RNA Purification from Fresh and Frozen Blood in Tempus™ Blood RNA Tubes Using the Maxwell® 16 LEV simplyRNA Blood Kit

Maxwell® 16 LEV simplyRNA Blood Kit Application Note

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Sample Type:
- Whole blood in Tempus™ Blood RNA Tubes

Instrument Requirements:
- Maxwell® 16 Instrument (Cat.# AS2000) with firmware version ≥4.9 **or**
- Maxwell® 16 Instrument (Cat.# AS3000) with firmware version ≥1.4
- High-Strength Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070)

Maxwell® 16 LEV simplyRNA Blood Kit
- Maxwell® 16 LEV simplyRNA Blood Kit (Cat.# AS1310)

Additional Materials Required:
- 50ml conical tubes
- 1X phosphate-buffered saline (PBS)
- centrifuge

A protocol for isolating RNA from whole blood in Tempus™ Blood RNA Tubes using the Maxwell® 16 LEV simplyRNA Blood Kit

Introduction

Clinical labs studying RNA expression face challenges when dealing with blood samples. The method of blood collection, storage and shipping conditions, and time between collection and analysis all can contribute to RNA degradation by RNases and unintentional expression of individual genes after blood collection. Tempus™ Blood RNA Tubes are designed for collection and stabilization of RNA for gene expression analysis. The tubes immediately lyse whole blood cells and freeze global gene expression profiles by stabilizing RNA.

Here, we describe an application for the Maxwell® 16 LEV simplyRNA Blood Kit for use with Tempus™ Blood RNA Tubes on the Maxwell® 16 Instrument. The Maxwell® 16 LEV simplyRNA Blood Kit is used with the Maxwell® 16 Instrument configured with the LEV High-Strength Magnetic Rod and Plunger Bar Adaptor. This RNA purification procedure is a simple method with minimal lysate handling prior to automated purification. The low elution volume results in concentrated, high-quality RNA suitable for use in several downstream applications. The instrument processes up to 16 samples in about 1 hour.

Reagent Preparation

Homogenization Solution: To prepare a working solution, add 20µl of 1-Thioglycerol per milliliter of Homogenization Solution. 1-Thioglycerol is viscous, so careful pipetting is required for accurate measurement. Alternatively, add 600µl of 1-Thioglycerol to the 30ml bottle of Homogenization Solution. Two hundred microliters of 1-Thioglycerol/Homogenization Solution is needed for each sample. Before use, chill the 1-Thioglycerol/Homogenization Solution on ice or at 2–10°C. Store the 1-Thioglycerol/Homogenization Solution at 2–10°C, where it is stable for up to 30 days.

DNase I: Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I. Invert the vial to rinse DNase I off the underside of the cap, and swirl gently to mix; do not vortex. Add 5µl of Blue Dye to the reconstituted DNase I as a visual aid in later steps to confirm that DNase I was added to each well. Dispense the DNase I solution into single-use aliquots in nuclease-free tubes. Each purification requires 10µl of DNase I solution. Store reconstituted DNase I at –30°C to –10°C. Do not freeze-thaw reconstituted DNase I more than three times.

Cartridge Preparation

Cartridges can be prepared during the centrifugation in Step 4 of the protocol (see below).

1. Wearing clean gloves, remove seal from each Maxwell® 16 LEV Cartridges (MCF) placed in the Maxwell® LEV Cartridge Rack. Ensure that all sealing tape and any residual adhesive are removed.
2. Place a plunger in well #8 of each cartridge. Well #8 is the well furthest from the cartridge label.
3. Place 0.5ml Elution Tubes in the front of the Maxwell® 16 LEV Cartridge Rack. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube. For a less concentrated eluate, up to 100µl of Nuclease-Free Water may be added to the elution tube.
4. Shortly before running the instrument, add 10µl of DNase I (prepared as described above) to well #4 of each simplyRNA Blood Cartridge (well #4 contains yellow reagent). The contents of well #4 will turn green following addition of the blue DNase I solution.

Protocol

Follow the manufacturer's instructions for drawing blood in Tempus™ Blood RNA Tubes (1), ensuring that tubes are vigorously shaken immediately after the blood draw. For frozen samples, be sure that blood is thawed at room temperature for at least 2 hours prior to RNA purification.

Before beginning the protocol, confirm that the correct firmware (version 4.90 or greater for AS1000 and AS2000 series instruments; version 1.40 or greater for AS3000 series instruments) is loaded on the instrument and that the LEV High Strength Magnetic Rod and Plunger Bar (Cat.# SP1070) are installed. Confirm that the instrument is set for LEV mode.

Note: The simplyRNA Blood Kit contains two reagents with the word lysis in their name: Cell Lysis Solution (A793A, 100ml) and Lysis Buffer (MC501C, 20ml). The Cell Lysis Solution (A793A) is **not used** in this protocol.

1. Pour contents of one Tempus™ Blood RNA Tube into a 50ml conical tube.
2. Add 1X PBS to bring the total volume up to 12ml (usually about 3ml). Close the tube cap.
3. Vortex for 30 seconds, ensuring that the solution reaches the top of the conical tube.
4. Centrifuge at 4,000 *x g* for 30 minutes at 4°C to pellet the RNA.

   Notes:

   1. The RNA pellet may not be visible. Make note of where the pellet should be, and take care to not dislodge the pellet.
   2. During this centrifugation step, prepare the cartridges as directed above.
5. Decant the supernatant, and blot the top of excess liquid.
6. Dry the RNA pellet at room temperature for 2 minutes.

   Note: The 2-minute drying time is essential. Do not dry the RNA for any other length of time, as yields will be decreased significantly.
7. Resuspend the RNA pellet in 200µl of Homogenization Solution with 2% 1-Thioglycerol.
8. Add 200µl of Lysis Buffer.
9. Vortex for 15 seconds.
10. Add the entire volume to well #1 of the Maxwell® 16 LEV Cartridge (MCF) .
11. Confirm that each cartridge contains 10µl of DNase I in well #4 (solution will be green if DNAse I was added) and has a plunger in well #8 and that an elution tube containing 50–100µl of Nuclease-Free Water is in the elution position.

12. If using the Maxwell® 16 MDx Instrument (Cat.# AS3000), verify that the Home screen indicates "LEV" and the LEV hardware is installed. Press "Run" to continue. At the Protocols screen, select "RNA", then select "simplyRNA Blood". Place the LEV rack with the cartridges containing the samples in the Maxwell® 16 MInstrument when directed.

    If using the Maxwell® 16 Instrument (Cat.# AS1000 and AS2000), verify that the instrument settings indicate an "LEV" hardware configuration and "Rsch" operational mode setting. Select "Run" on the Menu screen, and press the Run/Stop button to select the method. Select "RNA", then select "simplyRNA Blood" on the Menu screen. Next select "OK" at the Verification screen. Open the door when prompted. Press the Run/Stop button to extend the platform. Place the LEV rack with the cartridges containing the samples in the Maxwell® 16 Instrument.

13. Press the Run/Stop button. The platform will retract. Close the door.

14. After RNA isolation, store purified RNA at –80°C until ready to use.

Results

Blood was drawn from three different donors into four Tempus™ Blood RNA Tubes per donor. Two samples were processed as fresh blood, and the remaining two were frozen for 24 hours before being processed. The protocol described above was used to process all samples. RNA samples were quantitated using a NanoDrop® 1000 spectrophotometer. Results are shown in Table 1 and Figure 1.

Table 1. Results of RNA Isolation from Fresh and Frozen Blood Samples.

Fresh Blood	RNA Concentration (ng/µl)	A_{260}/A_{280} Ratio	A_{260}/A_{230} Ratio	Frozen Blood	RNA Concentration (ng/µl)	A_{260}/A_{280} Ratio	A_{260}/A_{230} Ratio
Donor 1	171.54	2.09	2.11	Donor 1	164.31	2.09	2.07
Donor 1	162.53	2.10	2.14	Donor 1	163.77	2.11	2.08
Donor 2	157.54	2.10	2.08	Donor 2	113.60	2.12	1.98
Donor 2	153.53	2.09	2.09	Donor 2	107.56	2.09	1.91
Donor 3	238.31	2.10	2.10	Donor 3	188.64	2.12	2.08
Donor 3	227.40	2.10	2.04	Donor 3	200.32	2.10	2.06
Average	**185.14**	**2.10**	**2.09**	**Average**	**156.37**	**2.11**	**2.03**
Standard Deviation	37.60	0.01	0.03	Standard Deviation	38.22	0.01	0.07

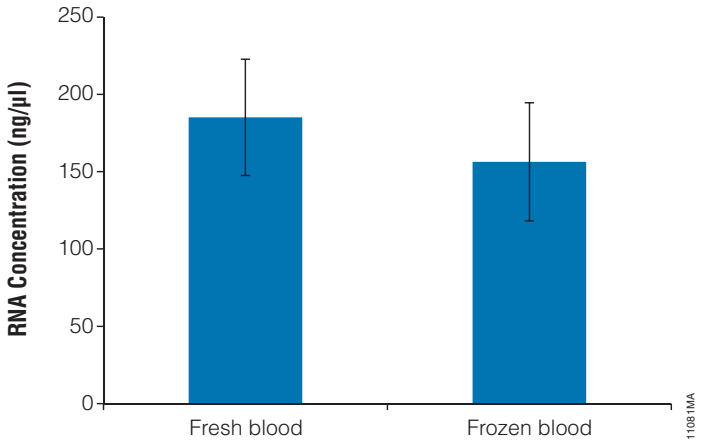

Figure 1. Concentration of RNA purified from fresh and frozen blood samples using the Maxwell® 16 LEV simplyRNA Blood Kit.

Conclusions

RNA can be purified successfully from fresh and frozen blood collected in Tempus™ Blood RNA Tubes using the Maxwell® 16 LEV simplyRNA Blood Kit. The 2-minute dry time is essential for optimal yield, as seen in previous experiments (data not shown).

Reference

1. *Tempus™ Blood RNA Tube and Tempus™ 12-Port RNA Isolation Kit Protocol*, Applied Biosystems.

Ordering Information

Product	Cat.#
Maxwell® 16 Instrument*	AS2000
Maxwell® 16 MDx Instrument*	AS3000
Maxwell® 16 High-Strength LEV Magnetic Rod and Plunger Bar Adaptor	SP1070
Maxwell® 16 LEV simplyRNA Blood Kit	AS1310
Nuclease-Free Water*	P1193
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	Z5341

*For Laboratory Use.