applied biosystems

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

USER GUIDE

for use with:

Cat. No. 4342792 and Cat. No. 4380204

Publication Number 4379232

Revision E

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Manufacturer: Life Technologies Corporation | 2130 Woodward Street | Austin, TX 78744

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Revision history: Revision history of Pub. no. 4379232

Revision	Date	Description
E	22 June 2018	Remove mention of AbsoluteRNA Wash Solution and optional gDNA removal step.

Contents

Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

Gene expression measurements in human whole blood is an 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.

The Tempus™ Blood RNA Tube, enables you to 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.

This user guide describes the protocol for collecting blood with Tempus™ Blood RNA Tubes (Cat. No. 4342792) followed by sample processing using the Tempus™ Spin RNA Isolation Kit (Cat. No. 4380204). The protocol can also be used with the Tempus™ Blood RNA Isolation Sample Kit (Cat. No. 4380202)

About Tempus™ Blood RNA Tubes

Tempus™ Blood RNA Tubes contain 6 mL of stabilizing reagent, which effectively lyses blood cells and stabilizes RNA in a single step. No pretreatment of blood is required before purification of RNA from the sample. After 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. The gene expression profile is immediately preserved, and remains stable for up to five days at room temperature and at least one week at 4°C.

About the Tempus™ Spin RNA Isolation Kit

The Tempus™ Spin RNA Isolation Kit makes it possible to isolate RNA conveniently from larger starting volumes of blood using standard laboratory centrifuges. The kit allows 6 to 25 µg of RNA to be isolated from 3 mL blood. The extracted RNA is pure ($A_{260/280}$ ratio > 1.9), with very low levels of protein and gDNA contamination.

Contents and storage

Tempus™ Blood RNA Tube

Item	Amount	Storage
Tempus™ Blood RNA Tube	50 tubes	Room temperature

Tempus™ Spin RNA Isolation Kit

Item	Amount	Storage
RNA purification filters	50 filters	Room temperature
2-mL collection tubes	2 × 100 tubes	
1✕ PBS	2 × 80 mL	
RNA Purification Resuspension Solution	24 mL	
RNA Purification Wash Solution 1	2 × 22 mL	
RNA Purification Wash Solution 2	120 mL	
Nucleic Acid Purification Elution Solution	4 × 1.9 mL	

Required materials not supplied

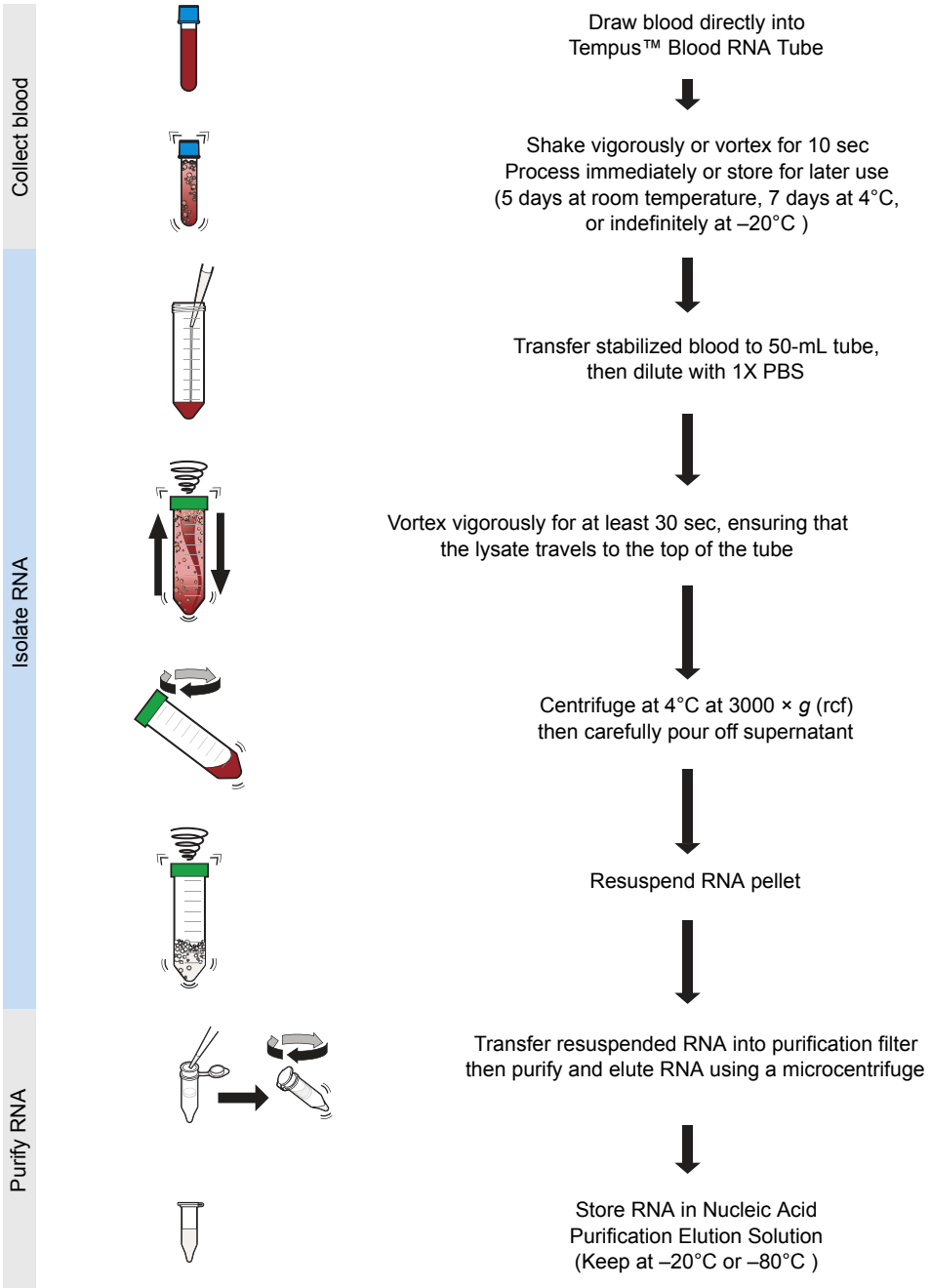
Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Sterile conical tubes, 50-mL	MLS
Pipette tips	MLS
Pipettes, 5-mL, 10-mL, 25-mL	MLS
Vortexer	MLS
Microcentrifuge	MLS
Heating block for microcentrifuge tubes	MLS
Centrifuge (>3,000 × *g* (rcf), temperature controlled)	MLS

Centrifuge protocol workflow

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.

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



Methods

Guidelines for drawing blood

Tempus™ Blood RNA Tube 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 VACUETTE™ Safety Blood Collection Set, go to the Greiner Bio-One™ web site for additional information on how to use the winged blood collection needle.

- Do not use Tempus™ Blood RNA Tubes if foreign matter is observed within the tube.
- 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™ Blood RNA 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™ Blood RNA 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™ Blood RNA Tubes if they are discolored or contain precipitates.
- Do not use the Tempus™ Blood RNA Tubes after their expiration date.

Guidelines for preventing backflow

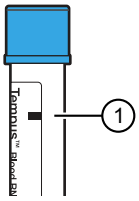
Tempus™ Blood RNA Tubes contain chemical additives. To prevent backflow from the tube into the individual's arm, observe the following precautions.

- 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.

Collect blood

IMPORTANT! Observe appropriate safety practices when collecting blood.

1. Draw 3 mL of blood directly into a Tempus™ Blood RNA Tube according to your laboratory's standard procedures for collecting blood from individuals into blood collection tubes containing liquid reagents.



① The black mark on the tube label indicates the level of approximately 3 mL of blood.

Note: If you are using the VACUETTE™ Safety Blood Collection Set, go to the Greiner Bio-One™ web site for additional information on how to use the winged blood collection needle.

2. Immediately after filling the tube, shake the tube vigorously or vortex the contents for 10 seconds to ensure that the stabilizing reagent is thoroughly mixed with the sample.

   Note: 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.

Storage and transport of blood in Tempus™ Blood RNA Tubes

store or ship Tempus™ Blood RNA Tubes containing stabilized samples in the following order of preference:

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

Guidelines for extracting RNA

Tempus™ Spin RNA Isolation Kit is used to isolate and purify RNA from whole blood collected with Tempus™ Blood RNA Tubes. The RNA isolated in this procedure contains very low levels of genomic DNA (<0.05% by weight).

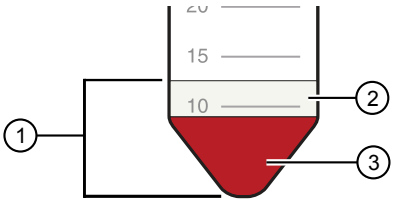
- Discard the blood-containing wastes following recognized disinfection procedures and in accordance with all local, state, and national bloodborne/infection regulations.
- Adjust the final concentration of the stabilizing reagent to a final concentration of 1X by diluting the stabilized blood with calcium- and magnesium-free phosphate-buffered saline (PBS) before extracting RNA for purification. Failure to do so results in significantly lower RNA yields.
- Keep the samples on ice as much as possible to prevent possible decrease in RNA yields.

Isolate RNA

1. If the sample is frozen, thaw the sample in the Tempus™ Blood RNA Tube at room temperature (18–25°C).

2. Remove the cap from the Tempus™ Blood RNA Tube, then pour the contents into a clean 50-mL conical tube.

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



① 12 mL total volume
② 3 mL 1X PBS
③ 3 mL blood mixed with 6 mL 2X stabilizing reagent

Note: If the initial blood sample is less than 3 mL, make up the difference by adding enough 1X PBS to bring the total volume to 12 mL.

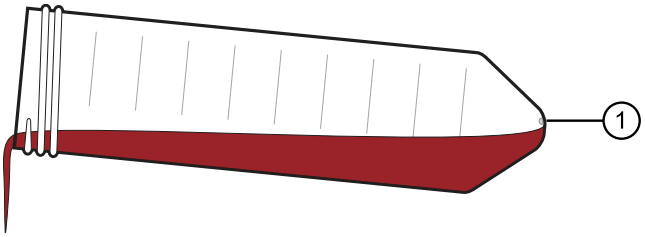
4. Replace the cap on the conical tube, then vortex the tube vigorously (at maximum vortex speed) for 30 seconds to ensure proper mixing of the contents.

   Note: Make sure the conical tube is capped properly to prevent the contents from leaking or spraying out during vortexing.

   Note: Vortexing for less than 30 seconds may cause clogging of the purification column. A layer of froth over the sample after vortexing is normal.

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

6. Carefully pour off the supernatant. Handle the conical tube carefully so that you do not dislodge the RNA pellet off the bottom of the tube.



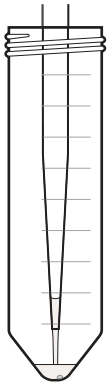
① RNA pellet (transparent and invisible)

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

8. Blot the remaining drops of liquid off the rim of the conical tube with clean absorbent paper.

9. Add 400 µL of RNA Purification Resuspension Solution into the conical tube, then vortex briefly to resuspend the RNA pellet.

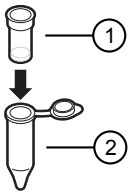
   Note: To prevent washing any blood residue down the inside of the tube, insert the pipette tip into the tube and add the resuspension solution to the bottom of the tube.



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

Purify RNA

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

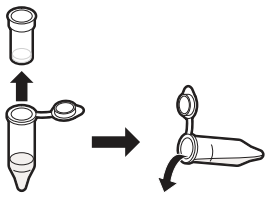


① RNA purification filter
② Waste collection tube

2. Pre-wet the filtration membrane by adding 100 µL of RNA Purification Wash Solution 1 into the purification filter.

3. Add ~400 µL of the resuspended RNA into the purification filter, then centrifuge for 30 seconds at 16,000 × *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 **15**for ordering information.

5. Add 500 µL of RNA Purification Wash Solution 1 into the purification filter, then centrifuge for 30 seconds at 16,000 × *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. Add 500 µL of RNA Purification Wash Solution 2 into the purification filter, then centrifuge for 30 seconds at 16,000 × *g*.

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. Add 500 µL of RNA Purification Wash Solution 2 into the purification filter, then centrifuge for 30 seconds at 16,000 × *g*.

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

11. Centrifuge for 30 seconds at 16,000 × *g* to dry the membrane.

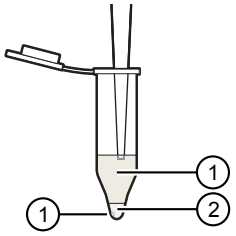
12. Transfer the purification filter to a new, labeled collection tube to collect the eluate.

13. Add 100 µL Nucleic Acid Purification Elution Solution into the purification filter, close the cap, incubate the entire tube for 2 minutes at 70°C, then centrifuge for 30 seconds at 16,000 × *g*.

14. Add ~100 µL of the collected RNA eluate back into the purification filter, then centrifuge for 2 minutes at 16,000–18,000 × *g*. No incubation is necessary.

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

    Note: When transferring the RNA eluate, carefully remove the liquid from the collection tube starting from the top of the liquid to ensure that the pellet is not disturbed.



① ~90 µL of eluate (transfer to new tube)
② ~10 µL (do not disturb)
③ Pellet

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

Troubleshooting

Observation	Possible cause	Recommended action
Filters appear black or brownish-red (even after using wash solutions)	Insufficient mixing of sample after drawing blood.	Immediately after drawing the blood into a Tempus™ Blood RNA Tube, shake the filled tube vigorously or vortex for 10 to 20 seconds.
	The sample was contaminated with blood residue when the resuspension solution was added.	Pipet 500 µL of RNA Purification Wash Solution 1 into the purification filter, then centrifuge for 30 seconds at 16,000 × *g*.
		Pipet 500 µL of RNA Purification Wash Solution 2 into the purification filter, then centrifuge for 30 seconds at 16,000 × *g*.
Sample leaks from tube	The tube was not capped properly before vortexing.	Make sure each tube is capped properly before vortexing.
RNA is degraded	Residual protein with RNase activity in sample.	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 a short period causing the RNA to go back into solution.	Freeze any remaining lysate, then thaw the lysate and repurify.
	Insufficient mixing after drawing blood and during dilution.	Vortex the sample for 10 seconds after drawing blood.
		Vortex the sample for 30 seconds after diluting with 1X PBS.
Filters appear black or brownish-red (even after using wash solutions)	Insufficient mixing of sample after drawing blood.	Immediately after drawing the blood into a Tempus™ Blood RNA Tube, shake the filled tube vigorously or vortex for 10 to 20 seconds.
	The sample was contaminated with blood residue when the resuspension solution was added.	Pipet 500 µL of RNA Purification Wash Solution 1 into the purification filter, then centrifuge for 30 seconds at 16,000 × *g*.
		Pipet 500 µL of RNA Purification Wash Solution 2 into the purification filter, then centrifuge for 30 seconds at 16,000 × *g*.

Accessory products

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Tempus™ Blood RNA Isolation Sample Kit	4380202
Tempus™ 12-Port RNA Isolation Kit	4378672
2-mL collection tubes, 100 count	AM12480
RNase-free water	MLS
Ethanol, 100%	MLS
High-Capacity cDNA Reverse Transcription Kit	4368813 4368814
High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor	4374967 4374966
TaqMan® One-Step RT-PCR Master Mix Reagents Kit	4309169 4313803
TaqMan® Gold RT-PCR Kit	N8080233 N8080232 4304133
TaqMan® EZ RT-PCR Core Reagents	N8080235 N8080236 403028
GLOBINclear™ Whole Blood Globin Reduction Kit	AM1980
MessageAmp™ aRNA Amplification Kit	AM1750
MessageAmp™ II aRNA Amplification Kit	AM1751
MessageAmp™ II-96 aRNA Amplification Kit	AM1819

A Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of 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.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number
Tempus™ Blood RNA Tube and Tempus™ Spin RNA Isolation Kit Quick Reference Card	4379233

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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