Maxwell® RSC simplyRNA Blood Kit

Instructions for Use of Products AS1380 and ASB1380.

Quick Protocol

This Quick Protocol provides instructions for use of the Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380, ASB1380) with Maxwell® Instruments to isolate total RNA from fresh (not frozen) whole blood collected in EDTA tubes. For detailed instructions, including information on instrument setup and troubleshooting, please refer to the *Maxwell® RSC simplyRNA Blood Kit Technical Manual* #TM417, available at: **www.promega.com/protocols/**

Preparing Total RNA from Whole Blood

Solution Preparation

1-Thioglycerol/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. A volume of 200µl of 1-Thioglycerol/Homogenization Solution is needed for each sample. Before use, chill the 1-Thioglycerol/Homogenization Solution on ice or at +2°C to +10°C.

DNase I Solution: Add 275µl 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 5µl 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 10µl of DNase I Solution. Store reconstituted DNase I at –30°C to –10°C Do not freeze-thaw reconstituted DNase I more than ten times.

Preparing White Blood Cell Pellets

Materials to Be Supplied by the User

- fresh (not frozen) whole blood in EDTA collection tubes
- vortex mixer
- 15ml tubes (sterile)
- centrifuge with swinging-bucket rotor
- RNase-free, sterile, aerosol-resistant pipette tips

Note: The simplyRNA Blood Kit contains two reagents with the word lysis in their name: **Cell Lysis Solution** (Part# A793A, 100ml) and **Lysis Buffer** (Part# MC501C, 20ml). Please check that you use the correct reagent at each step.

1. Transfer 2.5ml of well mixed, fresh (not frozen) whole blood from the EDTA collection tube into a sterile 15ml tube.
2. Add 7.5ml of Cell Lysis Solution (Part# A793A), and invert the tube 5–6 times to mix. This is a differential lysis step; the red blood cells are lysed, leaving the white blood cells intact.
3. Incubate lysates for 10 minutes at room temperature. Twice during the incubation, invert to mix.
4. Centrifuge tube at 3,000 × *g* for 10 minutes.
5. Remove and discard as much of the supernatant as possible without disturbing the visible white pellet. Briefly spin to collect residual liquid at the bottom of the tube, and remove and discard the supernatant with a pipette.
6. Add 200µl of chilled 1-Thioglycerol/Homogenization Solution to the pellet. Mix well with a pipette or vortex or both to ensure complete resuspension of the pellet.
7. Add 200µl of Lysis Buffer (Part# MC501C) and 25µl of Proteinase K to the resuspended pellet. Mix by vortexing for 20 seconds.
8. Incubate at room temperature for 10 minutes. During this time, prepare the Maxwell® RSC simplyRNA Blood Cartridges.
9. Add 10µl of DNase I Solution (blue solution) to well #4 of the Maxwell® RSC simplyRNA Blood Cartridge (well #4 contains yellow reagent). After adding the blue DNase I Solution, the reagent in well #4 will be green.
10. Add lysate to well #1 of the Maxwell® RSC simplyRNA Blood Cartridge (the well closest to the printed side of the cartridge).

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Automated RNA Purification

Preparing the Cartridge

1. Change gloves before handling Maxwell® RSC Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
2. Place one plunger into well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
3. Place an empty 0.5ml Elution Tube into the elution tube position for each cartridge in the deck tray. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube.

Figure 1. Setup and configuration of the deck tray. The CSC/RSC plunger is placed in well #8 of the cartridge (the well closest to the Elution Tube), lysate is placed into well #1 of the cartridge and DNase I Solution (blue solution) is dispensed into well #4.

Notes:

a. If Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.

b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® RSC Instrument.

4. Proceed to the next section, Running the Method on Maxwell® Instruments.

Running the Method on the Maxwell® Instruments (Cat.# AS4500, AS8500)

1. To run the Maxwell RSC simplyRNA Blood method, the appropriate Maxwell® RSC simplyRNA Blood method must installed on your instrument. Methods are available at: **www.promega.com/resources/software-firmware/**
2. Follow the instrument run instructions in the *Maxwell® RSC simplyRNA Blood Kit Technical Manual* #TM417.

Additional protocol information in Technical Manual #TM417, available online at: **www.promega.com**

