Tempus™ Blood RNA Tube and Tempus™ 12-Port RNA Isolation Kit

Protocol

Applied Biosystems

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Preface and Safety Information

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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

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; **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

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

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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™ 12-Port 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™ 12-Port RNA Isolation Kit allow you to isolate 2 to 8 µg of high quality RNA per milliliter of whole blood from up to 12 samples using the ABI PRISM® 6100 Nucleic Acid PrepStation.

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™ 12-Port RNA Isolation Kit

The 12-Port Kit consists of:

- 2 splash guards
- 2 12-port adapter plates
- 24 slip-fit cap plugs
- 24 RNA filter vials
- 72 15-mL reservoirs
- 24 1.5-mL collection tubes
- 1 80-mL bottle of 1✕ PBS
- 3 95-mL bottles of RNA Purification Wash Solution 1
- 1 1-L bottle of RNA Purification Wash Solution 2
- 3 1.9-mL tubes 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 12-Port Kit makes it possible to isolate RNA conveniently from larger starting volumes of blood using the 6100 PrepStation. This workflow requires only one microcentrifugation step.
- 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.

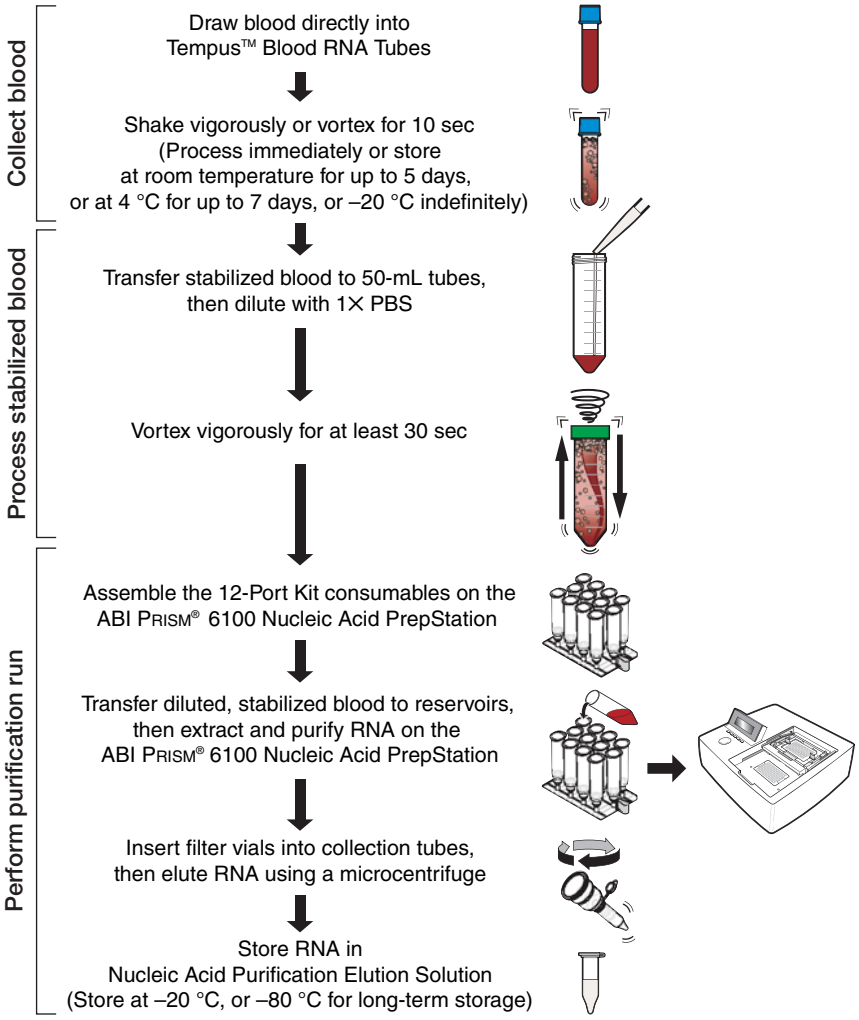
Protocol Overview

About This Protocol

This protocol describes the steps required to purify RNA from 3-mL samples of whole blood collected in Tempus tubes using the 12-Port Kit and the ABI PRISM® 6100 Nucleic Acid PrepStation.

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™ 12-Port RNA Isolation Kit • 2 splash guards • 2 12-port adapter plates • 24 slip-fit cap plugs • 24 RNA filter vials • 72 15-mL reservoirs • 24 1.5-mL collection tubes • 1 80-mL bottle of 1× PBS • 3 95-mL bottles of RNA Purification Wash Solution 1 • 1 1-L bottle of RNA Purification Wash Solution 2 • 3 1.9-mL tubes of Nucleic Acid Purification Elution Solution	Applied Biosystems	4378672
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™ Spin RNA Isolation Kit	Applied Biosystems	4380204
Optional Consumables and Reagents		
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
ABI PRISM® 6100 Nucleic Acid PrepStation	See your Applied Biosystems sales representative
Vortexer	MLS
Microcentrifuge	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™ 12-Port RNA Isolation Kit Quick Reference Card	Applied Biosystems	4379229
ABI PRISM® 6100 Nucleic Acid PrepStation User Guide	Applied Biosystems	4326242

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.

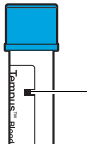
Collecting Blood

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 on dry ice. (Recommended) **IMPORTANT!** Avoid direct contact of sample with dry ice!	-20 to -80
Store or ship refrigerated within 7 days or less.	4
Store or ship at room temperature within 5 days or less.	18 to 25

Isolating RNA from Whole Blood Using the Tempus™ 12-Port RNA Isolation Kit

RNA isolation involves passing processed, stabilized blood across an RNA filter and eluting trapped RNA into 1.5-mL elution tubes.

RNA Isolation Procedure Overview

To isolate RNA from stabilized blood:

1. Process stabilized blood before purification (see below).
2. Assemble the purification consumables (see page 12).
3. Create or edit the *Tempus RNA* method, if necessary (see Appendix A on page 23).
4. 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

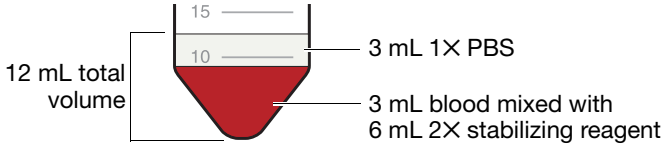
During the blood collection process, the blood is stabilized by mixing it with the Applied Biosystems Stabilizing Reagent contained in the Tempus tube. The stabilizing reagent must have a final concentration of 1X. To adjust the concentration of the stabilizing reagent for purification, dilute the stabilized blood with calcium- and magnesium-free phosphate-buffered saline (PBS) before extracting RNA. Failure to do so results in significantly lower RNA yields.

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 process stabilized blood:

Note: If you are processing a large batch of samples, keep the samples on ice as much as possible. Otherwise, RNA yields may decrease significantly.

1. If the samples are frozen, thaw the samples in the Tempus tubes at room temperature (18 to 25 °C).
2. Remove the caps from the Tempus tubes, then pour the contents of the tubes into clean 50-mL tubes (such as 50-mL Ambion conical tubes).
3. Pipet 3 mL of 1× PBS (Ca^{2+}/Mg^{2+}-free) into each tube to bring the total volume to 12 mL.

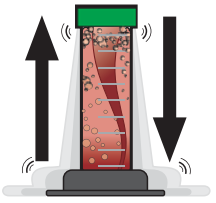


IMPORTANT! If the initial blood sample was less than 3 mL, make up the difference by adding enough 1× PBS to bring the total volume to 12 mL. Otherwise, RNA yields decrease significantly.

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

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

   Note: To prevent the tubes from leaking and spraying the samples during vortexing, make sure the tubes are capped properly.



   Note: Frothing of the samples after vortexing is normal.

5. Keep the processed samples on ice.

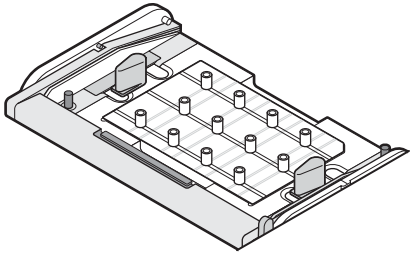
Proceed to "Assembling the Purification Consumables" on page 12.

Assembling the Purification Consumables

You can process a maximum of 12 samples at one time using the 12-Port Kit with the ABI PRISM® 6100 Nucleic Acid PrepStation. RNA filter vials and reservoirs have press-fit connections.

To assemble the purification consumables on the ABI PRISM® 6100 Nucleic Acid PrepStation:

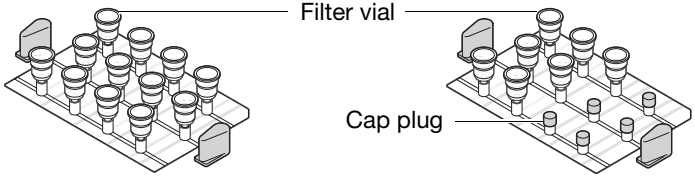
1. Place a new splash guard onto the waste station of the 6100 PrepStation.
2. Assemble the 12-Port Kit consumables on the 6100 PrepStation:
   a. Place the 12-port adapter plate onto the purification tray carriage.



   b. Label the filter vials, then insert a filter vial into each port on the adapter plate.

      If you have fewer than 12 samples: Cover the unused ports with cap plugs.

      Note: Cap plugs are included in the 12-Port Kit.

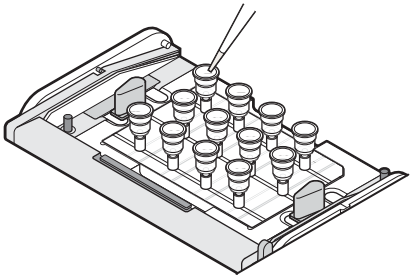


For 12 samples

For fewer than 12 samples

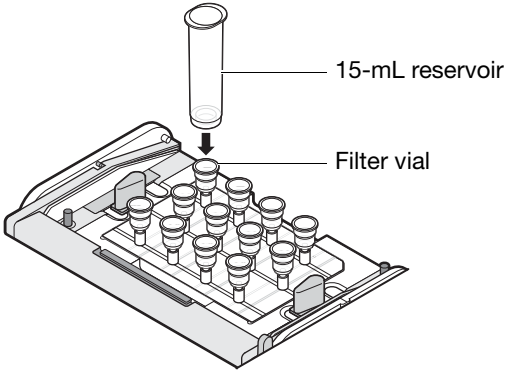
c. Pre-wet the membranes by pipeting RNA Purification Wash Solution 1 into each filter vial.

Wash Solution 1	Time	Vacuum
350 µL	—	—



d. Attach a 15-mL reservoir to the top of each of the filter vials.

IMPORTANT! Make sure each reservoir sits properly on the filter vial by pressing firmly to ensure a tight seal.



3. Move the carriage over the Waste position, then push the carriage handle down until it locks into position.
4. Press down on the corners of the adapter plate to ensure that it is properly seated, then lock the plate in position by rotating the locking knobs on both sides.

Proceed to "Performing the Purification Run."

Performing the Purification Run

Purifying 12 samples on the ABI PRISM® 6100 Nucleic Acid PrepStation takes approximately 45 to 50 minutes. For further information on running protocols on the 6100 PrepStation, refer to the *ABI PRISM® 6100 Nucleic Acid PrepStation User Guide.*

IMPORTANT! If you have not created the *Tempus RNA* method, create it before proceeding. If you have a pre-programmed Tempus RNA method, confirm that it is correct and edit as necessary. See Appendix A on page 23.

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.

CAUTION CHEMICAL HAZARD. RNA Purification Wash Solution 1 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.

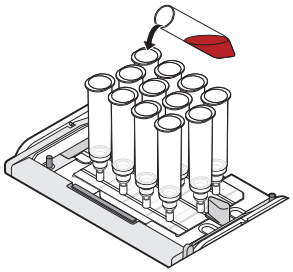
To perform the purification run:

Note: Make sure the carriage of the 6100 PrepStation is in the Waste position.

1. Transfer the diluted blood lysate by pouring the entire contents of each 50-mL tube into separate, pre-wetted reservoirs, then apply vacuum.

   Note: You can manually stop the vacuum at any time after all the samples have passed through the filters.

Blood Lysate	Time	Vacuum
~ 12 mL	300 sec	80%



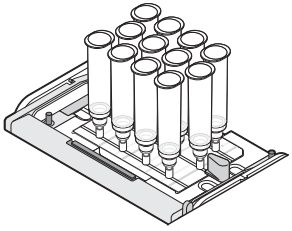
   Note: If all the samples have not passed through the filters in the specified time, run the vacuum for additional time until all samples are completely evacuated.

2. Pipet RNA Purification Wash Solution 1 into each reservoir, then apply vacuum.

   Note: To rinse the remaining lysate off the tube, pipet the wash solution into the 50-mL tube, then pour it into the reservoir.

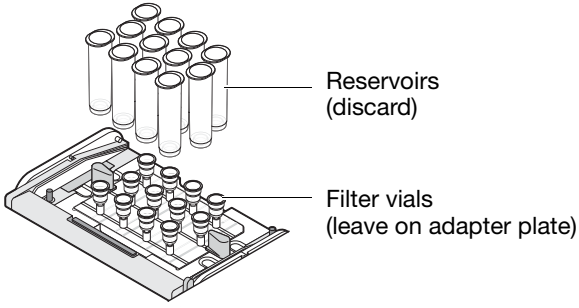
   Note: You can manually stop the vacuum at any time after all the samples have passed through the filters.

Wash Solution 1	Time	Vacuum
5 mL	300 sec	80%



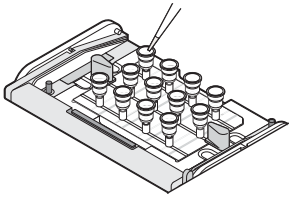
   Note: If all the samples have not passed through the filters in the specified time, run the vacuum for additional time until all samples are completely evacuated.

3. Replace the reservoirs:
   a. Leaving the filter vials on the adapter plate, remove and discard the reservoirs.



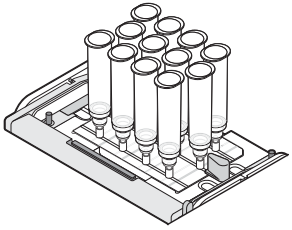
b. Clean the filter vials, including the rims, by pipeting RNA Purification Wash Solution I into each filter vial, then apply vacuum until all wash solution is completely evacuated.

Wash Solution 1	Time	Vacuum
~700 µL	Until evacuated	80%



c. Attach a new set of reservoirs to the filter vials.

Note: Make sure the filters are clean before attaching the new reservoirs. If necessary, wipe the rims with a clean, lint-free paper.



IMPORTANT! Make sure each reservoir sits properly on the filter vial by pressing firmly to ensure a tight seal.

4. Pipet RNA Purification Wash Solution 1 into each reservoir, then apply vacuum.

Note: You can manually stop the vacuum at any time after all the samples have passed through the filters.

Wash Solution 1	Time	Vacuum
5 mL	600 sec	80%

Note: If all the samples have not passed through the filters in the specified time, run the vacuum for additional time until all samples are completely evacuated.

5. Pipet RNA Purification Wash Solution 2 into each reservoir, then apply vacuum until the wash solution is evacuated completely and the filters are dry.

   IMPORTANT! When a DNase treatment is required, run the vacuum step for a minimum of 180 seconds to remove the wash solution completely.

Wash Solution 2	Time	Vacuum
5 mL	≥180 sec	80%

6. Remove and discard the reservoirs, checking that each membrane is completely white. If a membrane is not completely white, reattach the reservoir, then repeat steps 4 and 5.
   - If a DNase treatment is required, go to step 7.
   - If a DNase treatment is not required, attach a new set of reservoirs to the filter vials, then go to step 8.

   IMPORTANT! When attaching the reservoirs, make sure each one sits properly on the filter vial by pressing firmly to ensure a tight seal.

7. (*Optional*) Perform a DNase treatment:
   a. Pipet AbsoluteRNA Wash Solution (not provided) into each filter vial, then incubate for 15 minutes.

AbsoluteRNA Wash Solution	Time	Vacuum
350 µL	900 sec	—

   b. Attach a new set of reservoirs to the filter vials.

      IMPORTANT! Make sure each reservoir sits properly on the filter vial by pressing firmly to ensure a tight seal.

   c. Pipet RNA Purification Wash Solution 2 into each reservoir, incubate for 5 minutes, then apply vacuum.

Wash Solution 2	Time	Vacuum
5 mL	300 sec	—
	180 sec	80%

8. Pipet RNA Purification Wash Solution 2 into each reservoir, then apply vacuum.

Wash Solution 2	Time	Vacuum
5 mL	180 sec	80%

9. Pipet more RNA Purification Wash Solution 2 into each reservoir, then apply vacuum.

Wash Solution 2	Time	Vacuum
5 mL	180 sec	80%

10. Leaving the filter vials on the adapter plate, remove the reservoirs.

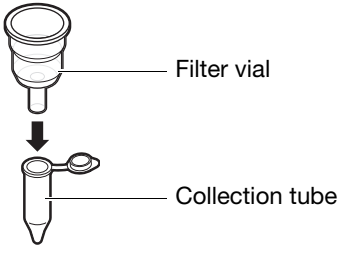
11. Apply vacuum to evacuate the wash solution and dry the filters, pressing **F2** (**Turbo**) during the last 10 seconds of the vacuum step.

    IMPORTANT! To completely dry the filters, press **F2** (**Turbo**) during the last 10 seconds of the step.

Solution	Time	Vacuum
—	300 sec	90% (Turbo during last 10 sec)

12. Elute the RNA using a microcentrifuge:
    a. Remove the filter vials from the adapter plate and insert them into the collection tubes.

       IMPORTANT! Ensure that the filters and the tips of the vials are completely dry before inserting them into the collection tubes. Wipe off any liquid with a clean, lint-free paper.

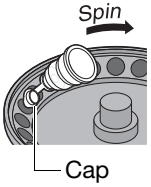


b. Pipet Nucleic Acid Purification Elution Solution into each filter vial and allow the elution solution to completely soak the filters.

Elution Solution	Time	—
200 µL	60 sec	—

c. Load the filter vial-collection tube assemblies into a microcentrifuge, then centrifuge.

IMPORTANT! In the microcentrifuge, make sure the collection tube caps are pointing away from the direction of the spin. For example, if the microcentrifuge spins in the clockwise direction, point the tube cap counterclockwise.



Solution	Time	Centrifuge
—	60 sec	4,000 x *g*

d. Pipet the collected RNA eluate back into the filter vial, then centrifuge.

RNA Eluate	Time	Centrifuge
~200 µL	60 sec	4,000 x *g*

13. Discard the filter vials, replace the caps on the collection tubes, then store the RNA at –20 °C, or –80 °C for long-term storage.

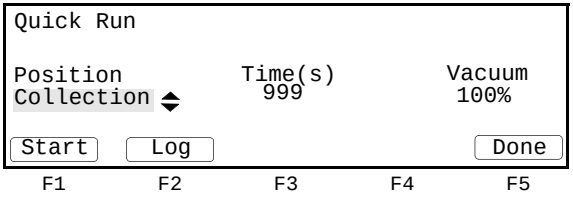
Cleaning the ABI PRISM® 6100 Nucleic Acid PrepStation

Because large volumes of blood lysate pass through the ABI PRISM® 6100 Nucleic Acid PrepStation, clean the Waste position after every run.

IMPORTANT! Do not use bleach to clean the 6100 PrepStation. Use a non-bleach virocide, such as Lysol® or Vesphene®, to clean the equipment.

To clean the ABI PRISM® 6100 Nucleic Acid PrepStation:

1. Remove and discard the large volume adapter plate.
2. Move the carriage to the Collection position.
3. Remove and discard the splash guard from the Waste position.
4. Use the Quick Run feature to flush the Waste position thoroughly with water and cleaning agent, if necessary:
   a. In the main menu, press **F1** (**Quick**) to display the Quick Run screen.



   b. Enter the values shown below, then press **F1** (**Start**).

Position	Time	Vacuum
Waste	180 sec	50%

   Note: Take care that water does not spill over the capture area and the waste container does not overfill.

   c. Flush the Waste position with the chosen cleaning agent.

5. If the white plastic piece that holds the splash guard in place is heavily contaminated, remove and clean it:

   Note: If the waste station is cleaned immediately after each use, this step may not be necessary.

   a. Unscrew the two Allen screws that hold the piece in place.
   b. Lift the plastic piece out.
   c. Wash the piece and the area underneath it thoroughly with Lysol, Vesphene, or 70% alcohol, detergent, and water.
   d. Allow the piece and the area to dry before replacing the plastic piece.

6. Clean the area around the vacuum carriage and gasket with 70% alcohol.

Appendix A: Creating the Tempus RNA Method

Method Definition

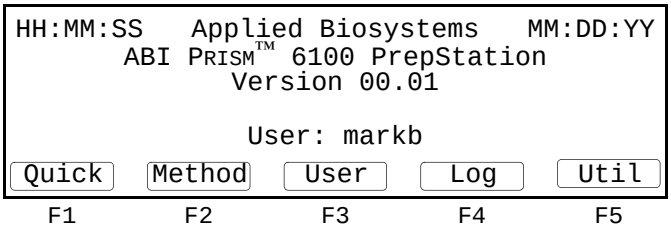
A *method* is a list of steps you perform on the ABI PRISM® 6100 Nucleic Acid PrepStation for a purification protocol.

Tempus RNA Method

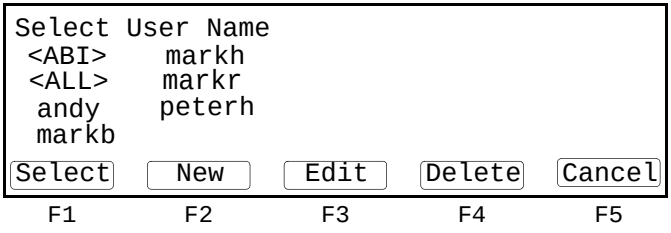
For the Tempus™ Blood RNA Tube protocol, you must program a new method for the purification run. If desired, you can edit the pre-programmed *Tempus RNA* method. For further information on programming the 6100 PrepStation, refer to the *ABI PRISM® 6100 Nucleic Acid PrepStation User Guide.*

To create the Tempus RNA method:

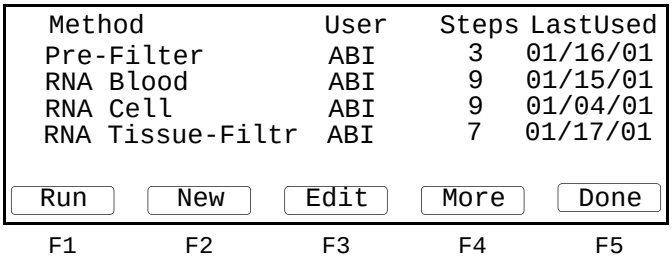
1. In the main menu, press **F3** (**User**) to display the Select User Name screen.



2. Use the arrow keys to highlight the user and press **F1** (**Select**) to display the user or press **F2** (**New**) to go to User Set-Up and enter a new name.



3. Press **F2** (**Method**) to display the Method Select 1 screen.



4. Press **F2** (**New**) and enter parameters for the Tempus RNA method.

   Note: If a pre-programmed Tempus RNA method already exists, use the arrow keys to highlight the method, then press **F3** (**Edit**) and enter the correct parameters.

Step	Position	Time (s)	Vacuum
1	Waste	300	80%
2	Waste	300	80%
3	Waste	*Until evacuated*	80%
4	Waste	600	80%
5	Waste	180	80%
6	Waste	900	—
7	Waste	300	—
8	Waste	180	80%
10	Waste	180	80%
11	Waste	180	80%
13	Waste	300	90%

5. Save the new method as **Tempus RNA**, then continue with "Performing the Purification Run" on page 14.

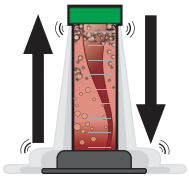
Appendix B: Troubleshooting Tips

Problem	Possible Cause	Solution
Filter appears to be clogged	Air is trapped underneath the blood lysate at the membrane surface	Stop the vacuum step and restart the vacuum at 100%.
		Place the tip of a 1.0 mL pipet in the bottom of the reservoir and gently pipet the lysate up and down to release the trapped air.
	Insufficient mixing immediately after 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.
	Insufficient mixing during sample dilution with PBS	1. Remove the remaining sample from the reservoir. 2. Vortex the sample, then replace the filter vial. 3. Restart the purification procedure.
	Proteins and other blood components have clogged the filter pores	1. Pipet the remaining blood lysate out of the reservoir, then attach a new reservoir. 2. Process a fraction of the remaining blood lysate (for example, 5 mL).
	The sample has too much RNA	Blood from human donors (or other animal species such as rats and mice) with very high leukocyte counts yields large amounts of RNA. 1. Remove the remaining blood lysate from the reservoir, then attach a new reservoir to the filter vial. 2. Process a fraction of the remaining blood lysate (for example, 5 mL).
	Vacuum leak	Check the seals and instrument lines on the ABI PRISM® 6100 Nucleic Acid PrepStation.

Problem	Possible Cause	Solution
Some samples have completely evacuated before the others.	Some filters are clogged or evacuating more slowly	1. Remove the reservoir-filter vial assemblies of the samples that have completely evacuated. 2. Cover the open ports of the adapter plate with cap plugs. 3. Continue to run the vacuum for the rest of the samples. 4. Replace the reservoir-filter vial assemblies when all samples have evacuated.
Sample leaks	The tube was not capped properly before vortexing	Make sure each tube is capped properly before vortexing.
	Improper fitting of filter vial and reservoir	Press the reservoir firmly onto the filter vial until the fitting is tight and stable.
Vacuum does not reach set point and vacuum leak is detected	The adapter plate is not properly seated in the 6100 PrepStation	1. Reposition adapter plate. 2. Press down on the corners of the adapter plate to ensure that it is properly seated.
	Unused ports on the adapter plate are not covered	Cover the unused ports with cap plugs.
	The 6100 PrepStation is set to Collection instead of Waste	Set the vacuum to Waste.

Problem	Possible Cause	Solution
Filter membranes are not completely white after the first Wash Solution 2 step (refer to step 6c on page 18).	Insufficient washing	1. Reattach the reservoirs. 2. Repeat the wash steps: a. Pipet 5 mL of RNA Purification Wash Solution 1 into each reservoir, then apply 80% vacuum for 600 seconds or until all the samples have passed through the filters. b. Pipet 5 mL of RNA Purification Wash Solution 2 into each reservoir, then apply 80% vacuum until the wash solution is evacuated completely and the filters are dry. **IMPORTANT!** When a DNase treatment is required, run the vacuum step for a minimum of 180 seconds to remove the wash solution completely.
RNA is degraded	Residual protein (RNase activity)	Increase the number of wash steps with RNA Purification Solution Wash 1 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 blood draw and during dilution	Vortex the sample: • For 10 seconds after blood draw • For 30 seconds after diluting with 1× PBS

Problem	Possible Cause	Solution
Excessive gDNA contamination	The filter was not completely dry when the 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 vacuum time in step 5 on page 18 to at least 180 seconds to remove all wash solutions and dry the membrane completely.
	The vacuum was turned on too early after adding the AbsoluteRNA Wash Solution	After adding the AbsoluteRNA Wash Solution, incubate for the entire 15 minutes before proceeding to the vacuum step.
	Air is trapped underneath 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 1× PBS to bring the total volume of the diluted blood lysate to 12 mL.
	The Applied Biosystems Stabilizing Reagent did not reach 1× final concentration	Add enough 1× 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.

Problem	Possible Cause	Solution
No RNA or low RNA yield *(continued)*	Insufficient mixing during sample dilution with PBS	1. Remove the sample from the reservoir. 2. Vortex the sample, then replace the filter vial.  3. Restart purification procedure.
	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.
	The filter membrane was not completely wet with the Nucleic Acid Purification Elution Solution	Ensure that no air bubbles are trapped in the filter.
	Residual RNA is trapped on the membrane	1. Reload ~ 200 µL of RNA eluate to the filter vial. 2. Centrifuge for 1 minute at 4,000 x *g*.

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