

Isolating RNA from Whole Blood Collected in PAXgene[®] Tubes

Maxwell[®] 16 LEV simplyRNA Blood Kit Application Note

Promega Corporation

Sample Type:

- Whole blood in PAXgene[®] tubes

Instrument Requirements:

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

Maxwell[®] 16 LEV simplyRNA Blood Kit Components Required:

- Maxwell[®] 16 LEV Cartridges (MCF)
- Homogenization Solution (Z305H)
- Lysis Buffer (MC501)
- DNase I (Z358A)
- 1-Thioglycerol (A208B)
- Blue Dye (C888A)
- Nuclease-free Water (P119C)
- LEV Plungers
- Elution Tubes

Additional Materials Required:

- Nuclease-free Water (Cat.# P1193)
- Vortex mixer
- 1.5ml Microcentrifuge tubes (optional)
- Centrifuge with swinging-bucket rotor
- RNase-free, sterile, aerosol-resistant pipette tips

A protocol for isolation of RNA from whole blood in PAXgene[®] tubes using the Maxwell[®] 16 LEV simplyRNA Blood Kit

Introduction

This Application Note describes a method for purification of total RNA from whole blood stored in PAXgene[®] tubes using the Maxwell[®] 16 Instrument and the Maxwell[®] 16 LEV simplyRNA Blood Kit.

Please refer to the Maxwell[®] 16 LEV simplyRNA Blood Kit Technical Manual, #TM372, for information on storage of kit components, instrument set-up and safety information.

Reagent Preparation

Homogenization Solution: To prepare a working solution, add 20 μ l of 1-Thioglycerol per milliliter of Homogenization Solution. 1-Thioglycerol is viscous, so careful pipetting is required for accurate measurement. Alternatively, add 600 μ l of 1-Thioglycerol to the 30ml bottle of Homogenization Solution. A volume of 200 μ l 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 275 μ l of Nuclease-Free Water to the vial of lyophilized DNase I. Invert to rinse DNase off the underside of the cap and swirl gently to mix; do not vortex. Add 5 μ l of Blue Dye to the reconstituted DNase I as a visual aid for pipetting. Dispense the DNase I solution into single-use aliquots in nuclease-free tubes. Each purification requires 10 μ l of DNase I solution. Store reconstituted DNase I at –30 to –10°C. Do not freeze-thaw reconstituted DNase I more than three times.

Cartridge Preparation

Cartridges can be prepared during the centrifugation at Step 5 of the protocol.

1. Wearing clean gloves, remove the seals from the Maxwell[®] 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.

Maxwell® 16 simplyRNA Blood Kit

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 more or less concentrated eluate, 50–100µl of nuclease-free water may be added to the elution tube.
4. Shortly before running the instrument, add 10µl DNase (prepared as described above) to well #4 of the simplyRNA Blood Cartridge (well #4 contains yellow reagent). The reagent will be green after addition of the blue DNase solution.

Protocol

Before beginning the protocol, confirm that the correct firmware (version 4.90 or greater for 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.

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. Centrifuge the PAXgene® tubes at 3,000 x *g* for 10 minutes at room temperature to pellet the white blood cells.
2. Remove and discard the supernatant.
3. Vortex the tube to resuspend the cells in the remaining liquid.
4. Add 5ml nuclease-free water and vortex again to mix.
5. Centrifuge at 3,000 x *g* for 10 minutes at room temperature. During the centrifugation, prepare the Maxwell® 16 simplyRNA Blood Cartridges as described above.
6. After centrifugation, remove and discard all of the supernatant.
7. Add 200µl 1-Thioglycerol/Homogenization Solution and completely resuspend the pellet by pipetting and vortexing.
8. Transfer the lysate to a 1.5ml microcentrifuge tube, or continue processing in the PAXgene® tube. The smaller tube may reduce foaming during mixing.
9. Add 200µl Lysis Buffer (MC501) and mix by vortexing for 20 seconds.
10. Add all of the lysate to well #1 of the Maxwell® 16 simplyRNA Blood cartridge (well #1 is the well closest to the cartridge label).
11. Check that the plunger and DNase have been added to the cartridge, and that the elution tube with nuclease-free water is loaded on the LEV rack.
12. Select “Run” on the Maxwell® Instrument LCD screen.
13. Next, select the sample type “RNA” and the method “simplyRNA Blood”. On the verification screen, click OK if “simplyRNA Blood” is the method listed.
14. Load the rack into the Maxwell® Instrument and follow the on-screen instructions.
15. When the run is complete, the LCD screen will display a message that the method has ended.
16. Follow the on-screen instructions to open the door. Verify that the plungers are located in well #8.
17. Press the “Run/Stop” button to extend the platform out of the instrument.
18. Remove the Maxwell® 16 LEV Cartridge Rack from the instrument. Remove and cap the elution tubes.
19. If paramagnetic particles are present in the elution tubes, centrifuge at 10,000 x *g* for 2 minutes. Alternatively, an additional particle capture may be performed with the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341) or Maxwell® 16 LEV Magnet (Cat.# AS1261).
20. Discard cartridges following local guidelines.

Store the RNA at –10 to –80°C. Consult the protocol for your downstream application for specific storage and handling recommendations.

Please refer to the Maxwell® 16 LEV simplyRNA Blood Kit Technical Manual, #TM372, for more detailed information on storage of kit components, instrument set-up and safety information.

Ordering Information

Product	Cat.#
Maxwell® 16 Instrument*	AS2000
Maxwell® 16 MDx Instrument*	AS3000
High-Strength Magnetic Rod and Plunger Bar Adaptor	SP1070
Maxwell® 16 LEV simplyRNA Blood Kit*	AS1310
Maxwell® 16 LEV simplyRNA Cells Kit*	AS1270
Maxwell® 16 LEV simplyRNA Tissue Kit*	AS1280
Nuclease-Free Water*	P1193
MagneSphere® Technology Magnetic Separation Stand	Z5341
Maxwell® 16 LEV Magnet*	AS1261

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