TECHNICAL MANUAL

Maxwell® 16 LEV Plant RNA Kit

Instructions for Use of Product **AS1430**

Note: To run the Plant RNA protocol, you must have Maxwell® 16 firmware version ≥4.97 installed on your Maxwell® 16 AS2000 Instrument, and you must use the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070). Using the original LEV magnetic rod will result in low yields.

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Maxwell® 16 LEV 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® 16 LEV Plant RNA Kit[a] is used with the Maxwell® 16 Instrument (Cat.# AS2000) to purify RNA from plant tissue samples. To run the Plant RNA protocol, you must have Maxwell® 16 firmware version ≥4.97 installed on your Maxwell® 16 AS2000 Instrument, and you must use the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070). 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® 16 Instrument can process from 1 to 16 samples in under an hour.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® 16 LEV Plant RNA Kit	**48 preps**	**AS1430**

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
- 50 LEV Plungers
- 50 Elution Tubes, 0.5ml

Storage Conditions: Upon receipt, remove 1-Thioglycerol and store 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. 1-Thioglycerol is toxic. Guanidine thiocyanate and guanidine hydrochloride (which are components of the Homogenization Solution and Lysis Buffer) are 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.

Note: Bleach reacts with guanidine thiocyanate and should not be added to any sample waste containing the Homogenization Solution.

3. Before You Begin

3.A. Maxwell® 16 Instrument Hardware and Firmware Setup

To use the Maxwell® 16 LEV Plant RNA Kit, the Maxwell® 16 Instrument must be configured with LEV hardware. If your Maxwell® 16 Instrument contains standard elution volume (SEV) hardware or the original LEV Magnetic Rod, **it must be reconfigured using the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070**; Figure 1). Using the original LEV magnetic rod will result in low yields. Reconfiguring the instrument is simple. Refer to the *Maxwell® 16 Instrument (AS2000) Operating Manual* #TM295 for directions.

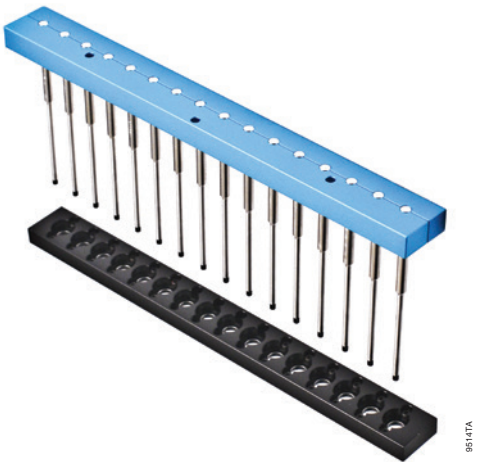

Figure 1. Maxwell 16® High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070).

Failure to change the Maxwell® 16 Instrument hardware to the LEV configuration could result in instrument damage. Using the standard elution volume (SEV) hardware configuration with LEV-configured reagent products will cause damage to the instrument.

Important: To run the Plant RNA protocol, you must have Maxwell® 16 firmware version ≥4.97 installed on your AS2000 instrument.

Capacity: Each cartridge in the Maxwell® 16 Plant RNA Kit can process up to 100mg of plant leaf material.

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

3.C. Cartridge Preparation

To maintain an RNase-free environment during processing, change gloves before handling cartridges, LEV Plungers and Elution Tubes. Place the cartridges to be used in the Maxwell® 16 LEV Cartridge Rack (Cat.# AS1251). Place each cartridge in the 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 Cartridge Rack.

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.

   For setup of plant leaf samples, see Section 4.

Figure 2. The Elution Tubes are placed in the front of the Maxwell® 16 LEV Cartridge Rack, and 50µl of Nuclease-Free Water is dispensed into each tube.

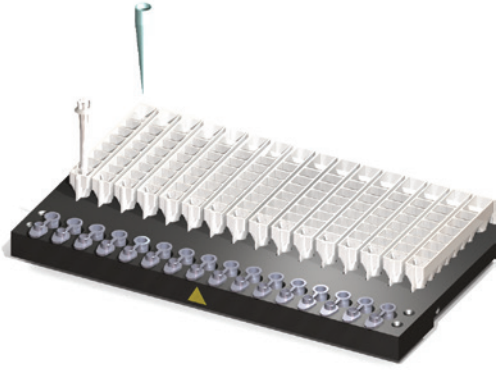

Figure 3. The LEV plunger is placed in well #8 of the cartridge (the well closest to the Elution Tube), and lysates are placed into well #1 of the cartridge.

4. Purification of Total RNA from Plant Leaf Tissue

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
- vortex mixer
- tube for homogenization
- liquid nitrogen
- microcentrifuge tube, 1.8ml
- RNase-free, sterile, aerosol-resistant pipette tips
- microcentrifuge

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 (see Section 3.B) to the tube. If plant material is on the tube cap or on the 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. Homogenize in 15- to 30-second increments if needed, then place the sample on ice.
6. With a wide-bore pipette, transfer 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® 16 Instrument, add 200µl of Lysis Buffer (Part #MC501C) 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. 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.
10. Add 5µl of DNase I (see Section 3.B) to well #4 (yellow reagent). After the blue DNase I solution is added, the reagent in well #4 will be green.

    Proceed to Section 5 for instructions on loading samples on the instrument and beginning the automated purification.

5. Instrument Run: Maxwell® 16 AS2000 Instrument

Refer to the *Maxwell® 16 Instrument (AS2000) Operating Manual* #TM295 for more detailed information.

Important: To run the Plant RNA protocol, you must have Maxwell® 16 firmware version **4.97** or higher installed on your instrument, and you must use the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070). Using the original LEV magnetic rod will result in low yields.

1. Turn on the Maxwell® 16 Instrument. The instrument will power up, display the firmware version number, proceed through a self-check and home all moving parts.

   Note: Ensure that you are running firmware version 4.97 or higher.

2. Verify that the instrument settings indicate an "LEV" hardware configuration and "Rsch" operational mode setting.

3 Select "Run" on the Menu screen, and press the Run/Stop button to select the method.

4. Select "RNA" then select "RNA" again then select"Plant" on the Menu screen. Next select "OK" at the Verification screen.

5. Open the door when prompted. Press the Run/Stop button to extend the platform.

Warning: Pinch point hazard.

6. Transfer the Maxwell® 16 LEV Cartridge Rack containing prepared cartridges on the Maxwell® 16 Instrument platform. Ensure that the rack is placed in the Maxwell® 16 Instrument with the Elution Tubes closest to the door. The rack will only fit in the instrument in this orientation. If you have difficulty fitting the rack on the instrument platform, check that the rack is in the correct orientation. Ensure that the cartridge rack is level on the instrument platform.

   Note: Hold the Maxwell® 16 LEV Cartridge Rack by the sides to avoid dislodging cartridges from the rack.

7. Verify that samples were added to well #1 of the cartridges, cartridges in the rack are loaded on the instrument, Elution Tubes are present with 50µl of Nuclease-Free Water and LEV Plungers are in well #8. Well #4 should be green to indicate that DNase was added.

Note: Failure to add DNase will result in DNA in the eluate.

8. Press the Run/Stop button. The platform will retract. Close the door.

Warning: Pinch point hazard.

9. The Maxwell® 16 Instrument will immediately begin the purification run. The screen will display the steps performed and the approximate time remaining in the run.

   Notes:

   1. Pressing the Run/Stop button or opening the door will pause the run.
   2. If the run is abandoned before completion, the instrument will wash the particles off the plungers and eject the plungers into well #8 of the cartridge. To continue processing the samples, rinse any particles off the plunger into the last well used. Discard the used plungers. Put new plungers into well #8, and start the run from the beginning.

10. When the automated purification run is complete, the LCD screen will display a message that the method has ended.

End of Run

11. Follow the on-screen instructions at the end of the method to open door. Verify that the plungers are located in well #8 of the cartridge at the end of the run. If the plungers are not removed from the magnetic plunger bar, push them down by hand to remove them.
12. Press the Run/Stop button to extend the platform out of the instrument.
13. Remove the Maxwell® 16 LEV Cartridge Rack from the instrument. Remove Elution Tubes containing total RNA, and close the tubes.
14. 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) or Maxwell® 16 LEV Magnet (Cat.# AS1261). Transfer the supernatant to a clean tube (not provided). Avoid transferring paramagnetic particles.
15. Remove the cartridges and plungers from the Maxwell® 16 LEV Cartridge Rack, and discard following the recommended guidelines. Do not reuse reagent cartridges, LEV plungers or Elution Tubes.

Storing Eluted RNA

If sample eluates are not processed immediately, the eluted RNA should be stored 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.

6. 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 rehomogenize the sample. The maximum volume of homogenate that can be processed in a single Maxwell® Cartridge (RSCO) is 400µl.
Low RNA yield, RNA degradation or poor reproducibility between samples	1-Thioglycerol was not added to the Homogenization Solution.
	Lysis Buffer was not added.
	Lysates were not mixed by vortexing for long enough.
	Homogenization was incomplete. Incomplete homogenization of samples results in loss of RNA within the particulates and clumps of debris.
	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.
	The wrong magnet bar was used. Use the High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070).

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Low RNA yield, RNA degradation or poor reproducibility between samples (continued)	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.
	RNase 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 7.A, Creating a Ribonuclease-Free Environment.
	The wrong method was run with the Maxwell® 16 Instrument.
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 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.
	The wrong method was run with the Maxwell® 16 Instrument.
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.

7. Appendix

7.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.

7.B. Related Products

Instrument/Instrument Accessories	Size	Cat.#
Maxwell® 16 Instrument	1 each	AS2000
Maxwell® 16 LEV Hardware Kit	1 each	AS1250
Maxwell® LEV Cartridge Rack	1 each	AS1251
Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor	1 each	SP1070

Accessory Products		Cat.#
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	0.5ml	Z5341
Maxwell® 16 LEV Magnet	1 each	AS1261

8. Summary of Change

The following change was made to the 10/19 revision of this document:

1. Updated Maxwell® RSC Cartridge name.

[a]U.S. Pat. No. 6,855,499, European Pat. No. 1368629, Japanese Pat. No. 4399164 and other patents pending.