Maxwell® RSC miRNA Tissue Kit

Instructions for Use of Product AS1460.

Quick Protocol

Solution Preparation and Sample Preprocessing

1-Thioglycerol/Homogenization Solution

A volume of 200µl of 1-Thioglycerol/Homogenization Solution is needed for each sample. 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. We recommend adding 600µl of 1-Thioglycerol to the 30ml bottle of Homogenization Solution. Before use, chill the 1-Thioglycerol/Homogenization Solution on ice or at 2–10°C.

DNase I Solution

Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I. Invert to rinse any 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. Store reconstituted DNase I at –30°C to –10°C. DNase I solution maintains activity for up to 10 freeze-thaw cycles.

Materials to Be Supplied By the User

- small tissue homogenizer (e.g., Tissue-Tearor™ homogenizer [PRO Scientific], or any homogenizer capable of handling small volumes)
- vortex mixer
- tube for homogenization
- RNase-free, sterile, aerosol-resistant pipette tips

1. Working as quickly as possible, homogenize the tissue sample in the chilled 1-Thioglycerol/Homogenization Solution until no visible tissue fragments remain. Homogenize for an additional 15–30 seconds to ensure complete homogenization. If foaming occurs, let the sample settle on ice. Only 200µl of homogenate can be processed per cartridge. The final volume of the homogenate to be added to the cartridge should be 200µl. Add more homogenization solution as needed to bring the sample to a final volume of 200µl.

   Note: After homogenization, samples may be stored frozen at –80°C for later processing. Thaw frozen homogenates on ice or at 2–10°C to avoid RNA degradation.
2. Add 200µl of Lysis Buffer, 200µl of Lytic Enhancer and 30µl of Proteinase K to the homogenized sample. Mix by vortex for 20 seconds.
3. Incubate at room temperature for 10 minutes. During this time, prepare the Maxwell® RSC Cartridges (RSCN).
4. Transfer 630µl of lysate to well #1 (the largest well) of the Maxwell® RSC Cartridge (RSCN).
5. Add 10µl of blue DNase I Solution to well #4 (yellow reagent) of the Maxwell® RSC Cartridge (RSCN). After the blue DNase I Solution is added, the reagent in well #4 will be green.
6. Load samples onto the instrument and begin the automated purification run.

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Method Setup and Cartridge Preparation

Maxwell® Method Setup

Before using the Maxwell® RSC RNA miRNA Tissue Kit for the first time, the miRNA Tissue method must be installed on your instrument.

Cartridge Preparation

1. Place the cartridges to be used in the deck tray with well #1 (the largest well) 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 into the instrument. **Caution:** Handle cartridges with care. Seal edges may be sharp.
2. Place a plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
3. Place 0.5ml Elution Tubes in the front of the deck tray.
4. Add 60µl of Nuclease-Free Water to the bottom of each Elution Tube.

   Note: Use only the RSC Plungers, Elution Tubes and Nuclease-Free-Water supplied with the Maxwell® RSC miRNA Tissue Kit. Other elution tubes may not be compatible with supported Maxwell® Instruments and may affect performance. Use of other elution buffers may affect RNA purification performance or downstream use.

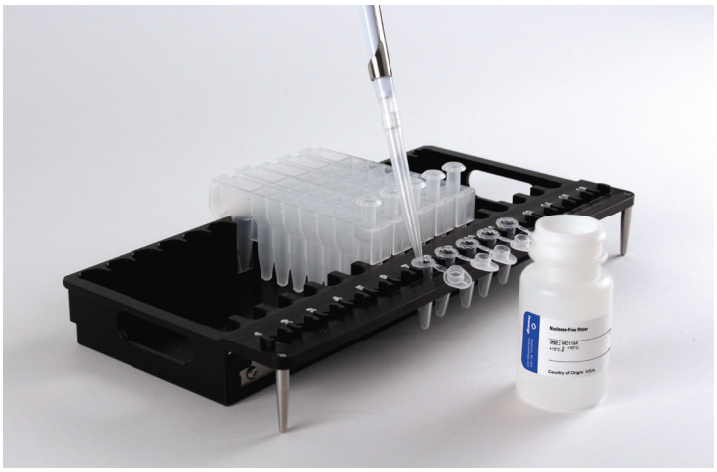

Figure 1. Setup and configuration of the deck tray.

Maxwell® Instruments Run

Follow the instrument run instructions in the *Maxwell® RSC miRNA Tissue Kit Technical Manual* #TM441.

Additional protocol information in Technical Manual #TM441, available online at: **www.promega.com**

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