Tempus™ Spin RNA Isolation Kit

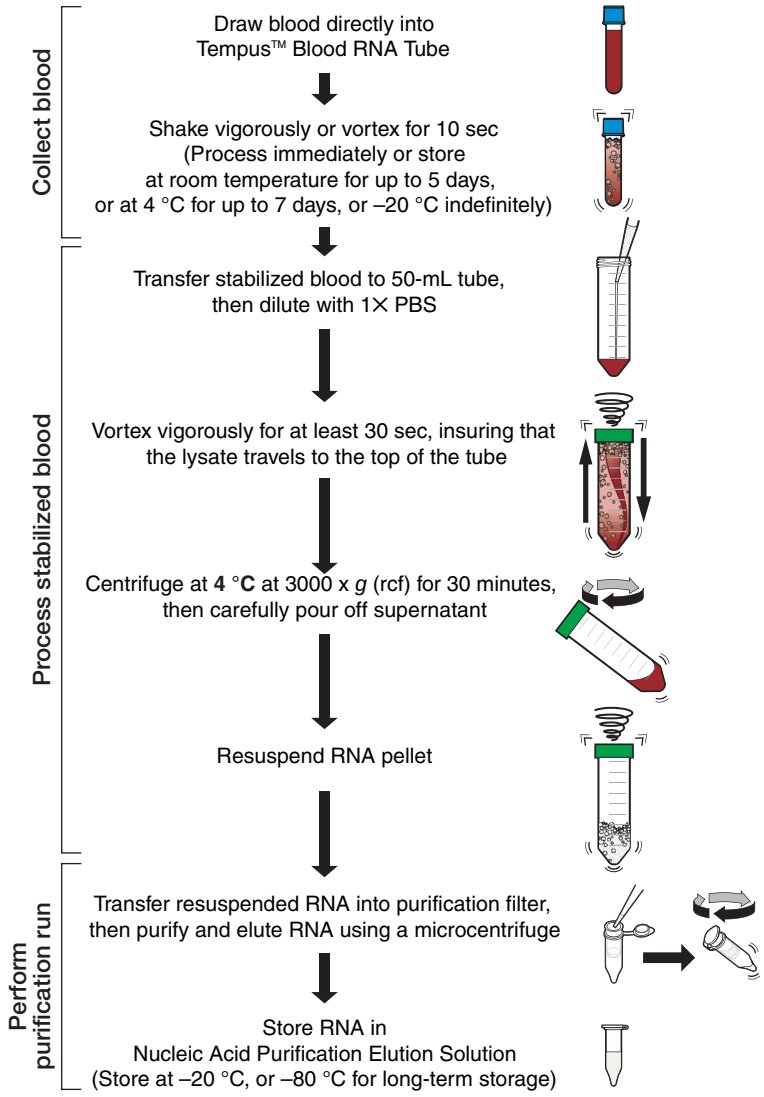
Quick Reference Card

For safety and biohazard guidelines, refer to the "Safety" section in the *Tempus™ Blood RNA Tube and Tempus™ Spin RNA Isolation Kit Protocol* (PN 4379232). 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™ Spin RNA Isolation Kit to isolate RNA from human whole blood collected in a Tempus Blood RNA Tube.

Note: The procedure described in this quick reference card can also be used with the Tempus™ Blood RNA Isolation Sample Kit.



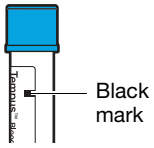
Collecting and Storing Blood in Tempus™ Blood RNA Tubes

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:

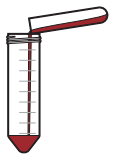
Storage / Shipping Options	Temperature Requirement (°C)
Store or ship refrigerated within 7 days or less. (Recommended)	4
Store of ship on dry ice. **IMPORTANT!** Avoid direct contact of sample with dry ice!	–20 to –80
Store or ship at room temperature within 5 days or less.	18 to 25

Processing Stabilized Blood Before Purification

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

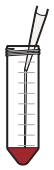
1. If the sample is frozen, thaw the sample in the Tempus tube at room temperature (18 to 25 °C).

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× phosphate-buffered saline (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.

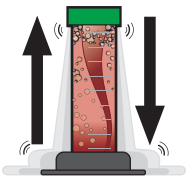


4. Replace the cap on the tube, then vortex the tube vigorously (at maximum vortex speed) for 30 seconds to ensure proper mixing of the contents.

   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.

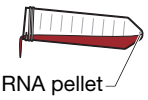


5. Centrifuge the tube at **4 °C** at 3,000 x *g* (rcf) for 30 minutes.

6. Carefully pour off the supernatant.

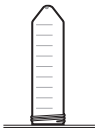
   Note: The RNA pellet is transparent and invisible.

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



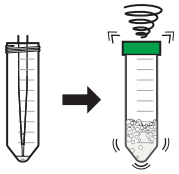
7. Leave the tube inverted on absorbent paper for 1 to 2 minutes.

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.

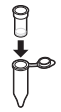


10. The resuspended RNA can be kept on ice while preparing for the next steps.

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 noted.

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



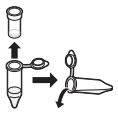
2. Pre-wet the filtration membrane by pipeting **RNA Purification Wash Solution 1** into the purification filter.

Wash Solution 1	Time	Centrifuge
100 µL	—	—

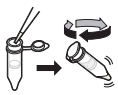
3. Pipet the resuspended RNA into the purification filter, then centrifuge.

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.

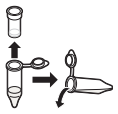


5. Pipet RNA Purification Wash Solution 1 into the purification filter, then centrifuge.

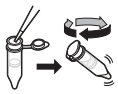


Wash Solution 1	Time	Centrifuge
500 µL	30 sec	16,000 x *g*

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.

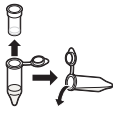


IMPORTANT! When a DNase treatment is required, extend the centrifuge time to 1 minute to remove all wash solutions and dry the membrane completely.

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

‡ 60 sec, if a DNase treatment is required

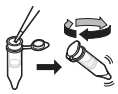
8. Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.



9. (*Optional*) Perform a DNase treatment:
   a. Pipet AbsoluteRNA Wash Solution (not provided) into the purification filter, then incubate at room temperature.

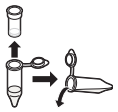
AbsoluteRNA Wash Solution	Time	Centrifuge
100 µL	15 min	—

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

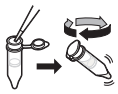


Wash Solution 2	Time	Centrifuge
500 µL	5 min	—
	30 sec	16,000 x *g*

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

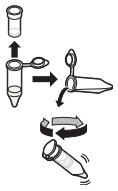


10. Pipet RNA Purification Wash Solution 2 into the purification filter, then centrifuge.



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

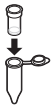
11. Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.



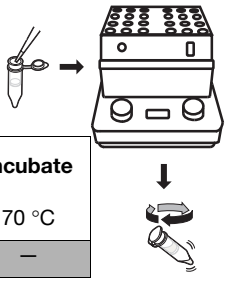
12. Centrifuge to dry the membrane.

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

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

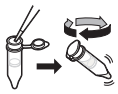


14. Pipet **Nucleic Acid Purification Elution Solution** into the purification filter, close the cap, incubate the entire tube, then centrifuge.



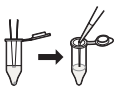
Elution Solution	Time	Centrifuge	Incubate
100 µL	2 min	—	70 °C
	30 sec	16,000 x *g*	—

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

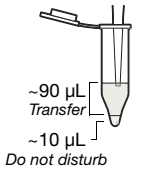


RNA Eluate	Time	Centrifuge
~100 µL	2 min	Maximum (16,000 to 18,000 x *g*)

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.

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™ 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	–
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 Protocol **Note:** To download this and additional protocols, see the Applied Biosystems Web site. Go to **docs.appliedbiosystems.com/search.taf**.	Applied Biosystems	4379232

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