in your lab be 18 to 25°C. If the temperature in your lab is <18°C, perform the digestion in a 25°C incubator.
	During the TURBO™ DNase digestion, the incubation time and/or mixing speed were not sufficient	After adding the TURBO™ DNase mixture to the sample, incubate the sample for 10 minutes with vigorous mixing.
	Excessive amounts of the debris pellet were aspirated with the RNA-rich supernatant	To minimize aspiration of the debris pellet in subsequent experiments, follow these guidelines: • If the debris pellet was disturbed, re-centrifuge the sample in the 2.0 mL Tube at 16,000 × g for 3 minutes. • Aspirate the supernatant slowly. • Keep the pipette tip openings away from the debris pellet when aspirating the supernatant.
The purified RNA sample is a clear reddish-brown color	During the collection step, sample lysis was not complete	Immediately after collecting the blood sample, gently invert the PAXgene® tube 8 to 10 times to thoroughly mix the RNA stabilizing additive with the blood sample.
		After collecting the blood sample, incubate the sample for at least 2 hours at room temperature.

Observation	Possible cause	Recommended action
The purified RNA does not perform in downstream applications	There was salt carryover	Verify that the correct type and volume of alcohol is added to each Wash Solution Concentrate.
		Remove the supernatant completely after each binding step or wash step.
	There was alcohol carryover	Allow the RNA sample to dry completely before adding the Elution Buffer.
	Inhibitors are present (the eluate is a green or reddish color)	Use a quantitative PCR or genotyping master mix that functions in the presence of inhibitors (for example, the TaqMan® GTXpress™ Master Mix [Life Technologies Part no. 4403311]).
		Centrifuge the RNA samples at 16,000 × g for 3 minutes, then remove the clean eluate fraction to a new plate or tube.

A High-Throughput Applications

You can use this MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit with Applied Biosystems MagMAX™ Express-96 Deep Well Magnetic Particle Processor.

The MagMAX Express-96 processor automates the nucleic acid isolation process. A brief protocol for the MagMAX Express-96 processor is provided in this appendix. Contact **automation@ambion.com** to obtain scripts for the MagMAX Express-96 processor.

User-supplied materials

To use this MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit with the MagMAX Express-96 processor, you need the materials listed in the following table.

Item	Life Technologies part number
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	4400077
MagMAX™ Express-96 Deep Well Tip Combs	4388487
MagMAX™ Express-96 Deep Well Plates	4388476
MagMAX™ Express-96 Standard Plates	4388475

Before you begin

- Prepare your thermomixer, as described on page 9.
- Determine your plate shaker compatibility and settings, as described on page 9.
- Prepare Wash Solution 1 and Wash Solution 2, as described on page 10. Store Wash Solution 1 and Wash Solution 2 at room temperature until needed.
- In addition, follow the guidelines to prevent RNase contamination described on page 10.

Prepare the PAXgene®-stabilized blood samples

WARNING! Biological samples have the potential to transmit infectious diseases. For safety and biohazard guidelines, see "Biological hazard safety" on page 26.

Centrifuge the samples and wash the crude RNA pellets

Follow the steps in "Centrifuge the samples and wash the crude RNA pellets" on page 11.

Resuspend the washed crude RNA pellets and digest with protease

1. Just before use, prepare the resuspension mixture:
   a. Per the table below, calculate the total volume required for each component:
   volume per sample × the total number of samples

   Include 5% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Component	Volume per sample
Resuspension Solution†	174 µL
Proteinase†	6 µL
Total volume required for 1 sample	**180 µL**

† Compatible with PAXgene® Blood RNA Tubes, not compatible with Tempus® Blood RNA Tubes.

   b. Add the components to a microcentrifuge tube, then mix well by vortexing at moderate speed.
   c. Briefly (2 to 3 seconds) centrifuge to collect the resuspension mixture at the tube bottom.
   d. Place the resuspension mixture on ice until needed.
2. To each PAXgene® tube containing the washed crude RNA pellet, add 180 µL of the prepared resuspension mixture, then securely re-cap the tubes.
3. Vortex the tubes at vigorous speed until the crude RNA pellets are fully resuspended.
4. For *each* PAXgene® tube, transfer the total volume of resuspended crude RNA pellet to one 2.0 mL Tube (provided with the kit), then securely cap and label each 2.0 mL Tube with the sample identification.
5. Place the 2.0 mL Tubes in the pre-heated thermomixer, then mix at 1000 rpm for 10 minutes at 55°C to digest.

   IMPORTANT! It is critical that you perform the protease digestion at the specified temperature and mixing speed. Otherwise, RNA yields and quality may decrease significantly.
6. Centrifuge the 2.0 mL Tubes at 16,000 × *g* for 3 minutes to clarify the digested samples.
7. For *each* 2.0 mL Tube: Without disturbing the debris pellet, transfer the total volume (~180 to 200 µL) of RNA-rich supernatant to one well of a MagMAX™ Express-96 Deep Well Plate.

   Note: Small amounts of the debris pellet may be aspirated with the RNA-rich supernatant. This will not interfere with RNA purification.

STOPPING POINT You can store the RNA-rich supernatant at room temperature (18 to 25°C) for up to 4 hours. Place a cover over all wells that contain the RNA-rich supernatant.

Purify the RNA

Note: You can prepare the TURBO™ DNase mixture (below) and set up the MagMAX™ Express-96 Standard Plates (below) during the protease digestion and subsequent centrifugation steps described in "Prepare the PAXgene®-stabilized blood samples" on page 21.

Prepare the TURBO™ DNase mixture

Just before use, prepare the TURBO™ DNase mixture:

1. Per the table below, calculate the total volume required for each component:
   volume per sample × the total number of samples

   Include 10% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Component	Volume per sample
Nuclease-free water	35 µL
10× TURBO™ DNase Buffer	5 µL
TURBO™ DNase	10 µL
Total volume required for 1 sample	**50 µL**

2. Add the components to a microcentrifuge tube, then mix well by vortexing at gentle speed.
3. Briefly (2 to 3 seconds) centrifuge to collect the TURBO™ DNase mixture at the tube bottom.
4. Place the TURBO™ DNase mixture on ice until needed.

Set up the MagMAX™ Express-96 Standard Plates

Set up the MagMAX™ Express-96 Standard Plates for the MagMAX Express-96 processor according to the table below.

Plate		Reagent	Volume per well (µL)	Plate type
ID	Position			
Wash 1	2	Wash Solution 1	150	MME-96 Standard Plate / 4388475
Wash 2	3	Wash Solution 2	150	
DNase	4	TURBO™ DNase mixture	50	
First Wash 2	5	Wash Solution 2	150	
Second Wash 2	6	Wash Solution 2	150	
Elution	7	Elution Buffer	90	

Prepare the MagMAX™ Express-96 Deep Well Plate

1. Vortex the RNA Binding Beads at vigorous speed until fully resuspended.
2. To each well of the Express-96 Deep Well Plate (containing RNA-rich supernatant, from step 7 on page 22), add 20 µL of the vortexed RNA Binding Beads, then shake the plate on an orbital shaker at moderate speed (settings 6 to 7) for 3 minutes.
3. Add 200 µL of 100% isopropanol to each well.

Proceed immediately to "Perform the run" below.

Perform the run

1. Power on the MagMAX Express-96 processor.
2. Combine the MagMAX™ Express-96 Deep Well Tip Comb and an unused Express-96 Standard Plate.
3. Using the keypad or MagMAX™ Express Software, select the appropriate protocol, then start the run.
4. If the lid is in place, open the sliding door.
5. When prompted, load the plates into the loading station. Press **Start** after loading each plate.
6. When prompted, load reagents into the DNase plate as indicated in the table below.

Plate		Reagent	Volume per well (µL)
ID	Position		
DNase	4	RNA Rebinding Buffer	50
		100% Isopropanol	100

The run takes ~35 minutes to complete. The eluted RNA will be in position 7.

STOPPING POINT Store the purified RNA on ice for immediate use, at –20°C for up to 6 months, or at –80°C for long-term storage.

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.

Biological hazard safety

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/**

Documentation and Support

Related documentation

Document	Part number	Description
MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Compatible with PAXgene® Blood RNA Tubes) Quick Reference	4452005	Provides at-a-glance procedures for preparing samples that were taken with PAXgene® Blood RNA Tube.
MagMAX™ Express-96 Deep Well Magnetic Particle Processor scripts	Email **automation@ambion.com**.	

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.appliedbiosystems.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, go to:

www.lifetechnologies.com/support

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

4452006 0511

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.invitrogen.com/support or email techsupport@invitrogen.com

www.lifetechnologies.com

life technologies™