TECHNICAL MANUAL

Maxwell® RSC Plant RNA Kit

Instructions for Use of Product
AS1500

Note: To use the Maxwell® RSC Plant RNA Kit, you must have the "Plant RNA" method loaded on the Maxwell® RSC or Maxwell® RSC 48 Instrument.

Caution: Handle cartridges with care; seal edges may be sharp.

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TM459

Maxwell® RSC Plant RNA Kit

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The Maxwell® RSC Plant RNA Kit[a] is designed for automated isolation of total RNA from plant tissue samples. The Maxwell® RSC (Cat.# AS4500) and Maxwell® RSC 48 (Cat.# AS8500) Instruments are supplied with preprogrammed purification methods and are designed for use with predispensed reagent cartridges, maximizing simplicity and convenience. The RNA purification procedure is a simple method with minimal lysate handling before automated purification. The low elution volume is used to generate concentrated high-quality RNA suitable for use in downstream applications such as quantitative RT-PCR. The kit provides the reagents required for processing the samples and uses prefilled cartridges for purification, maximizing simplicity and convenience. The Maxwell® RSC Instrument can process up to 16 samples, and the Maxwell® RSC 48 Instrument can process up to 48 samples, both in approximately 50 minutes.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Plant RNA Kit	**48 preps**	**AS1500**

Sufficient for 48 automated isolations from plant tissue samples. Includes:

- 48 Maxwell® RSC Cartridges (RSCO)
- 30ml Homogenization Solution
- 20ml Lysis Buffer
- 2 vials DNase I (lyophilized)
- 900µl 1-Thioglycerol
- 50µl Blue Dye
- 25ml Nuclease-Free Water
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes, 0.5ml

Storage Conditions: Upon receipt, remove the 1-Thioglycerol and store it at 2–10°C. Store the remaining kit components at room temperature (15–30°C). 1-Thioglycerol also can be stored at room temperature (15–30°C), where it is stable for up to 9 months.

Safety Information: The reagent cartridges contain ethanol, which is flammable and an irritant. 1-Thioglycerol is toxic. Guanidine thiocyanate and guanidine hydrochloride (which are components of the Homogenization Solution and Lysis Buffer) are toxic, harmful and irritants. The Lysis Buffer also has a possible risk of harm to an unborn child. Wear gloves and follow standard safety procedures while working with these substances. Refer to the SDS for detailed safety information.

Note: Bleach reacts with guanidine thiocyanate and should not be added to any sample waste from these cartridges.

3. Before You Begin

3.A. Maxwell® Method Setup

Before using the Maxwell® RSC Plant RNA Kit for the first time, the Plant RNA method must be installed on your instrument. The method is available at: **www.promega.com/resources/tools/maxwellrscmethod/** and **www.promega.com/resources/software-firmware/maxwell-rsc48-methods/**

See the *Maxwell® RSC Instrument Operating Manual* #TM411 or the *Maxwell® RSC 48 Instrument Operating Manual* #TM510 for instructions.

3.B. Preparation of Solutions

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. Before use, chill 1-Thioglycerol/Homogenization Solution on ice or at 2–10°C.

Note: Store the 1-Thioglycerol/Homogenization Solution at 2–10°C, where it is stable for up to 30 days.

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. Store reconstituted DNase I at −30 to −10°C. Do not freeze-thaw reconstituted DNase I more than three times.

3.C. Preparation of Samples

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

1. Weigh plant material and grind to a fine powder in liquid nitrogen with a mortar and pestle or other mechanical device.
2. Decant the 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 (Section 3.B) to the tube. If plant material is on the tube cap or sides of the tube above the homogenization buffer level, pulse spin the sample at maximum speed in a microcentrifuge.
5. Homogenize samples with a small tissue homogenizer for 30–60 seconds, then place on ice. If foaming occurs, let the sample settle on ice and homogenize in 15- to 30-second increments. Place the sample on ice.
6. With a wide-bore pipette, transfer 400µl of homogenate to a 1.8ml microcentrifuge tube. Discard the remainder of the homogenate.

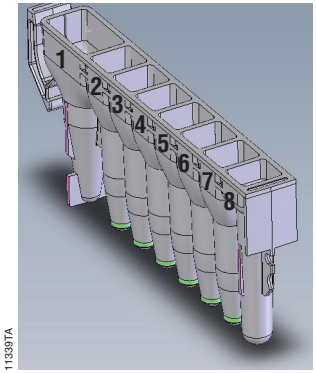
   Note: Samples may be stored frozen at −70°C after homogenization for later processing. Thaw homogenates on ice or at 2–10°C to avoid RNA degradation.
7. Shortly before processing samples on the Maxwell® Instrument, add 200µl of Lysis Buffer to 400µl of homogenate. Vortex vigorously for 15 seconds to mix.
8. Incubate at room temperature for 10 minutes.
9. Spin the sample at maximum speed in a microcentrifuge for 2 minutes.

3.D. Maxwell® RSC Plant RNA Cartridge Preparation

1. To maintain an RNase-free environment during processing, change gloves before handling cartridges, Plungers and Elution Tubes. Place each cartridge 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.

   Note: Use only the plungers provided in the Maxwell® RSC Plant RNA Kit.

2. Place a Maxwell® RSC Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tubes. See Figure 1.



User Adds to Wells:
1. Preprocessed samples
4. DNase I Solution
8. Plunger

Figure 1. Maxwell® RSC Cartridge (RSCO).

3. Place an empty Elution Tube into the elution position for each cartridge in the deck tray(s).

   Note: Use only the 0.5ml Elution Tubes provided in the kit; other tubes may not work with the Maxwell® Instruments.

4. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube. See Figure 2. 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.

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

Figure 2. Setup and configuration in the deck tray(s). Nuclease-Free Water is added to the Elution Tubes as indicated.

5. Transfer the supernatant (Section 3.C, Step 9) to well #1 (the largest well) of the cartridge. Do not transfer any pelleted debris.

6. Add 5µl of DNase I (Section 3.B) to well #4 (yellow reagent). After the blue DNase I solution is added, the reagent in well #4 will be green.

4. Maxwell® Instrument Setup and Run

Refer to the *Maxwell® RSC Instrument Operating Manual* #TM411 or the *Maxwell® RSC 48 Instrument Operating Manual* #TM510 for detailed information.

1. Turn on the Maxwell® Instrument and Tablet PC. Sign in to the Tablet PC, and start the Maxwell® software by double-touching the icon on the desktop. The instrument will power up, proceed through a self-check and home all moving parts.
2. Touch **Start** to access the extraction 'Methods' screen.
3. On the 'Methods' screen, select a method using one of the following two options:
   a. Touch the Plant RNA method.
   b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate method.

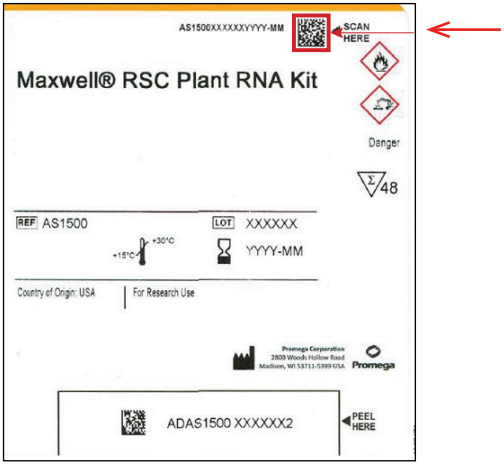


Figure 3. Kit label indicating the method bar code. Scan this bar code to automatically select the method for a purification run.

4. Verify that the Plant RNA method has been selected, and touch the **Proceed** button. If requested by the software, enter any kit lot and expiration information that has been required by the Administrator.
5. On the 'Cartridge Setup' screen (if shown), touch the cartridge positions to select/deselect any positions to be used for this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.

   Note: When using the Maxwell® RSC 48 Instrument, use the **Front** and **Back** buttons to select/deselect cartridge positions on each deck tray.

6. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with Nuclease-Free Water and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® Instrument platform.

   Inserting the Maxwell® deck tray(s): Hold the deck tray by the sides to avoid dislodging cartridges from the deck tray. Ensure that the deck tray is placed in the Maxwell® Instrument with the elution tubes closest to the door. Angle the back of the deck tray downward and place into the instrument so that the back of the deck tray is against the back of the instrument platform. Press down on the front of the deck tray to firmly seat the deck tray on the instrument platform. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation. Ensure the deck tray is level on the instrument platform and fully seated.

   Note: When using the Maxwell® RSC 48 Instrument, check the identifier on the Maxwell® RSC 48 deck tray to determine whether it should be placed in the front or back of the instrument.

7. Touch the **Start** button to begin the extraction run. The platform will retract, and the door will close.

   Warning: Pinch point hazard.

   The Maxwell® Instrument will immediately begin the purification run. The screen will display information including the user who started the run, the current method step being performed and the approximate time remaining in the run.

   Notes:

   1. When using the Maxwell® RSC 48 Instrument, if the Vision System has been enabled, the deck trays will be scanned as the door retracts. Any errors in deck tray setup (e.g., plungers not in well #8, elution tubes not present and open) will cause the software to return to the 'Cartridge Setup' screen; problem positions will be marked with an exclamation point in a red circle. Resolve all error states, and press the **Start** button again to repeat deck tray scanning and begin the extraction run.
   2. Touching **Abort** will abandon the run. All samples from an aborted run will be lost.
   3. If the run is abandoned before completion, you will be prompted to check whether plungers are still loaded on the plunger bar. If plungers are present on the plunger bar, you should perform Clean Up when requested. If plungers are not present on the plunger bar, you can choose to skip Clean Up when requested. In all cases, the samples will be lost.

8. Follow the on-screen instructions at the end of the method to open the door. Verify that plungers are located in well #8 of the cartridge at the end of the run. If plungers are not removed from the plunger bar, follow the instructions in the *Maxwell® RSC Instrument Operating Manual* #TM411 or the *Maxwell® RSC 48 Instrument Operating Manual* #TM510 to perform a Clean Up process to attempt to unload the plungers.

9. Remove the deck tray(s) from the instrument. Remove elution tubes containing RNA, and cap the tubes. After the run has been completed, the extraction run report will be displayed. From the 'Report View' screen, you can print or export this report or both.

   Note: To remove the deck tray from the instrument platform, hold onto the sides of the deck tray.

4. Maxwell® Instrument Setup and Run (continued)

10. If paramagnetic particles are present in the elution tubes, centrifuge at 10,000 × *g* for 2 minutes, and transfer the supernatant to a clean tube (not provided). Alternatively, if desired, an additional particle capture step may be performed using the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341). Transfer the supernatant to a clean tube (not provided). Avoid transferring paramagnetic particles.
11. Remove the cartridges and plungers from the deck tray(s).

Discard the cartridges and plungers as hazardous waste following your institution's recommended guidelines. Do not reuse reagent cartridges, plungers or elution tubes.

Ensure samples are removed before performing any required UV light treatment to avoid damage to the nucleic acid.

Storing Eluted RNA

If sample eluates are not processed immediately, store the eluted RNA at −70°C, or at −20°C for up to 24 hours. Consult the protocol for your downstream application for specific storage and handling recommendations.

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Sample foams during homogenization	Some homogenizers will generate foam when samples are homogenized. Allow the foam to dissipate prior to pipetting. Homogenize for shorter periods of time until visible particles and tissue fragments are eliminated. Keep rotor submerged whenever the homogenizer is on.
	Sample was homogenized in the Lysis Buffer instead of the Homogenization Solution.
Homogenate is too viscous to pipet	The homogenate was too concentrated and became viscous while sitting on ice. Reduce the homogenate viscosity by increasing the amount of Homogenization Solution from 1.5- to 2-fold and briefly rehomogenizing the sample. The maximum volume of homogenate that can be processed in a single Maxwell® RSC Cartridge (RSCO) is 400µl.
	Use wide-bore tips.

Symptoms	Causes and Comments
RNA degradation	1-Thioglycerol was not added to the Homogenization Solution. Keep all reagents on ice until Lysis Buffer is added.
	Samples were not properly prepared or stored. Samples must be flash frozen or immediately homogenized in Homogenization Solution to halt RNA degradation. Delays during sample collection may result in RNA degradation and lower yields. Freeze samples immediately and store at –70°C if they cannot be processed immediately. Homogenates should be stored at –70°C and thawed on ice.
	Frozen lysate was heated to thaw. Thaw frozen lysates on ice or at 2–10°C.
	RNase was introduced by handling. Use sterile, disposable plasticware or baked glassware when handling RNA. Wear clean gloves at all times. RNases introduced during or after purification will degrade the RNA. See Section 6.A, Creating a Ribonuclease-Free Environment.
Low RNA yield, contaminates or poor reproducibility between samples	Sample contains a low amount of RNA. The amount of RNA present in a sample depends on the metabolic state, stage of growth, type of sample and growth conditions. Sample types vary in the amount of total RNA.
	Lysis Buffer was not added.
	Lysates were not mixed by vortexing long enough.
	Homogenization was incomplete. Incomplete homogenization of samples results in reduction in yield. Centrifuge the sample well before adding it to the cartridge and only pipet the clear lysates; inclusion of the pellet or debris floating on the surface may increase the risk of contaminates in the eluate.
	The wrong method was run with the Maxwell® Instrument.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
DNA contamination seen when performing RT-PCR or PCR	DNase was not added to the correct well in the cartridge or was not added at all. Check the color of the liquid in well #4. If the blue DNase Solution was added, the reagent in well #4 will be green, not yellow.
	Too much sample was processed. Reduce the starting sample amount by twofold.
	Sample has an excessive amount of genomic DNA. Reduce the starting material or increase the amount of DNase added.
	Possible cross-contamination. RT-PCR and PCR are extremely sensitive techniques. Use aerosol-resistant pipette tips. Set up reactions and analyze samples in separate locations.
	Too much sample was used in RT-PCR. Reduce the total RNA input to 50–100ng in RT-PCR. Generally a rare message can be detected in 50ng of total RNA by RT-PCR.
In a gel, eluate floats out of the well when loading	Alcohol carryover in the eluate may cause it to float. Allow eluate to air-dry or dry in a Speed Vac® before loading on a gel.

6. Appendix

6.A. Creating a Ribonuclease-Free Environment

Ribonucleases are extremely difficult to inactivate. Take care to avoid introducing RNase activity into your RNA samples during and after isolation. This is especially important if the starting material was difficult to obtain or is irreplaceable. The following notes may help prevent accidental RNase contamination of your samples.

1. Two of the most common sources of RNase contamination are the user's hands and bacteria or molds that may be present on airborne dust particles. To prevent contamination from these sources, use sterile technique when handling the reagents supplied with this system. Wear gloves at all times. Change gloves whenever ribonucleases may have been contacted.
2. Whenever possible, sterile, disposable plasticware should be used for handling RNA. These materials generally are RNase-free and do not require pretreatment to inactivate RNase.
3. Treat nonsterile glassware, plasticware and electrophoresis chambers before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plasticware with 0.1N NaOH, 1mM EDTA, followed by RNase-free water. Commercially available RNase removal products also may be used following the manufacturer's instructions.

   Note: Electrophoresis chambers may be contaminated with ribonucleases, particularly RNase A, from analysis of DNA samples. Whenever possible, set aside a new or decontaminated apparatus for RNA analysis only.

4. Treat solutions not supplied with the system by adding diethyl pyrocarbonate (DEPC) to 0.1% in a fume hood. Incubate overnight with stirring at room temperature in the hood. Autoclave for 30 minutes to remove any trace of DEPC.

   Caution: DEPC is a suspected carcinogen and should only be used in a chemical fume hood. DEPC reacts rapidly with amines and cannot be used to treat Tris buffers.

Note: For all downstream applications, it is essential that you continue to protect your RNA samples from RNases. Continue to wear clean gloves and use solutions and centrifuge tubes that are RNase-free.

6.B. Related Products

Product	Size	Cat.#
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	0.5ml	Z5341
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
Maxwell® RSC Plunger Pack	1 each	AS1670

7. Summary of Changes

The following change was made to the 1/21 revision of this document:

1. Updated names of Cat.# AS8401, AS8402 in Section 6.B, Related Products.