ambion®
by life technologies™

MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit

(Compatible with Tempus® Blood RNA Tubes)

Protocol

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About This Guide

Safety information

Note: For general safety information, see this section and Appendix C, "Safety" on page 33. When a hazard symbol and hazard type appear by a chemical name, see the "Safety" Appendix for the complete alert on the chemical.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This alert word is to be limited to the most extreme situations.

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 34.

IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

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

Product information

Purpose of the product

This MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit is designed to purify total RNA that includes microRNAs from 3.0 mL of human whole blood that has been collected and stabilized in a Tempus® Blood RNA Tube. (The Tempus tubes are available from Applied Biosystems.)

Procedure overview

This MagMAX for Stabilized Blood Tubes RNA Isolation Kit protocol begins with blood that has been drawn into Tempus Blood RNA Tubes (3.0 mL of blood per Tempus tube). After collection, the blood is diluted, pelleted, and washed. The washed pellet is re-collected by a second centrifuge step. The re-collected RNA pellet is dissolved in a proprietary solution, 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 re-suspended pellets are extracted for RNA using a combined protease and DNase digestion and the streamlined MagMAX™ magnetic bead-based protocol provided with this kit. The magnetic beads can be fully dispersed in solution, allowing thorough nucleic acid binding, washing, and elution. This protocol consistently delivers maximum yields (approximately 3 to 25 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 this 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 27.

Kit contents and storage

Applied Biosystems ships this MagMAX for Stabilized Blood Tubes RNA Isolation Kit in two boxes; the boxes contain the components listed in the table below. Upon receipt, open the boxes, then store each component as indicated below.

Kit part number	Kit name	Kit contents			Storage conditions
		Box	Component	Quantity	
4451893	MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Compatible with Tempus® Blood RNA Tubes)	1	Tempus® Resuspension Solution	12 mL	Room temperature
			Binding Solution Concentrate	5 mL	
			Wash Solution 1 Concentrate†	2 × 16 mL	
			Wash Solution 2 Concentrate‡	30 mL	
			Elution Buffer	10 mL	
			Processing Plate (96-well)	1 plate	
			Tempus® Pre-Digestion Wash	2 × 200 mL	2 to 8 °C Do not freeze.
			RNA Binding Beads	2 mL	
			Tempus® 1X PBS	2 × 150 mL	
		2	Tempus® Proteinase	0.25 mL	–15 to –25 °C
			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 otherwise indicated, all items are available from major laboratory suppliers (MLS).

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

Sample

Item	Source
Tempus® Blood RNA Tubes (50 tubes)	Applied Biosystems (PN 4342792)
Blood samples, collected in Tempus® Blood RNA Tubes	---

Reagents

Reagent	Source
100% Ethanol, ACS reagent grade or equivalent	MLS
100% Isopropanol, ACS reagent grade or equivalent	MLS
RNAse*Zap*® surface decontamination solution	Ambion (PN AM9780, AM9782, AM9784)

Laboratory consumables and equipment

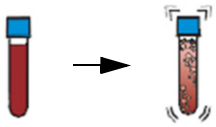
Item	Source
Disposable gloves	MLS
Pipette tips, aerosol-resistant, nuclease-free	MLS
Pipettes: • Disposable serological pipettes (3- to 4-mL range), or equivalent • Pipetting device for serological pipettes • Single- and multichannel pipettes	MLS
50-mL Conical Tubes (Certified RNase- and DNase-free)	Ambion (PN AM12501)
Non-Stick RNase-free Microfuge Tubes (1.5-mL)	Ambion (PN AM12450)
Processing Plates (recommended if you process <96 samples at a time)	Fisher Scientific (Cat# AB-1127)
Liquid reservoirs (RNase-free)	MLS

Item	Source
Magnetic stand: • DynaMag™-2 magnet • 6 Tube Magnetic Stand • Magnetic Stand-96 • 96 well Magnetic-Ring Stand	 • Invitrogen (Cat# 123.21D) • Ambion (PN AM10055) • Ambion (PN AM10027) • Ambion (PN AM10050)
Microcentrifuge or picocentrifuge	MLS
MicroAmp® Clear Adhesive Film (for protecting unused wells of the 96-well Processing Plate)	Applied Biosystems (PN 4306311)
Orbital shaker (for the 96-well Processing Plate)	MLS
Vortexer	MLS
Vortex Adapter-60 (for the 1.5-mL microfuge tubes)	Ambion (PN AM10014)
Centrifuge, with a swing bucket rotor and 50-mL conical tube adapters (capable of 4 °C and 5000 × g)	MLS

Workflow

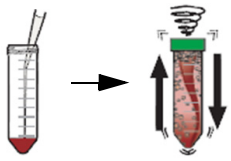
The figure below provides a simplified workflow for using this MagMAX for Stabilized Blood Tubes RNA Isolation Kit to purify RNA from human whole blood collected in a Tempus Blood RNA Tube.

Collecting blood samples in the Tempus® Blood RNA Tubes

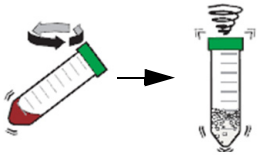


Prepare the Tempus®-stabilized blood samples

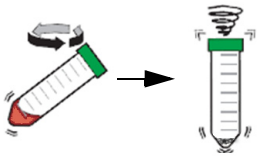
Dilute the samples with Tempus® 1X PBS



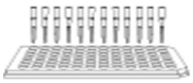
Centrifuge the samples and wash the crude RNA pellets



Resuspend the washed crude RNA pellets and digest with protease and TURBO™ DNase

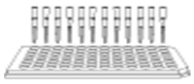


(Workflow continued on the next page.)

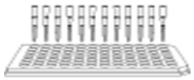


Purify the RNA

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

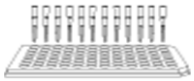


Wash twice with Wash Solution 1



Wash 2×

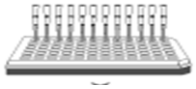
Wash twice with Wash Solution 2



Wash 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 ~1.9 hours. (The Processing Plate is provided in this MagMAX for Stabilized Blood Tubes RNA Isolation Kit.)

Note: This time estimate does not include the time needed for collecting the blood sample or thawing the sample.

Before you begin

Determine plate shaker compatibility and settings

When performing this protocol, Applied Biosystems used a Thermo Scientific Barnstead Titer Plate Shaker with the following settings (based on a range of 1 to 10):

- Binding and wash steps – Moderate shaker speed, settings 6 to 7
- Protease and DNase digestion, bead drying, and RNA elution steps – Vigorous shaker speed, settings 8 to 9

If you are using a different plate shaker:

- Verify that the 96-well Processing Plate fits securely on your shaker.
- Determine the maximum settings for your 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	130 µL of water	Use this speed for the protease and 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, you need a vortexer that can accommodate the 1.5-mL microfuge tubes. When performing this protocol, Applied Biosystems used 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
- Protease and 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

To determine a...	Perform these steps:	When to use
Vigorous vortex adapter speed	1. Add 130 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 protease and 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).

Prepare the wash solutions

Prepare Wash Solution 1

Each RNA isolation requires 300 µL of prepared Wash Solution 1. To prepare Wash Solution 1:

1. To each 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 labels 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 300 µL of prepared Wash Solution 2. To prepare Wash Solution 2:

1. To the 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 label 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.

Collecting blood samples in the Tempus® Blood RNA Tubes

This section provides brief procedures for collecting blood samples with Tempus® Blood RNA Tubes. For additional information, refer to the *Tempus® Blood RNA Tube and Large-Volume Consumables Protocol* (see "Related documentation" on page 37) and to the product documentation for your blood collection set.

Note: If you are using the Greiner Vacuette® Safety Blood Collection Set, go to the Vacuette Web site for additional information (**www.vacuette.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 36.

1. Draw 3 mL of blood directly into each Tempus Blood RNA Tube according to your laboratory's standard procedures.

   Note: The black mark on each tube label indicates approximately 3 mL.

2. Immediately after filling each Tempus tube, vigorously shake or vortex the tube for 10 seconds to ensure that the Applied Biosystems Stabilizing Reagent makes uniform contact with the sample.

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

Storage conditions

Applied Biosystems recommends that you store Tempus tubes that contain stabilized blood samples as follows:

Storage option	Temperature
Store at room temperature for up to 5 days.	18 to 25 °C
Store refrigerated for up to 7 days.	4 °C
Freeze or keep on dry ice for long-term storage. **IMPORTANT!** Do not let the samples come into direct contact with the dry ice.	–20 to –80 °C

Prepare the Tempus®-stabilized blood samples

Dilute the samples with Tempus® 1× PBS

IMPORTANT! Keep the samples on ice as much as possible. Otherwise, RNA yields may decrease significantly.

During the blood collection process, the blood is stabilized when mixed with the Applied Biosystems Stabilizing Reagent contained in the Tempus tube. The stabilizing reagent must have a final concentration of 1×. To adjust the concentration of the stabilizing reagent for purification, dilute the stabilized blood with Tempus® 1× PBS (provided in the kit) before extracting RNA. Failure to do so results in significantly lower RNA yields.

For *each* blood sample being processed:

1. If the sample is frozen, thaw the sample in the Tempus tube for 10 to 30 minutes on ice.
2. Label a clean 50-mL conical tube with the sample identification.
3. Pour the entire contents of the Tempus tube into the conical tube.
4. Add 3 mL of Tempus 1× PBS to the conical tube to bring the total volume to 12 mL.

   IMPORTANT! If the initial blood sample was less than 3 mL, make up the difference by adding enough Tempus 1× PBS to bring the total volume to 12 mL. Otherwise, RNA yields decrease significantly.
5. Securely cap the conical tube, then vortex at vigorous speed for 30 seconds to ensure proper mixing of the contents.

   IMPORTANT! To prevent the tube from leaking and spraying the sample during vortexing, make sure that the tube is securely capped.

   Note: Frothing of the sample after vortexing is normal.

Proceed immediately to "Centrifuge the samples and wash the crude RNA pellets" on page 17.

Centrifuge the samples and wash the crude RNA pellets

1. Centrifuge the conical tubes at 5000 × *g* for 15 minutes at 4 °C to pellet the crude RNA.

   Note: The crude RNA pellet in each tube is transparent and invisible.

   IMPORTANT! Handle each tube carefully so that you do not dislodge the crude RNA pellet from the tube bottom.

2. Carefully pour the supernatant from each tube.

3. Add the Tempus® Pre-Digestion Wash:

   IMPORTANT! Before using the Tempus Pre-Digestion Wash, mix well by gently inverting the bottle 3 to 4 times to ensure it is a homogenous solution.

   a. To each conical tube, add 4 mL of the Tempus Pre-Digestion Wash, then securely re-cap the conical tubes.

   b. Vortex the conical tubes at moderate speed for 5 seconds.

      Note: The vortex speed setting should gently draw the Tempus Pre-Digestion Wash three-quarters of the way up the inside of the conical tube.

4. Centrifuge the conical tubes at 5000 × *g* for 10 minutes at 4 °C to re-pellet the crude RNA.

5. Carefully pour the supernatant from each tube, being careful not to dislodge the crude RNA pellet.

6. Drain residual supernatant by inverting the tubes on absorbent paper for 2 minutes.

7. Using clean, absorbent paper, blot any remaining liquid from the tube rims.

8. Place the conical tubes on ice until needed.

Proceed immediately to "Resuspend the washed crude RNA pellets and digest with protease and TURBO™ DNase" on page 18.

Resuspend the washed crude RNA pellets and digest with protease and TURBO™ DNase

1. Just before use, prepare the resuspension mixture:
   a. Per the table below, calculate the total volume required for each component:
   volume for 1 sample × the total number of samples
   Include 5% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Component	Volume for 1 sample
Tempus® Resuspension Solution	117.5 µL
Tempus® Proteinase	2.5 µL
Total volume required for 1 sample	**120 µL**

   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 conical tube (from step 8 on page 17), add 120 µL of the prepared resuspension mixture, then securely re-cap the tubes.

3. Vortex the tubes at gentle speed for 5 to 10 seconds to fully resuspend the crude RNA pellets.

4. For *each* conical tube, transfer the total volume of resuspended crude RNA pellet to:
   - One 1.5-mL microfuge tub; securely cap and label each tube with the sample identification
     OR
   - One well of the 96-well Processing Plate

   STOPPING POINT You can store the resuspended crude RNA pellets on ice for up to 4 hours. If you are using the 96-well Processing Plate, place a cover over all wells that contain a resuspended pellet. Remove the resuspended pellets from the ice and remove the cover just before proceeding to step 5 below.

5. To each microfuge tube or to each well (containing a resuspended crude RNA pellet), add 10 µL of TURBO™ DNase, then mix at vigorous speed for 10 minutes:
   - Shake the microfuge tubes on a vortex adaptor (settings 6 to 7).
   - Shake the plate on an orbital shaker (settings 8 to 9).

Proceed immediately to "Purify the RNA" on page 19.

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 an RNA sample, from step 5 on page 18), add:
   - 50 µL of Binding Solution Concentrate
   - 20 µL of the vortexed RNA Binding Beads
3. 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).
4. To each microfuge tube or well, add 200 µL of 100% isopropanol, 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).
5. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 3 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 you perform the next step.

Wash twice with Wash Solution 1

1. To each microfuge tube or well, add 150 µL of prepared Wash Solution 1 (from page 14), 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.

Wash twice with Wash Solution 2

1. To each microfuge tube or well, add 150 µL of prepared Wash Solution 2 (from page 14), 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. Applied Biosystems recommends 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 the NanoDrop Web site: (**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 40 µg of RNA/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 protocol 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. Protocols are available at **www.ambion.com/techlib/append/supp**.

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 Tempus® 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 <3.0 mL	During the phlebotomy procedure, make sure that the Tempus® Blood RNA Tube is filled with blood up to the black mark on the tube label. If it is not, make up the difference after transferring the sample to the 50-mL conical tube by adding enough Tempus® 1X PBS to bring the total volume of the diluted blood lysate to 12 mL.
	The Applied Biosystems Stabilizing Reagent did not reach the 1X final concentration	Add enough Tempus 1X PBS to bring the total volume of the diluted blood lysate in the 50-mL conical tube to 12 mL.
	During sample dilution with Tempus 1X PBS, mixing was not sufficient	After diluting the samples with Tempus 1X PBS, vortex the samples for 30 seconds.
	The blood lysate was exposed to temperatures >37 °C for a short period, which caused the RNA to go back into solution	1. Freeze any remaining unused lysate. 2. Thaw the lysate, then repurify.
	When processing the stabilized blood sample before RNA purification, the centrifuge time and/or speed were not sufficient	Always perform the initial centrifuge step for the diluted sample at 5000 × g for 15 minutes at 4 °C.
		After washing the crude RNA pellet with the Tempus® Pre-Digestion Wash, always centrifuge the sample at 5000 × g for 10 minutes at 4 °C.
		Always centrifuge the sample in a 50-mL conical tube.
	When processing the stabilized blood sample before RNA purification, the crude RNA pellet was lost	After the centrifuge steps, pour the supernatant slowly and carefully without jarring the tube. **IMPORTANT!** Applied Biosystems recommends decanting the supernatant. The crude RNA pellet may be lost if you use a pipette to remove the supernatant.
		Use a centrifuge with a swing bucket rotor to form a compact crude RNA pellet at the tube bottom.
	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.

Observation	Possible cause	Recommended action
No RNA or low RNA yield *(Continued)*	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 sample before adding the isopropanol.
	During the RNA binding step, an incorrect volume of isopropanol was used	Use the volume recommended in this protocol.
	During the RNA binding step 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.
	During the RNA elution step, the RNA Binding Beads were not fully resuspended/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/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/tube, perform another collection on the magnetic stand, then transfer the purified RNA sample to a new plate/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.

Observation	Possible cause	Recommended action
Excess DNA content	During the combined protease and TURBO™ DNase digestion, the ambient temperature was too cold	Because the combined protease and TURBO DNase digestion is done at room temperature, it is important that the ambient temperature in your lab be 18 to 25 °C. If your lab is <18 °C, perform the digestion in a 25 °C incubator.
	During the combined protease and TURBO DNase digestion, the incubation time and/or mixing speed were not sufficient	After adding the TURBO DNase to the resuspended crude RNA pellet, incubate the mixture for 10 minutes with vigorous mixing.
	The Applied Biosystems Stabilizing Reagent was carried over to the combined protease and TURBO DNase digestion	To prevent carryover of the Applied Biosystems Stabilizing Reagent in subsequent experiments, follow these guidelines: • Wash the crude RNA pellet as recommended in this protocol. • Make sure that any residual wash supernatant is completely drained from the sides of the tube. • Blot any remaining drops of liquid from the tube rim using clean, absorbent paper. • Add the resuspension mixture to the tube bottom.
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 [Applied Biosystems PN 4403311]).
		Centrifuge the RNA samples at 16,000 × g for 3 minutes, then remove the clean eluate fraction to a new plate/tube.

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. You can download scripts from the Ambion automation resource page:

www.ambion.com/techlib/automation

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 table below.

Item	Applied Biosystems 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

Determine plate shaker compatibility and settings

Determine your plate shaker compatibility and settings, as described on page 13.

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).

Prepare the wash solutions

Prepare Wash Solution 1 and Wash Solution 2, as described in "Prepare the wash solutions" on page 14. Store Wash Solution 1 and Wash Solution 2 at room temperature until needed.

Prepare the Tempus®-stabilized blood samples

> ⚠ **WARNING!** Biological samples have the potential to transmit infectious diseases. For safety and biohazard guidelines, see "Biological hazard safety" on page 36.

Dilute the samples with Tempus® 1× PBS

Follow the steps in "Dilute the samples with Tempus® 1× PBS" on page 16.

Centrifuge the samples and wash the crude RNA pellets

Follow the steps in "Centrifuge the samples and wash the crude RNA pellets" on page 17.

Resuspend the washed crude RNA pellets and digest with protease and TURBO™ DNase

1. Just before use, prepare the resuspension mixture:
   a. Per the table below, calculate the total volume required for each component:
   volume for 1 sample × the total number of samples
   Include 5% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Component	Volume for 1 sample
Tempus® Resuspension Solution	117.5 µL
Tempus® Proteinase	2.5 µL
Total volume required for 1 sample	**120 µL**

   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 conical tube (from "Centrifuge the samples and wash the crude RNA pellets" above), add 120 µL of the prepared resuspension mixture, then securely re-cap the tubes.

3. Vortex the tubes at gentle speed for 5 to 10 seconds to fully resuspend the crude RNA pellets.

4. For *each* conical tube, transfer the total volume of resuspended crude RNA pellet to one well of a MagMAX™ Express-96 Deep Well Plate.

   STOPPING POINT You can store the resuspended crude RNA pellets on ice for up to 4 hours. Place a cover over all wells that contain a resuspended pellet. Remove the resuspended pellets from the ice and remove the cover just before proceeding to step 5 below.

5. To each well (containing a resuspended crude RNA pellet), add 10 µL of TURBO™ DNase, then shake the plate on an orbital shaker at vigorous speed (settings 8 to 9) for 10 minutes.

Proceed immediately to "Purify the RNA" below.

Purify the RNA

Set up the MagMAX™ Express-96 Standard Plates

During the TURBO™ DNase incubation (step 5 on page 29), 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 1	3	Wash Solution 1	150	
Wash 2	4	Wash Solution 2	150	
Wash 2	5	Wash Solution 2	150	
Elution	6	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 an RNA sample, from step 5 on page 29), add:
   - 50 µL of Binding Solution Concentrate
   - 20 µL of the vortexed RNA Binding Beads
3. Shake the plate on an orbital shaker at moderate speed (settings 6 to 7) for 1 minute.
4. 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.

The run takes ~25 minutes to complete. The eluted RNA will be in position 6.

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.

Benefits of Tempus® Blood RNA Tube Chemistry

Tempus® Blood RNA Tube chemistry is the combination of collecting blood in Tempus tubes and purifying RNA through Applied Biosystems Total RNA chemistry. Tempus Blood RNA Tubes chemistry has the following benefits:

- The Applied Biosystems Stabilizing Reagent in the Tempus tube lyses whole blood cells and stabilizes RNA in a single step. No pretreatment of blood is required before purification of RNA from the sample.
- This MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit makes it possible to isolate RNA conveniently from larger starting volumes of blood using standard laboratory centrifuges.
- The extracted RNA is pure ($A_{260}/_{280}$ ratio >1.8), with very low levels of protein and gDNA contamination.
- You can isolate ~3 to 25 µg of RNA from 3 mL of blood.
- The gene expression profile is immediately frozen. The profile remains stable for up to 5 days at room temperature or at least 1 week at 4 °C.

APPENDIX C

Safety

This appendix covers:

- Chemical safety 34
  - General chemical safety 34
  - SDSs 34
  - Chemical waste safety 35
  - Biological hazard safety 36

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- 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. (See "About SDSs" on page 34.)
- 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 SDS.
- 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 SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

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

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

Obtaining SDSs

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.

3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- 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 SDS.
- 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 SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel 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

General biohazard

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:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**).
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **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

Documentation and Support

Documentation

Kit documentation

The following documents are available for this MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit:

Document	Part number	Description
MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Compatible with Tempus® Blood RNA Tubes) Protocol	4452007	Provides detailed procedures for preparing samples that were taken with Tempus® Blood RNA Tube.
MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Compatible with Tempus® Blood RNA Tubes) Quick Reference Card	4452004	Provides at-a-glance procedures for preparing samples that were taken with Tempus® Blood RNA Tube.

Related documentation

When using this protocol, you may find the documents listed below useful. To obtain this and additional documentation, see "Obtaining support" on page 38.

Document	Part number
Tempus® Blood RNA Tube and Large-Volume Consumables Protocol	4345218
MagMAX™ Express-96 Deep Well Magnetic Particle Processor scripts	Go to: **www.ambion.com/techlib/automation**

Obtaining support

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

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

Providing feedback

To provide feedback on this product and/or protocol, please contact your local Applied Biosystems Technical Support or Sales facility. To find the contact information for your area, go to Applied Biosystems web site:

www.appliedbiosystems.com

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www.invitrogen.com/support