Maxwell® 16 LEV simplyRNA Blood Kit

INSTRUCTIONS FOR USE OF PRODUCT AS1310.

Preparation of Total RNA from Whole Blood

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 x *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 and/or vortex 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 vortex for 20 seconds.
8. Incubate at room temperature for 10 minutes. During this time prepare the simplyRNA Blood Cartridges.
9. Add 10µl of DNase I solution (blue solution) to well #4 of the simplyRNA Blood Cartridge (well #4 contains yellow reagent).
10. Add lysate to well #1 of the simplyRNA Blood cartridge (the well closest to the label on the cartridge).

Printed in USA. Revised 3/14. Part# 9FB132

Maxwell® 16 LEV simplyRNA Blood Kit

INSTRUCTIONS FOR USE OF PRODUCT AS1310.

Solution Preparation, Cartridge Preparation and Instrument Setup

Solution 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. 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 2–10°C.

DNase I: 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 –20°C. Do not freeze-thaw reconstituted DNase I more than three times.

Cartridge Preparation

Place the cartridges to be used in the Maxwell® 16 LEV Cartridge Rack with the label side 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.
Note: If you are processing fewer than 16 samples, center the cartridges on the platform.

1. Place an LEV Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
2. 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 concentrated eluate, as little as 30µl of nuclease-free water may be added to the elution tube, but the total amount of RNA recovered may be reduced.

   Notes:

   1. If Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
   2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may not work with the Maxwell® 16 Instrument.

Instrument Run on the Maxwell® 16 Instrument (Cat.# AS2000 or AS3000)

1. Refer to the *Maxwell® 16 Instrument Operating Manual* #TM295 (AS2000) or #TM320 (AS3000) for detailed information. To run the simplyRNA protocol, the Maxwell® 16 firmware version ≥4.95 (AS2000) or ≥1.50 (AS3000) must be installed on the instrument and the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070) must be used. Using the original LEV magnetic rod will result in low yields.
2. Follow the instrument run instructions in the *Maxwell® 16 LEV simplyRNA Blood Kit Technical Manual* #TM372. To run the simplyRNA Blood protocol for AS2000 instruments, select “RNA”, select “simplyRNA”, then select “simplyRNA Blood” on the Menu screen. To run the simplyRNA Blood protocol for AS3000 instruments, select “RNA”, then select “simplyRNA Blood” on the Menu screen.

Additional protocol information in Technical Manual #TM372, available online at:
www.promega.com/protocols/

Ordering and Technical Information
www.promega.com • Phone 608-274-4330
Toll free in USA 800-356-9526 • Fax 608-277-2601

Printed in USA. Revised 3/14. Part# 9FB132