Maxwell® RSC Plant RNA Kit

Instructions for Use of Product AS1500.

Promega

Quick Protocol

Preparation of Plant Leaf Samples for RNA Purification

This Quick Protocol provides intructions for use of the Maxwell® RSC Plant RNA Kit with Maxwell® RSC Instruments to purify RNA from plant leaf samples. For detailed instructions, including information on instrument setup and troubleshooting, please refer to the *Maxwell® RSC Plant RNA Kit Technical Manual* #TM459, available at: **www.promega.com/protocols/**

Materials to Be Supplied by the User

- small tissue homogenizer
- mortar and pestle or rapid bead-beater
- vortex mixer
- tube for homogenization
- liquid nitrogen
- microcentrifuge tube, 1.8ml
- RNase-free, sterile, aerosol-resistant pipette tips

Homogenization Solution Preparation: 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 provided in the kit. Before use, chill the 1-Thioglycerol/Homogenization Solution on ice or at 2–10°C.

DNase I Preparation: Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I prior to use. 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 for pipetting. Dispense the DNase I solution into single-use aliquots in nuclease-free tubes. Store reconstituted DNase I at –10°C to –30°C after use.

Preprocessing Samples

1. Weigh plant material and grind to a fine powder in liquid nitrogen with a mortar and pestle or other mechanical device.
2. Decant tissue powder and any remaining liquid nitrogen into an appropriately sized tube (allow liquid nitrogen to evaporate, if present). Immediately place on dry ice, or store at –70°C until ready to use.
3. Weigh and transfer 20–100mg of the plant tissue powder into a 1.8ml tube. Store samples on dry ice until the last sample is weighed then transfer to wet ice for homogenization.
4. Add 600µl of the chilled 1-Thioglycerol/Homogenization solution. If there is plant material on the cap or on the sides of the tube above the homogenization solution level, pulse-spin the sample at maximum speed in a microcentrifuge.
5. Homogenize with a small tissue homogenizer for 30–60 seconds, then place on ice. If foaming occurs, let the sample settle on ice. Homogenize in 15–30 second increments if needed, then place the sample on ice.
6. With a wide-bore pipet, aliquot 400µl of homogenate to a 1.8ml microcentrifuge tube.

   Note: Samples may be stored frozen at –70°C after homogenization for later processing. Thaw homogenates on ice or at 2–10°C to avoid RNA degradation.
7. Shortly before processing samples on the Maxwell® RSC Instrument, add 200µl of Lysis Buffer to 400µl of homogenate. Vortex vigorously for 15 seconds to mix.
8. Incubate at room temperature for 10 minutes. Spin the sample at maximum speed in a microcentrifuge for 2 minutes.
9. Set up the deck tray and cartridges (see Cartridge Preparation section).
10. Transfer the supernatant to well #1 of the Maxwell® RSC Cartridge (RSCO). Well #1 is closest to the cartridge label and farthest from the elution tube.
11. Add 5µl of DNase to well #4 (yellow reagent). After the DNase I solution is added, the reagent in well #4 will be green.

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Cartridge Preparation

1. Place the cartridges to be used in the Maxwell® RSC deck trays with the label side facing away from the Elution Tube.
2. 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 in the deck tray.
3. Place a plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.

   Note: Use only the plungers provided in the Maxwell® RSC Plant RNA Kit.
4. Place 0.5ml Elution Tubes in the front of the deck tray. 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

   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 not work with the Maxwell® RSC Instrument(s).

Figure 1. Setup and configuration of the Maxwell® RSC deck tray.

Instrument Run on the Maxwell® RSC (Cat.# AS4500) or RSC 48 (Cat.# AS8500) Instruments

1. See the *Maxwell® RSC Instrument Operating Manual* #TM411 or the *Maxwell® RSC 48 Instrument Operating Manual* #TM510 for detailed information. To run the Plant RNA protocol, the Maxwell® RSC Plant RNA method must be installed on the instrument. See the *Maxwell® RSC Methods Installation Technical Manual* #TM435 for instructions. The method is available at: **www.promega.com/resources/tools/maxwellrscmethod/**
2. Follow the instrument run instructions in the *Maxwell® RSC Plant RNA Kit Technical Manual* #TM459.

Additional protocol information in Technical Manual #TM459, available online at: **www.promega.com**

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