Tempus™ Blood RNA Tube and Tempus™ Spin RNA Isolation Kit

Protocol

Applied Biosystems

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Contents

Preface and Safety Information

This preface covers:

Safety

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.

Definitions

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 signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD.** Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death. Some of the chemicals referred to in this protocol may not have been provided with your kit. If the chemicals are not provided, they are not manufactured or sold by Applied Biosystems. Please obtain the material safety data sheets from their manufacturers or distributors.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

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

About MSDSs

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

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

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems or Ambion is available to you free 24 hours a day.

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

To obtain MSDSs supplied by Applied Biosystems:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field of the MSDS Search page:
   a. Enter the chemical name, part number, or other information that you expect to appear in the MSDS of interest.
   b. Select the language of your choice.
   c. Click **Search**.
3. To view, download, or print the document of interest:
   a. Right-click the document title.
   b. Select:
      - **Open** – To view the document
      - **Save Target As** – To download a PDF version of the document to a destination that you choose
      - **Print Target** – To print the document
4. To have a copy of an MSDS sent by fax or e-mail, in the Search Results page:
   a. Select **Fax** or **Email** below the document title.
   b. Click **RETRIEVE DOCUMENTS** at the end of the document list.
   c. Enter the required information.
   d. Click **View/Deliver Selected Documents Now**.

To obtain MSDSs supplied by Ambion:

1. Go to **http://www.ambion.com/techlib/index.html**
2. In the Restrict by Title Words or Keywords field of the Technical Resources page:
   a. Enter the chemical name or catalog number for the MSDS of interest.
   b. Select the **MSDSs** check box.
   c. Click **Find Documents**.
3. To view, download, or print the document of interest:
   a. Right-click the document title.
   b. Select:

- **Open** – To view the document
- **Save Target As** – To download a PDF version of the document to a destination that you choose
- **Print Target** – To print the document

Chemical Waste Hazards

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

WARNING **CHEMICAL WASTE HAZARD.** Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

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 Material Safety Data Sheets (MSDSs) 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 MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the 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 when you operate the instrument, 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 instrument 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

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; **http://bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **http://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:

http://www.cdc.gov

How to Obtain Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

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techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **http://www.appliedbiosystems.com**, then click the link for **Support**. (See "How to Obtain Support" above).

Tempus™ Blood RNA Tube and Tempus™ Spin RNA Isolation Kit Protocol

Product Overview

Purpose

Gene expression measurements in human whole blood are becoming an increasingly important research tool. However, isolating high quality RNA from human whole blood is complicated by the instability of gene expression profiles in blood collected in standard evacuated tubes and stored at room temperature.

With the Tempus™ Blood RNA Tube, you can draw blood directly into a reagent that stabilizes RNA at room temperature for up to five days. The reagents and consumables included in the Tempus™ Spin RNA Isolation Kit allow you to isolate 2 to 8 µg of high quality RNA per milliliter of whole blood

About Tempus™ Blood RNA Tubes

The Tempus tube contains 6 mL of Applied Biosystems Stabilizing Reagent, which effectively lyses blood cells. After the blood is drawn into the tube and mixed with the reagent, lysis occurs almost immediately. The stabilizing reagent inactivates cellular RNases and selectively precipitates RNA; genomic DNA (gDNA) and proteins remain in solution.

After drawing blood into the Tempus tube, you can use RNA isolation chemistry to purify high quality RNA without sample pretreatments such as leukocyte isolation or selective red blood cell (RBC) lysis.

About the Tempus™ Spin RNA Isolation Kit

The Spin Kit consists of:

- 1 bag of 50 RNA purification filters
- 2 boxes of 100 2-mL collection tubes
- 2 80-mL bottles of 1× PBS
- 1 24-mL bottle of RNA Purification Resuspension Solution
- 2 22-mL bottles of RNA Purification Wash Solution 1
- 1 120-mL bottle of RNA Purification Wash Solution 2
- 4 1.9-mL tubes of Nucleic Acid Purification Elution Solution

About the Tempus™ Blood RNA Isolation Sample Kit

The procedures in this protocol can also be used to isolate RNA using the Tempus™ Blood RNA Isolation Sample Kit.

The Tempus Sample Kit consists of:

- 6 Tempus™ Blood RNA Tubes
- 1 bag of 7 RNA purification filters
- 1 bag of 24 2-mL collection tubes
- 1 25-mL bottle of 1× PBS
- 1 24-mL bottle of RNA Purification Resuspension Solution
- 1 22-mL bottles of RNA Purification Wash Solution 1
- 1 25-mL bottle of RNA Purification Wash Solution 2
- 1 1.9-mL tube of Nucleic Acid Purification Elution Solution

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. It 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.
- The Spin Kit makes it possible to isolate RNA conveniently from larger starting volumes of blood using standard laboratory centrifuges.
- Extracted RNA is pure ($A_{260/280}$ ratio > 1.9), with very low levels of protein and gDNA contamination.
- You can isolate up to 6 to 25 µg of RNA from 3 mL blood.
- The gene expression profile of important gene targets is immediately frozen, and the profile remains stable for up to five days at room temperature and at least one week at 4 °C.

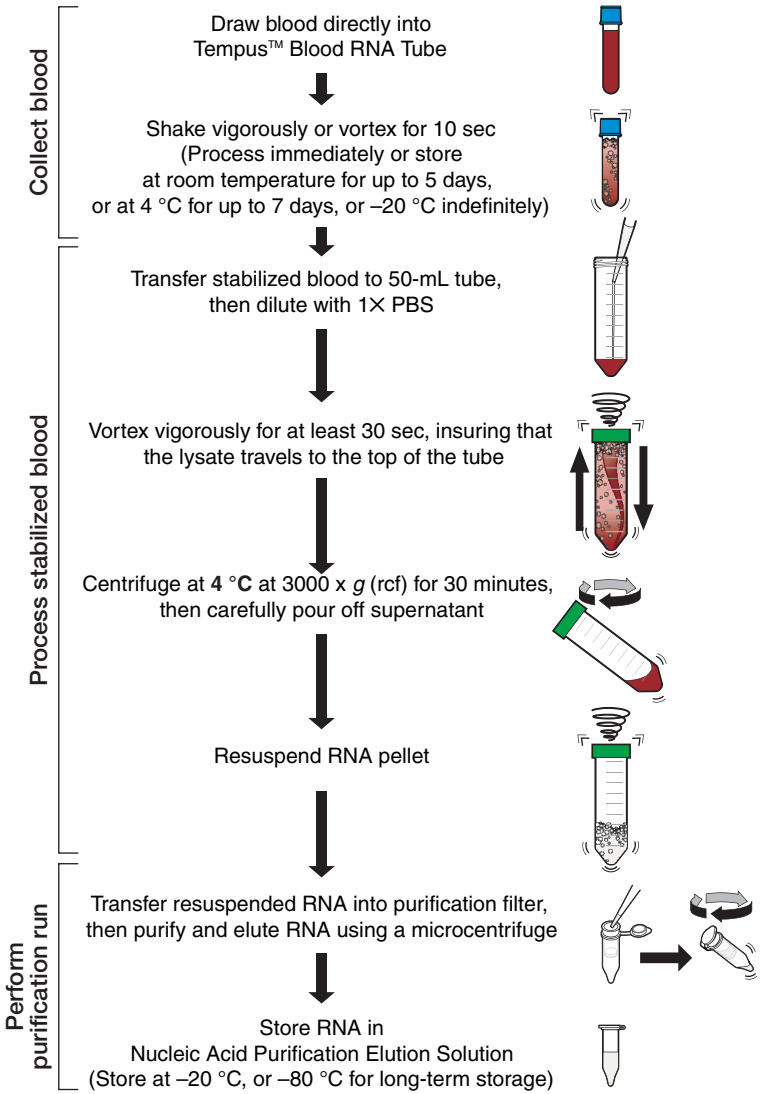
Protocol Overview

About This Protocol

This protocol describes the steps required to use the Spin Kit to purify RNA from a 3-mL sample of whole blood collected in a Tempus tube. This protocol can also be used with the Tempus Sample Kit.

Procedure Flowchart

The following diagram provides an overview of the procedure described in this protocol.



Materials and Equipment

Unless otherwise noted, many of the items listed can be obtained from a major laboratory supplier (MLS).

Consumables and Reagents

Item	Supplier	PN
Required Consumables and Reagents		
Tempus™ Spin RNA Isolation Kit • 1 bag of 50 RNA purification filters • 2 boxes of 100 2-mL collection tubes • 2 80-mL bottles of 1× PBS • 1 24-mL bottle of RNA Purification Resuspension Solution • 2 22-mL bottles of RNA Purification Wash Solution 1 • 1 120-mL bottle of RNA Purification Wash Solution 2 • 4 1.9-mL tubes of Nucleic Acid Purification Elution Solution	Applied Biosystems	4380204
Tempus™ Blood RNA Tube	Applied Biosystems	4342792
Sterile conical tubes, 50-mL • 200 count • 250 count	Ambion	 AM12501 AM12502
Pipette tips **Note:** See the Ambion Web site (**www.ambion.com**) for sizes and part numbers	Ambion	See the Ambion Web site
Pipettes, 5-mL, 10-mL, 25-mL	MLS	–

Item	Supplier	PN
Alternative Consumables and Reagents		
Tempus™ Blood RNA Isolation Sample Kit	Applied Biosystems	4380202
Tempus™ 12-Port RNA Isolation Kit	Applied Biosystems	4378672
Optional Consumables and Reagents		
2-mL collection tubes, 100 count	Ambion	AM12480
AbsoluteRNA Wash Solution	Applied Biosystems	4305545
RNase-free water **Note:** See the Ambion Web site (**www.ambion.com**) for quantities and part numbers.	Ambion	See the Ambion Web site
Ethanol, 100%	MLS	–

Required Equipment

Item	Supplier
Vortexer	MLS
Microcentrifuge	MLS
Heating Block for Microcentrifuge Tubes	MLS
Centrifuge (greater than 3,000 x *g* (rcf), temperature controlled)	MLS

Optional Materials

Item	Supplier	PN
High-Capacity cDNA Reverse Transcription Kit • 1000 reactions • 200 reactions • 1000 reactions, with RNase Inhibitor • 200 reactions, with RNase Inhibitor	Applied Biosystems	 4368813 4368814 4374967 4374966
TaqMan® One-Step RT-PCR Master Mix Reagents Kit • 200 reactions • 2000 reactions	Applied Biosystems	 4309169 4313803
TaqMan® Gold RT-PCR Kit • 200 reactions, with controls • 200 reactions, without controls • 2000 reactions, without controls	Applied Biosystems	 N8080233 N8080232 4304133
TaqMan® EZ RT-PCR Core Reagents • 200 reactions, with controls • 200 reactions, without controls • 2000 reactions, without controls	Applied Biosystems	 N8080235 N8080236 403028
GLOBINclear™ Whole Blood Globin Reduction Kit (Human), 40 reactions	Ambion	AM1980
MessageAmp™ aRNA Amplification Kit, 20 reactions	Ambion	AM1750
MessageAmp™ II aRNA Amplification Kit, 20 reactions	Ambion	AM1751
MessageAmp™ II-96 aRNA Amplification Kit, 100 reactions	Ambion	AM1819

Related Documentation

Document Title	Supplier	PN
Tempus™ Blood RNA Tube and Tempus™ Spin RNA Isolation Kit Quick Reference Card	Applied Biosystems	4379233

Note: For additional protocols, see the Applied Biosystems Web site. Go to **docs.appliedbiosystems.com/search.taf**.

Collecting and Storing Blood in Tempus™ Blood RNA Tubes

Standard Procedures for Drawing Blood

Tempus tubes are used for the collection of venous whole blood specimens to stabilize RNA prior to purification for gene expression analysis. Refer to the product documentation of your blood collection set for specific instructions on venipuncture technique and blood collection. If you are using the Greiner Vacuette® Safety Blood Collection Set, refer to the Vacuette Web site (**www.vacuette.com**) for additional information.

BIOHAZARD SAFETY. For additional safety information, follow the precaution, cautions, and prevention instructions listed below for specimen collection.

CHEMICAL HAZARD. Tempus Blood RNA Tube. Exposure to the contents causes eye, skin, and respiratory tract irritation. Contents are harmful if swallowed. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Precaution

Do not use Tempus tubes if foreign matter is present!

Cautions

- Handle all biological samples and blood collection “sharps” (lancets, needles, luer adapters, and blood collection sets) according to the policies and procedures of your facility.
- Obtain appropriate medical attention in the case of any exposure to biological samples (for example, through a puncture injury), since they may transmit HIV (AIDS), viral hepatitis, or other infectious disease.
- Discard all blood collection “sharps” in biohazard containers approved for their disposal.
- Transferring a sample from a syringe to a Tempus tube is not recommended. Additional manipulation of sharps increases the potential for needle stick injury. In addition, depressing the syringe plunger during transfer can create a positive pressure,

forcefully displacing the stopper and sample and causing a potential blood exposure. Using a syringe for blood transfer may also cause over or under filling of tubes, resulting in an incorrect blood-to-additive ratio and potentially incorrect analysis results.

- If blood is collected through an intravenous (IV) line, ensure that the line has been cleared of IV solution before beginning to fill the Tempus tubes. Clearing the line is critical to avoid erroneous laboratory data from IV fluid contamination.
- All liquid preservatives and anticoagulants are clear and colorless. Do not use the Tempus tubes if they are discolored or contain precipitates.
- Do not use the Tempus tubes after their expiration date.

Prevention of Backflow

Tempus tubes contain chemical additives. To prevent backflow from the tube into the individual's arm, observe the following precautions:

- Place the individual's arm in a downward position.
- Hold the tube with the cap up.
- Release the tourniquet as soon as the blood starts to flow into the tube.
- Make sure the tube contents do not touch the cap or the end of the needle during venipuncture.

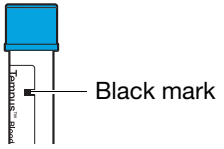
Collecting Blood

To collect blood in Tempus tubes:

1. Draw 3 mL of blood directly into the Tempus Blood RNA Tube, following your laboratory's standard procedures for drawing blood from individuals into blood collection tubes containing liquid reagents. Observe the appropriate safety practices when collecting blood.

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

   Note: Filling up the tube to the black mark on the tube label indicates the collection of approximately 3 mL of blood.



2. Immediately after the Tempus tube is filled, stabilize the blood by shaking the tube vigorously or vortexing the contents 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 clog the purification filter.

Storing and Transporting Blood in Tempus™ Blood RNA Tubes

Applied Biosystems recommends that you store or ship Tempus tubes containing stabilized samples in the following order of preference:

Storage/Shipping Options	Temperature Requirement (°C)
Store or ship refrigerated within 7 days or less. (Recommended)	4
Store or ship on dry ice. **IMPORTANT!** Avoid direct contact of sample with dry ice!	−20 to −80
Store or ship at room temperature within 5 days or less.	18 to 25

Isolating RNA from Whole Blood Using the Tempus™ Spin RNA Isolation Kit

Note: The following procedures can also be used with the Tempus Sample Kit.

RNA isolation involves centrifuging the diluted lysate to collect the RNA precipitate. The RNA is further purified in the spin columns, then eluted into 1.5-mL microcentrifuge tubes.

RNA Isolation Procedure Overview

To isolate RNA from stabilized blood:

1. Process stabilized blood before purification (see page 11).
2. Perform the purification run (see page 14).

The parameters and reagents specific to this protocol are provided in the procedures.

BLOODBORNE/INFECTIOUS WASTE HAZARD. Discard the blood-containing wastes following recognized disinfection procedures and in accordance with all local, state, and national bloodborne/infection regulations.

Processing Stabilized Blood Before Purification

During the blood collection process, the blood is stabilized by mixing it 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 calcium- and magnesium-free phosphate-buffered saline (PBS) before extracting RNA. Failure to do so results in significantly lower RNA yields.

WARNING **CHEMICAL HAZARD**. **Tempus Blood RNA Tube.** Exposure to the contents causes eye, skin, and respiratory tract irritation. Contents are harmful if swallowed. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

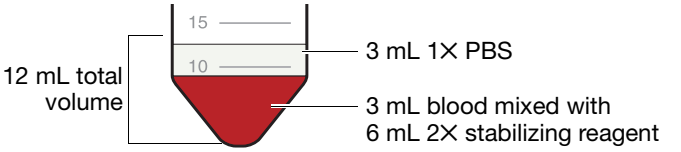
WARNING **CHEMICAL HAZARD. Resuspension Solution, RNA Purification** may be harmful if swallowed. Exposure may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To process stabilized blood:

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

1. If the sample is frozen, thaw the sample in the Tempus tube at room temperature (18 to 25 °C).
2. Remove the cap from the Tempus tube, then pour the contents of the tube into a clean 50-mL tube (such as a 50-mL Ambion conical tube).

3. Pipet 3 mL of 1× PBS (Ca^{2+}/Mg^{2+}-free) into the tube to bring the total volume to 12 mL.

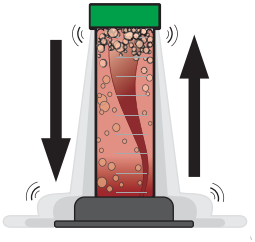


IMPORTANT! If the initial blood sample was less than 3 mL, make up the difference by adding enough 1× PBS to bring the total volume to 12 mL. Otherwise, RNA yields decrease significantly.

4. Replace the cap on the tube, then vortex the tube *vigorously* (at maximum vortex speed) for 30 seconds to ensure proper mixing of the contents.

Note: To prevent the tube from leaking and spraying the sample during vortexing, make sure the tube is capped properly.

IMPORTANT! Vortex the diluted sample for at least 30 seconds; vortexing for less than 30 seconds may cause clogging of the purification consumable.



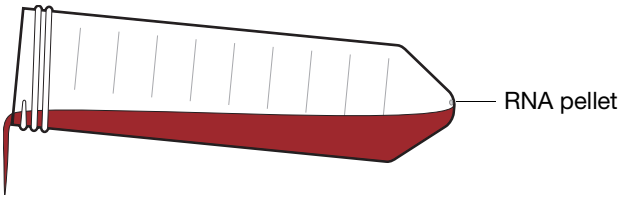
Note: Frothing of the sample after vortexing is normal.

5. Centrifuge the tube at **4 °C** at 3,000 x *g* (rcf) for 30 minutes.

6. Carefully pour off the supernatant.

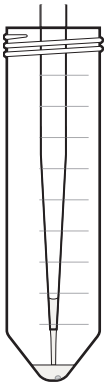
   Note: The RNA pellet is transparent and invisible.

   IMPORTANT! Handle the tube carefully so that you do not shake the RNA pellet off the bottom of the tube.



7. Leave the tube inverted on absorbent paper for 1 to 2 minutes.
8. Blot the remaining drops of liquid off the rim of the tube with clean absorbent paper.
9. Pipet 400 µL of RNA Purification Resuspension Solution into the tube, then vortex briefly to resuspend the RNA pellet.

   IMPORTANT! To prevent washing any blood residue down the inside of the tube, insert the pipet tip into the tube and add the resuspension solution to the bottom of the tube.



10. The resuspended RNA can be kept on ice while preparing for the next steps.

Proceed to "Performing the Purification Run" on page 14.

Performing the Purification Run

Note: The RNA isolated in this procedure contains very low levels of genomic DNA (less than 0.05% by weight). If you are using the RNA with assays for low-expressing genes, you may want to perform an optional DNase treatment to further reduce the trace amounts of DNA that might interfere with signal detection and mask signals. All steps are at room temperature unless otherwise stated.

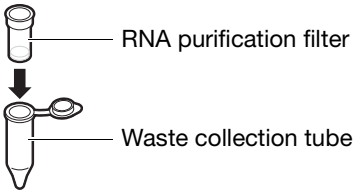
CAUTION CHEMICAL HAZARD. RNA Purification Wash Solution 1 and Nucleic Acid Purification Elution Solution may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING CHEMICAL HAZARD. RNA Purification Wash Solution 2 is a flammable liquid. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. It may cause central nervous system effects such as drowsiness, dizziness, and headache. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING CHEMICAL HAZARD. Tempus Blood RNA Tube. Exposure to the contents causes eye, skin, and respiratory tract irritation. Contents are harmful if swallowed. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To perform the purification run:

1. Label the RNA purification filter, then insert the filter into a waste collection tube.



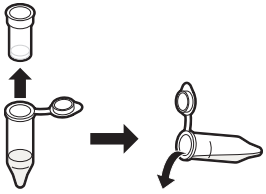
2. Pre-wet the filtration membrane by pipeting RNA Purification Wash Solution 1 into the purification filter.

Wash Solution 1	Time	Centrifuge
100 µL	—	—

3. Pipet the resuspended RNA into the purification filter, then centrifuge.

Resuspended RNA	Time	Centrifuge
~400 µL	30 sec	16,000 x *g*

4. Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.



Note: Each time you discard the liquid waste, instead of reusing the waste tube, you can transfer the purification filter into a new collection tube (not provided in the kit). See page 5 for ordering information.

5. Pipet RNA Purification Wash Solution 1 into the purification filter, then centrifuge.

Wash Solution 1	Time	Centrifuge
500 µL	30 sec	16,000 x *g*

6. Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.

7. Pipet RNA Purification Wash Solution 2 into the purification filter, then centrifuge.

   IMPORTANT! When a DNase treatment is required, extend the centrifuge time to 1 minute to remove all wash solutions and dry the membrane completely.

Wash Solution 2	Time	Centrifuge
500 µL	30 sec‡	16,000 x *g*

‡ 60 sec, if a DNase treatment is required

8. Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.
9. (*Optional*) Perform a DNase treatment:
   a. Pipet AbsoluteRNA Wash Solution (not provided) into the purification filter, then incubate at room temperature.

AbsoluteRNA Wash Solution	Time	Centrifuge
100 µL	15 min	—

   b. Pipet RNA Purification Wash Solution 2 into the purification filter, incubate, then centrifuge.

Wash Solution 2	Time	Centrifuge
500 µL	5 min	—
	30 sec	16,000 x *g*

   c. Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.
10. Pipet RNA Purification Wash Solution 2 into the purification filter, then centrifuge.

Wash Solution 2	Time	Centrifuge
500 µL	30 sec	16,000 x *g*

11. Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.

12. Centrifuge to dry the membrane.

Solution	Time	Centrifuge
—	30 sec	16,000 x *g*

13. Transfer the purification filter to a new, labeled collection tube to collect the eluate.

14. Pipet Nucleic Acid Purification Elution Solution into the purification filter, close the cap, incubate the entire tube, then centrifuge.

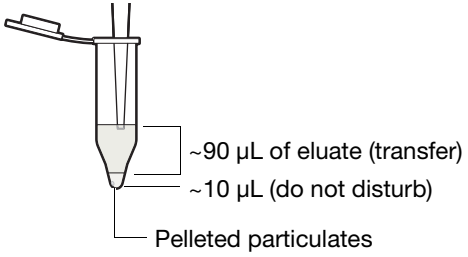
Elution Solution	Time	Centrifuge	Incubate
100 µL	2 min	—	70 °C
	30 sec	16,000 x *g*	—

15. Pipet the collected RNA eluate back into the purification filter, then centrifuge. No incubation is necessary.

RNA Eluate	Time	Centrifuge
~100 µL	2 min	Maximum (16,000 to 18,000 x *g*)

16. Discard the purification filter, then transfer approximately 90 µL of the RNA eluate to a new, labeled collection tube.

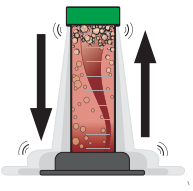
    IMPORTANT! When transferring the RNA eluate, carefully pipet the liquid out of the collection tube starting from the top of the liquid to ensure that the pelleted particulates are not disturbed.



17. Replace the cap on the new collection tube, then store the RNA at −20 ·C, or −80 ·C for long-term storage.

Appendix A: Troubleshooting Tips

Problem	Possible Cause	Solution
Filters appear black or brownish-red even after using Wash Solutions 1 and 2	Insufficient mixing immediately after the blood draw	Shake the filled Tempus tube vigorously or vortex the sample for 10 to 20 seconds immediately after drawing the blood into each tube.
	The sample was contaminated with blood residue when the resuspension solution was added	Repeat the wash steps: 1. Pipet 500 µL of RNA Purification Wash Solution 1 into the purification filter, then centrifuge for 30 seconds at 16,000 × *g*. 2. Pipet 500 µL of RNA Purification Wash Solution 2 into the purification filter, then centrifuge for 30 seconds at 16,000 × *g*. **IMPORTANT!** When a DNase treatment is required, extend the centrifuge time to 1 minute to remove all wash solutions and dry the membrane completely.
Sample leaks	The tube was not capped properly before vortexing	Make sure each tube is capped properly before vortexing.
RNA is degraded	Residual protein (RNase activity)	Increase the number of wash steps with RNA Purification Solution Wash1 and 2 in the next run until the membrane appears white.
	The blood lysate was exposed to >37 °C for short period	RNA has gone back into solution. 1. Freeze any remaining lysate. 2. Thaw lysate and repurify.
	Insufficient mixing after the blood draw and during dilution	Vortex the sample: • For 10 seconds after the blood draw • For 30 seconds after diluting with 1× PBS

Problem	Possible Cause	Solution
Excessive gDNA contamination	The filter was not completely dry when AbsoluteRNA Wash Solution was added	Ensure that the filter is completely dry before proceeding to the DNase treatment. **IMPORTANT!** When a DNase treatment is required, extend the centrifuge time in step 7 on page 16 to 1 minute to remove all wash solutions and dry the membrane completely.
	Insufficient incubation time after adding the AbsoluteRNA Wash Solution	After adding the AbsoluteRNA Wash Solution, incubate for the entire 15 minutes.
	Air is trapped underneath the AbsoluteRNA Wash Solution	Ensure that the membrane is wetted completely with AbsoluteRNA Wash Solution.
No RNA or low RNA yield	The blood sample was less than 3 mL	Make sure the Tempus tube is filled with blood up to the black mark on the tube label.
		Make up the difference after transferring the sample to the 50-mL tube by adding enough 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 1X PBS to bring the total volume of the diluted blood lysate in the 50-mL tube to 12 mL.
	The filters were not completely dry before the Nucleic Acid Purification Elution Solution was added	RNA remains on the membrane (in residual RNA Purification Wash Solution 2). Re-elute the RNA with another aliquot of Nucleic Acid Purification Elution Solution.
	Insufficient mixing during sample dilution with PBS	Vortex the samples for 30 seconds after diluting them with PBS. 

Problem	Possible Cause	Solution
No RNA or low RNA yield *(continued)*	The blood lysate was exposed to >37 °C for short period	RNA has gone back into solution. 1. Freeze any remaining lysate. 2. Thaw lysate and repurify.
	Insufficient centrifugation time and speed when processing the stabilized blood samples before purification	Centrifuge the samples for at least 30 minutes or longer at 4,000 x *g* or higher.
	The RNA pellet was lost during decanting	Pour off the supernatant slowly and carefully without jarring the tube.
	The filter membrane was not completely wet with the Nucleic Acid Purification Elution Solution	Allow the elution solution to wet the entire filter area.
		Increase the volume of elution solution or add another elution step using additional elution solution.
		Reload the same eluate back into the filter, then centrifuge for 2 minutes at 16,000 × *g*.
	Insufficient incubation of elution solution	Allow at least 2 minutes, at 70 °C, for the elution solution to soak through the membrane.
	Residual RNA is trapped on the membrane	Reload the same eluate back into the filter, then centrifuge for 2 minutes at 16,000 × *g*.

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