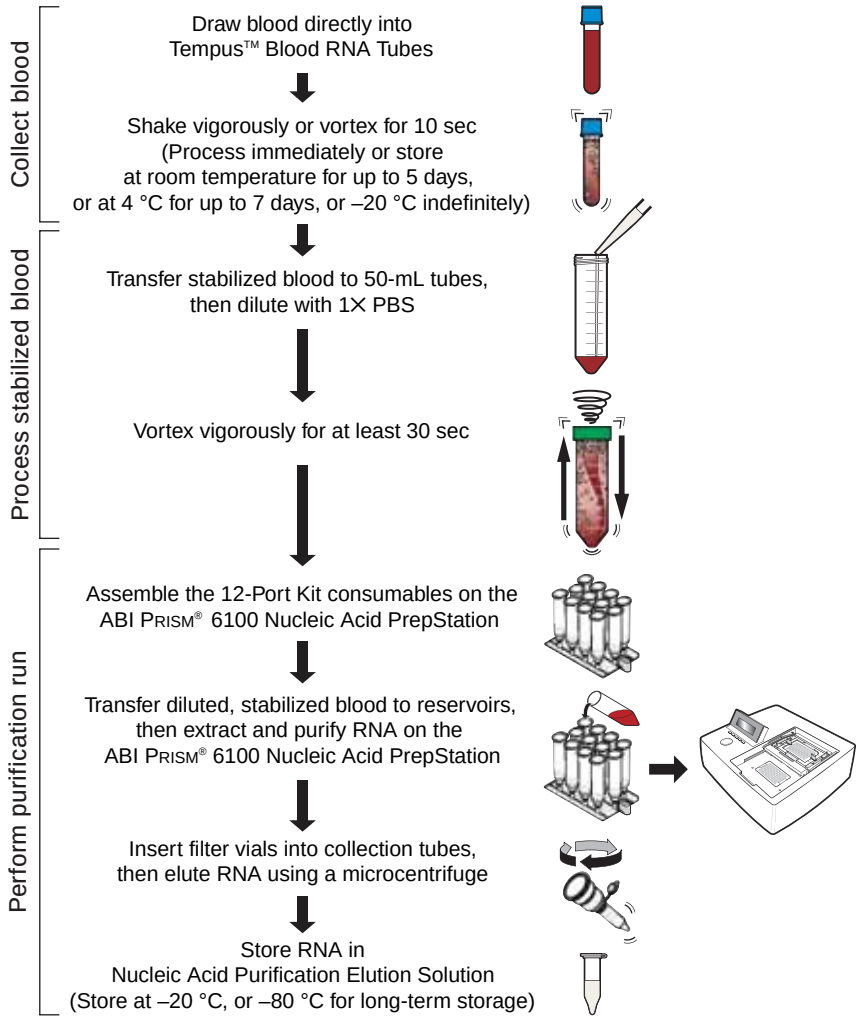
Tempus™ 12-Port RNA Isolation Kit

Quick Reference Card

For safety and biohazard guidelines, refer to the "Safety" section in the *Tempus™ Blood RNA Tube and Tempus™ 12-Port RNA Isolation Kit Protocol* (PN 4379228). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Procedure Overview

The following diagram provides an overview of the procedure for using the Tempus™ 12-Port RNA Isolation Kit to isolate RNA from human whole blood collected in Tempus™ Blood RNA Tubes.



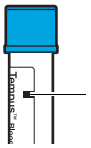
Collecting and Storing Blood in Tempus™ Blood RNA Tubes

Collecting Blood

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:

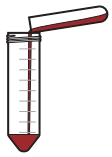
Storage / Shipping Options	Temperature Requirement (°C)
Store or ship on dry ice. (Recommended) IMPORTANT! Avoid direct contact of sample with dry ice!	−20 to −80
Store or ship refrigerated within 7 days or less.	4
Store or ship at room temperature within 5 days or less.	18 to 25

Processing Stabilized Blood Before Purification

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 1X phosphate-buffered saline (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 1X PBS to bring the total volume to 12 mL.

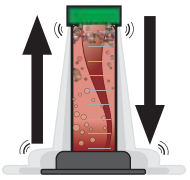
   

4. Replace the caps on the tubes, then vortex the tubes vigorously (at maximum vortex speed) for 30 seconds to ensure proper mixing of the contents.

   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.

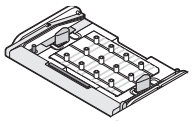
5. Keep the processed samples on ice.

Assembling the Purification Consumables

1. Place a new splash guard onto the waste station of the ABI PRISM® 6100 Nucleic Acid PrepStation.

2. Assemble the 12-Port Kit consumables on the 6100 PrepStation:

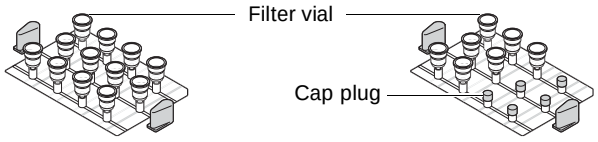
   a. Place the 12-port adapter plate onto the purification tray carriage.



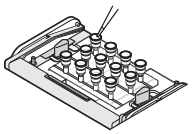
   b. Label the filter vials, then insert a filter vial into each port on the adapter plate.

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

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



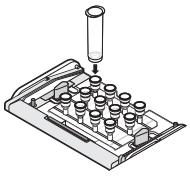
   c. Pre-wet the membranes by pipeting **RNA Purification Wash Solution 1** into each filter vial.



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.

Performing the Purification Run

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 in the *Tempus™ Blood RNA Tube and Tempus™ 12-Port RNA Isolation Kit Protocol*.

Note: For further information on running protocols on the 6100 PrepStation, refer to the *ABI PRISM® 6100 Nucleic Acid PrepStation User Guide*.

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.

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%

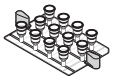
Note: If all the samples have not passed through the filters in the specified time, run the vacuum for additional time until all samples are completely evacuated.

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%

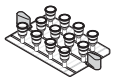
8. Pipet RNA Purification Wash Solution 2 into each reservoir, then apply vacuum.

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.

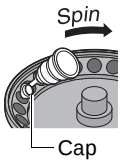
IMPORTANT! Ensure that the filters and the tips of the vials are completely dry before inserting them into the collection tubes. Wipe off any liquid with a clean, lint-free paper.

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

Clean the Waste position of the 6100 PrepStation after every run. Refer to the *Tempus™ Blood RNA Tube and Tempus™ 12-Port RNA Isolation Kit Protocol* for instructions.

Materials and Equipment

Unless otherwise noted, many 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 1X 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	–
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 Protocol Note: To download this and additional protocols, see the Applied Biosystems Web site. Go to **docs.appliedbiosystems.com/search.taf**.	Applied Biosystems	4379228
ABI PRISM® 6100 Nucleic Acid PrepStation User Guide	Applied Biosystems	4326242

06/2007