Tempus™ Blood RNA Tube
Stabilization and Isolation of RNA From Whole Blood
on the ABI PRISM™ 6100 Automated Nucleic Acid PrepStation

Quick Reference Card

WARNING! Biological samples have the potential to transmit infectious disease. For safety and biohazard guidelines, refer to the "Safety" section in the *Tempus Blood RNA Tube and Large Volume Consumables Protocol*, PN 4345218. Follow these guidelines and other safety provisions set forth in the protocol when using the Tempus Blood RNA Tubes and the ABI Prism™ 6100 Automated Nucleic Acid PrepStation. For each chemical in **bold** type below, read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note that this Quick Reference Card is to be used as a general guideline. Refer to the Tempus™ Blood RNA Tube and Large-Volume Consumbles Protocol, PN 4345218 for detailed information and protocols.

Collection and Isolation of RNA

WARNING! 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 safety information or detialed protocols, refer to the blood collection section in the *Tempus Blood RNA Tube and Large Volume Consumables Protocol*, PN 4345218.

Collect Blood:

Draw 3 mL of blood directly into **Tempus Blood RNA Tube** and shake vigorously for 10–20 sec. If you draw less than 3 mL of blood, or shake or vortex for less than 10–20 sec, you may get low recovery and clogging.

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Storage and Shipping Options:

- Short-term: room temperature for up to 5 days
- Medium-term: 4 °C for up to 7 days
- Long-term: –20 °C to –80 °C (vortex well before storage)

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Assemble and Load Consumables:

See "Reagents and Consumables" on page -4 for ordering information.

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Purify Samples:

1. Create a new method on the ABI PRISM™ 6100 Automated Nucleic Acid PrepStation, then save the method as *Tempus RNA*. (See Table 1 on page 2 and Table 2 on page 3 for methods to use.)
2. Dilute samples to a total volume of 12 mL with 1× PBS (Ca^{2+}/Mg^{2+}–free) and vortex at least 30 sec.
3. Pre-wet the RNA prep filters with RNA Purification Wash Solution 1.
4. Load the diluted blood samples into 20-mL reservoirs.
5. Run the Tempus RNA method.
   a. Wash samples with RNA Purification Wash Solution 1 and remove the 20-mL reservoirs and replace with 5-mL reservoirs.
   b. Wash samples with **RNA Purification Wash Solution 1 and 2**, then completely dry the filters.
   c. Replace reservoirs with new 5-mL reservoirs.
   d. Incubate in AbsoluteRNA Wash Solution to remove gDNA.
   e. Wash with **RNA Purification Wash Solution 2** two more times, then completely dry the filters.
   f. Elute the RNA. See the *Tempus™ Blood RNA Tube and Large-Volume Consumbles Protocol*, PN 4345218, for elution methods (centrifugation or vacuum). Remove reservoir before turning on the vacuum.

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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 rinse the Waste position thoroughly with water. Program a 50% vacuum for 3 minutes.
5. Clean the white plastic piece that holds the splash guard in place with Lysol®, Vesphene®, or 70% alcohol, detergent, and water.
6. Clean the area around the vacuum carriage and gasket with 70% alcohol.

Applied Biosystems

WARNING! Biological samples have the potential to transmit infectious disease. For safety and biohazard guidelines, refer to the "Safety" section in the *Tempus Blood RNA Tube and Large-Volume Consumables Protocol*, PN 4345218. Follow specific safety practices when using this instrument. For each chemical in **bold** type below, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

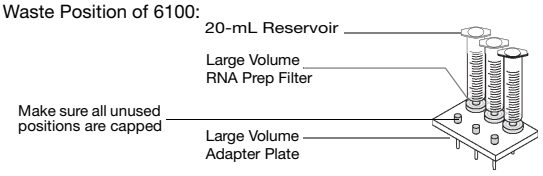
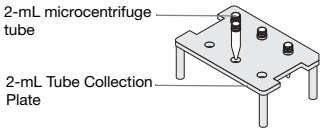
STEP	ACTION	
1	Collect blood	a. Draw 3 mL of blood directly into **Tempus Blood RNA Tubes** per your laboratory's normal procedures. b. Immediately after filling each tube during collection, shake or vortex vigorously for 10–20 sec. You can store the stabilized blood safely at room temperature for up to 5 days, at 4 ×C for 7 days, or at –20 °C to –80 °C for prolonged periods. Alternatively, you can dilute the samples with PBS and extract RNA.
2	Assemble the purification consumable	Assemble the purification consumables as shown in the following diagram: To Waste Position of 6100:  To Collection Position of ABI PRISM™ 6100 Nucleic Acid PrepStation: 
3	Purify samples with the *Tempus RNA* method	a. Load the purification consumables, placing a splash guard in the Waste position and the assembled Large Volume Adapter Plate in the purification tray carriage. Secure the tray. Do not load the 2-mL Tube Collection Tray in the collection compartment until you are ready to elute the RNA. Cap all unused positions. b. Create a new method named *Tempus RNA*. Enter the parameter values provided in Table 1 below or Table 2 on page 3. c. Dilute samples to a total volume of 12 mL with 1× PBS, and vortex for 20–30 sec. d. **Prewet the filters** with **350 µL** of **RNA Purification Wash Solution 1**. Ensure that the highlighter is at step 1 of the *Tempus RNA* method and that no air bubbles are trapped in the filter. e. Follow the steps in Table 1 below for vacuum-based elution or Table 2 on page 3 for centrifugation-based elution.

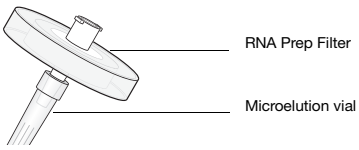
Table 1. Extracting RNA from whole blood collected in Tempus tubes using vacuum based elution (low concentration RNA):

Step	Description	Vol (mL)	Position	Incubation (sec)	Vacuum (%)	Time (sec)
1	Load the samples and turn on the vacuum.	12	Waste	0	80	300
2	Add **RNA Purification Wash Solution 1**.	4.5	Waste	0	80	300
3	Remove the 20 mL reservoir and wash the neck and filter with 1–3 mL of **RNA Purification Wash Solution 1** using a P1000 pipette. Wash until the blood lysate is completely washed off and no coloration remains.	1–3	Waste	0	80	300
4	Attach new 5-mL reservoirs and add **RNA Purification Wash Solution 1**.	4.5	Waste	0	80	300
5	Add **RNA Purification Wash Solution 2**. Repeat steps 3 and 4 until filter is heme-free. Make sure filter is dry (free of **RNA Purification Wash Solution 2**).	4.5	Waste	0	80	180
6	Attach new 5-mL reservoirs, add AbsoluteRNA Wash Solution, and incubate. IMPORTANT! Do not turn on the vaccum until step 7.	0.35	Waste	900	0	0
7	Add **RNA Purification Wash Solution 2** and incubate.	3	Waste	300	0	0
8	Turn on vacuum to remove wash solution 2.	–	Waste	0	80	120
9	Add **RNA Purification Wash Solution 2**.	3	Waste	0	80	120
10	Add **RNA Purification Wash Solution 2**.	3	Waste	0	80	120
11	Remove the 5-mL reservoirs, wash the tips of the adapter plate and splash guard and replace on the carriage.	–	Waste	–	–	–
12	Turn on the vacuum and dry filters.	–	Waste	–	90	400
13	Touch off at waste and ensure the filter tip does not have any residual liquid.	–	Waste	0	–	–
14	Load the assembled 2-mL Tube Collection Plate in the collection position, and move the carriage to the collection position.	–	Collection	0	–	–
15	Attach new 5-mL reservoirs, add Nucleic Acid Purification Elution Solution and incubate. If higher yields are desired, use a larger volume (for example 2× elution volume). Note: 0.5 mL is the minimum volume you can use.	0.5	Collection	120	0	0
16	Turn on the vacuum to elute RNA. Eluate volume should be approximately 400 µL. Repeat step at higher vacuum if necessary.	–	Collection	–	60	120

3 Run the *Tempus RNA* method *(continued)*

Table 2. Extracting RNA from whole blood collected in Tempus tubes using centrifugation based elution (high concentration RNA):

Step	Description	Vol (mL)	Position	Incubation (sec)	Vacuum (%)	Time (sec)
1	Load the samples and turn on the vacuum.	12	Waste	0	80	300
2	Add **RNA Purification Wash Solution 1**.	4.5	Waste	0	80	300
3	Remove the 20 mL reservoirs and wash the necks and filters with 1–3 mL of **RNA Purification Wash Solution 1** using a P1000 pipette. Wash until the blood lysate is completely washed off and no coloration remains.	1–3	Waste	0	80	300
4	Attach new 5-mL reservoirs and add **RNA Purification Wash Solution 1**.	4.5	Waste	0	80	300
5	Add RNA Purification Wash **Solution 2**. Repeat steps 2 and 3 until filter is heme-free. Make sure filter is completely dry (free of **RNA Purification Wash Solution 2**).	4.5	Waste	0	80	180
6	Attach new 5-mL reservoirs, add AbsoluteRNA Wash Solution and incubate. **IMPORTANT!** Do not turn on the vaccum until step 7.	0.35	Waste	900	0	0
7	Add **RNA Purification Wash Solution 2** and incubate.	3	Waste	300	0	0
8	Turn on vacuum to remove wash solution 2.	–	Waste	0	80	120
9	Add **RNA Purification Wash Solution 2**.	3	Waste	0	80	120
10	Add **RNA Purification Wash Solution 2**.	3	Waste	0	80	120
11	Remove 5-mL reservoirs.	–	Waste	–	–	–
12	Turn on vacuum and dry filter	–	Waste	0	90	400
13	Touch off at Waste.	–	Waste	–	–	–
14	Remove the RNA Prep Filters and ensure the tips of the filter are dry and free from Wash Solution 2.	–	–	–	–	–
15	Add a microelution vial (see starter kit, PN 4344440) to each RNA Prep Filter, and place each assembly in an Applied Biosystems 96-well splash-free base plate.	–	–	–	–	–



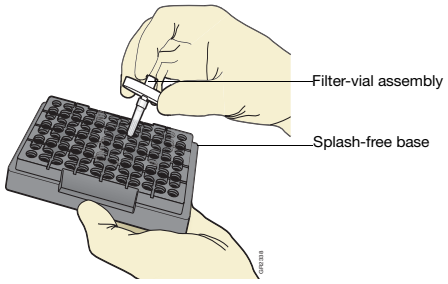
16 Add 1 µL of RNase Inhibitor (PN N8080119) to 100 µL of RNA elution solution and add solution to the tip of the RNA filter.

Note: The RNase Inhibitor concentration is 20 U/µL.

IMPORTANT! Allow the membrane to get wet, but do not introduce air bubbles.

17 Centrifuge the filter-vial assembly at 2100 × *g* (~3500 to 4000 rpm) in a splash-free base or tray retainer set on a base for 3 min. Adding an incubation time of 5 min prior to centrifugation, or increasing the volume of the elution solution to 130 µL may improve yields of RNA.

CAUTION The filter-vial assembly should only be placed in a centrifuge that is qualified to spin 96-well plates.



18 When the spin is finished, repeat steps 15–16 with the same eluate to ensure that all RNA is removed from the RNA prep filter.

Note: A 40% improvement in recovery is typically gained by repeating the elution. No further improvement is gained by recycling the eluate a third time.

4	Clean the 6100 PrepStation	a. Remove and discard the purification consumables. b. Use the Quick Run feature to flush the Waste position with water. Program a 50% vacuum for 3 minutes. c. Clean the white plastic piece that holds the splash guard in place with Lysol®, Vesphene®, or 70% alcohol, detergent, and water. d. Clean the area around the vacuum carriage and gasket with 70% alcohol.

Reagents and Consumables

Item	Part Number	Item	Part Number
Large Volume Adapter Plate	4344438	Absolute RNA Wash Solution	4305545
Large Volume RNA Prep Filter	4344439	Nucleic Acid Purification Elution Solution	4305893
Large Volume Upgrade Starter Kit	4344440	RNA Purification Wash Solution 1	4305891
5-mL Reservoir	4344437	RNA Purification Wash Solution 2	4305890
20-mL Reservoir	4344435	2-mL microcentrifuge tubes	4305936
Splash Guard	4311758	High Capacity cDNA Archive Kit	4322171
Tempus™ Blood RNA Tube	4342792	5-mL, 10-mL, 25-mL pipettes	MLS*
2-mL Tube Collection Plate	4344436	50-mL sterile tubes (BD Falcon)	MLS*

*Major laboratory supplier.

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

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