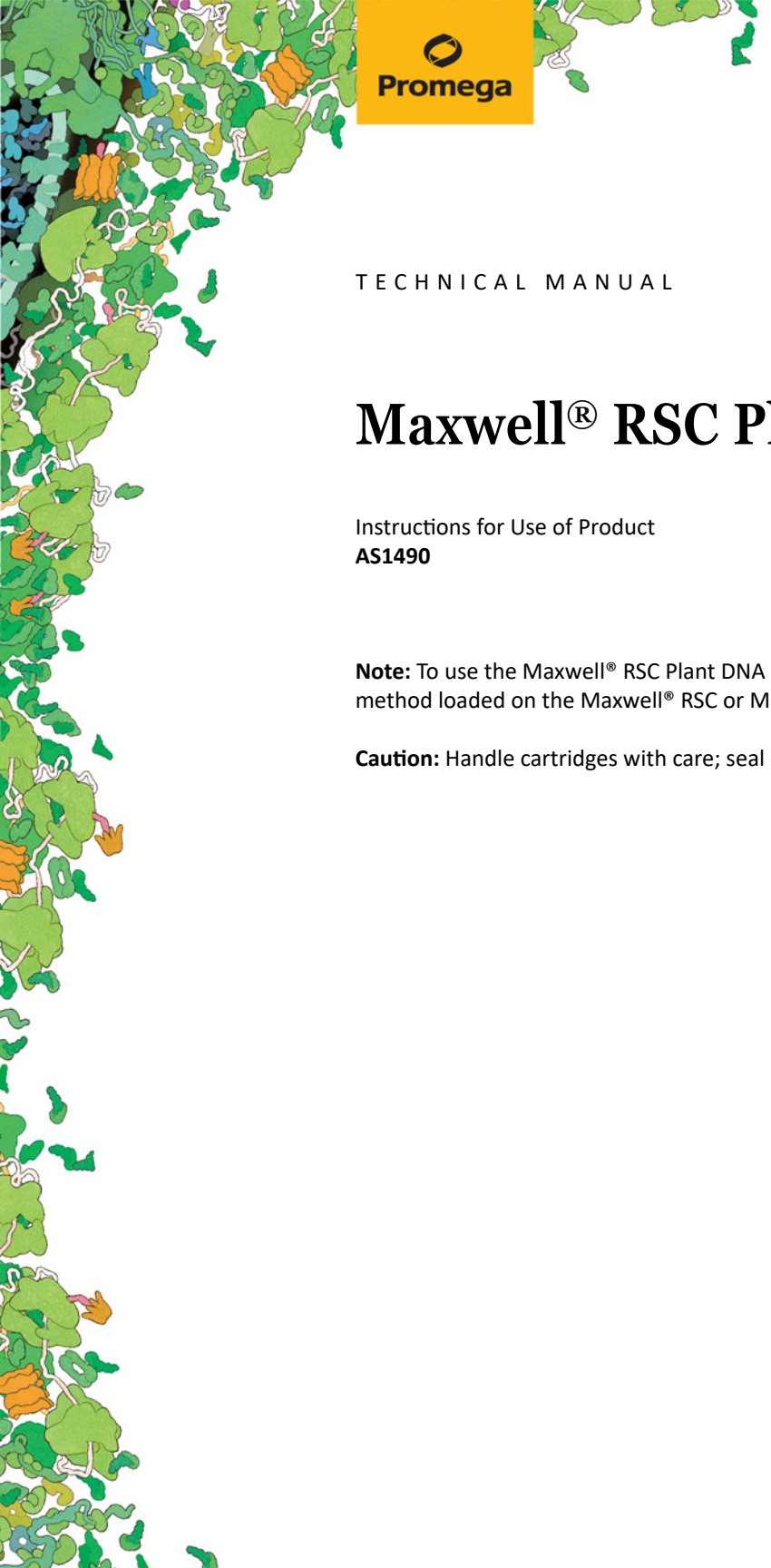


TECHNICAL MANUAL

Maxwell® RSC Plant DNA Kit

Instructions for Use of Product
AS1490

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

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

Revised 10/21
TM458

Promega

Maxwell® RSC Plant DNA 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 DNA Kit[a] is designed to provide an easy method for efficient, automated purification of genomic DNA (gDNA) 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 Maxwell® RSC Instrument can process up to 16 samples, and the Maxwell® RSC 48 Instrument can process up to 48 samples, both in about 40 minutes. The entire procedure, including sample disruption, can be completed in approximately 60 minutes, depending on the preprocessing method chosen. The purified DNA can be used directly in a variety of downstream applications, including amplification and agarose gel electrophoresis.

The Maxwell® RSC Plant DNA Kit purifies samples using a novel paramagnetic particle, the MagnaCel™ particle, which provides a mobile solid phase that optimizes sample capture, washing and purification of gDNA. This cellulose-based particle provides higher binding capacity and higher concentration eluates than silica-based DNA purification methods. The Maxwell® RSC and Maxwell® RSC 48 Instruments are magnetic particle handlers that efficiently bind gDNA to the paramagnetic particles in the first well of the reagent cartridge and mix during processing. This approach to magnetic capture avoids common problems such as clogged tips or partial reagent transfers that result in suboptimal purification by other commonly used automated systems.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Plant DNA Kit	**48 preps**	**AS1490**

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

- 25ml Tail Lysis Buffer (TLA)
- 25ml Nuclease-Free Water
- 1ml RNase A (4mg/ml)
- 48 Maxwell® RSC Cartridges (RSCB)
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® RSC Plant DNA Kit at 15–30°C.

Safety Information: The reagent cartridges contain ethanol and isopropanol, which are flammable and irritants. Wear gloves and follow standard safety procedures while working with these substances. Refer to the SDS for detailed safety information.

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

Available Separately: ClickFit Microtubes (recommended for use with the liquid nitrogen sample extraction method; Section 3.B).

PRODUCT	SIZE	CAT.#
ClickFit Microtube, 1.5ml	**1,000/pack**	**V4741**

3. Before You Begin

The Maxwell® RSC Plant DNA Kit can process up to 20mg of plant tissue per DNA isolation.

3.A. Maxwell® Method Setup

Before using the Maxwell® RSC Plant DNA Kit for the first time, the Plant DNA 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/**

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

3.B. Preparation of Plant Leaf Samples with a Mechanical Bead-Beating Device

This preprocessing protocol requires a mechanical bead-beating device with a bead and tube combination or a bead and sealable deep-well plate combination.

Materials to Be Supplied by the User

- bead-beating device (e.g., MP Biomedicals FastPrep®-24 Instrument)
- sterile, aerosol-resistant pipette tips for sample transfer into prefilled reagent cartridges
- microcentrifuge or plate-specific centrifuge

Sample Processing Notes

The total yield of genomic DNA from plant materials depends on the volume of material processed and the amount of genomic DNA in the plant material used. Each cartridge supplied in the Maxwell® RSC Plant DNA Kit is designed to purify genomic DNA from 310µl of plant lysate with up to 20mg of plant material. The reagents required to generate plant lysates are supplied in the kit. Nuclease-Free Water is provided to dilute the binding buffer in the first well of the cartridge to optimize binding of genomic DNA.

1. Place up to 20mg of leaf tissue in the bottom of each tube or well.
2. Place a bead (or beads, as recommended by manufacturer) into each tube or well.
3. Add 300µl of Tail Lysis Buffer (TLA) to each tube or well.
4. Add 10µl of RNase A to each well (optional, to eliminate RNA).

   Note: If you are processing a large number of samples, combine sufficient volume of Tail Lysis Buffer and RNase A immediately before use and add 310µl of this cocktail to each sample.
5. Run the bead-beating device using the time and speed recommended by the manufacturer. Some optimization may be required to generate sufficient sample lysis for the desired DNA yield.
6. Place the extraction tubes or plates into a centrifuge and spin for 2 minutes at maximum speed to pellet any solid particulates from the sample lysate.
7. Proceed to Section 3.D to set up the deck tray(s) and cartridges.

3.C. Preparation of Plant Leaf Samples with a Microtube, Pestle and Liquid Nitrogen

This preprocessing protocol uses a microtube and pestle for tissue grinding and liquid nitrogen to freeze the sample.

Materials to Be Supplied by the User

- Pellet Pestles (Sigma Aldrich Cat.# Z359947)
- ClickFit Microtubes, 1.5ml
- liquid nitrogen
- sterile, aerosol-resistant pipette tips for sample transfer into prefilled reagent cartridges
- microcentrifuge

Sample Processing Notes

The total yield of genomic DNA from plant materials depends on the volume of material processed and the amount of genomic DNA in the plant material used. Each cartridge supplied in the Maxwell® RSC Plant DNA Kit is designed to purify genomic DNA from 310µl of plant lysate with up to 20mg of plant tissue. The reagents required to generate plant lysates are supplied in the kit. Nuclease-Free Water is provided to dilute the binding buffer in the first well of the cartridge to optimize binding of genomic DNA.

1. Place up to 20mg of leaf tissue in the bottom of a ClickFit Microtube, 1.5ml.
2. Add liquid nitrogen to the plant tissue sample. Allow the liquid to evaporate, freezing the sample.
3. Using a pellet pestle, grind the frozen plant tissue against the tube wall as thoroughly as possible.
4. Add 300µl of Tail Lysis Buffer (TLA) to each tube.
5. Add 10µl of RNase A to each tube (optional, to eliminate RNA).

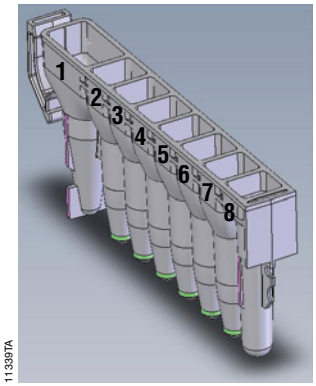
   Note: If you are processing a large number of samples, combine sufficient volume of Tail Lysis Buffer and RNase A immediately before use and add 310µl of this cocktail to each sample.
6. Vortex tubes for 10 seconds.
7. Place tubes with lysate into a microcentrifuge and spin for 2 minutes at maximum speed to pellet solid particulates from the lysate.
8. Proceed to Section 3.D to set up the deck tray and cartridges.

3.D. Maxwell® RSC Plant DNA Cartridge Preparation

1. Change gloves before handling cartridges, Plungers and Elution Tubes. Place each cartridge in the deck tray(s) with the labeled 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: Sample or reagent spills on any part of a deck tray should be cleaned with a detergent-water solution, followed by a bactericidal spray or wipe and then water. Do not use bleach on any instrument parts.

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



User Adds to Wells
1. 300µl of Nuclease-Free Water + preprocessed samples
8. Plunger

Figure 1. Maxwell® RSC Cartridge.

3.D. Maxwell® RSC Plant DNA Cartridge Preparation (continued)

3. Place empty Elution Tubes into the front of the deck tray(s). Add 50µl of Elution Buffer to the bottom of each Elution Tube. See Figure 2.

Notes:

1. If Elution Buffer 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 be incompatible with the Maxwell® Instruments.

Figure 2. Setup and configuration in the deck tray(s). Elution Buffer is added to the Elution Tubes as shown. Plungers are in well #8 of the cartridge.

4. Add 300µl of Nuclease Free Water to well #1 (the largest well) of the Maxwell® RSC Plant DNA cartridge.
5. Transfer each plant lysate sample from Step 7 of Section 3.B or Step 8 of Section 3.C into well #1 of the Maxwell® RSC Plant DNA cartridge. Transfer the clear liquid, being careful not to transfer any solid material at the bottom of the tube or on the surface of the liquid.

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 DNA method.
   b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate method.

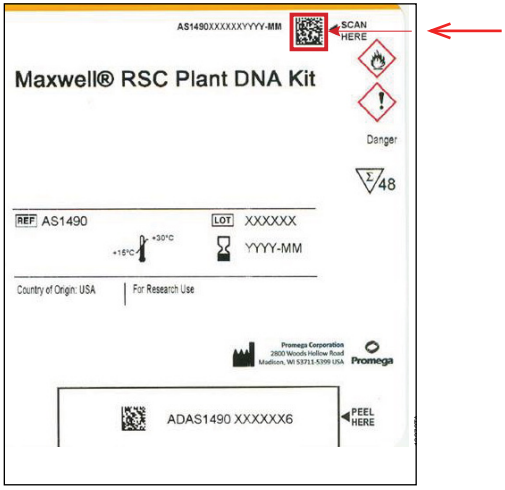


Figure 3. Kit label indicating the method bar code. Scan this bar code to automatically select the method for a purification run.

4. Verify that the Plant DNA 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.

4. Maxwell® Instrument Setup and Run (continued)

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 Elution Buffer 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. Pressing **Abort** will abandon the run.
   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, 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 the plungers are located in well #8 of the cartridge at the end of the run. If the 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 DNA, and cap the tubes. For short-term storage or frequent use of the DNA, store at 2–10°C; for long-term storage, store at –30 to –10°C. Avoid multiple freeze-thaw cycles.

   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: Following the automated purification procedure, the deck tray(s) will be warm. It will not be too hot to touch. To remove the deck tray from the instrument platform, hold onto the sides of the deck tray.

10. Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bactericidal spray or wipe and then water. All cartridges and tubes should be removed before cleaning. Do not use bleach on any instrument parts.

11. Remove the cartridges and plungers from the deck tray.

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.

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
Lower than expected A_{260} (yield)	Insufficient lysis. Consider optimization of the extraction protocol. If using a mechanical bead-beating device, consider increasing the number of strokes/minute or the amount of processing time.
	Sample is relatively poor in DNA content. Use more starting material.
Resin fines are present in the eluate	Briefly centrifuge and transfer the eluate to a clean tube.
Lower than expected absorbance (A_{260}/A_{280} or A_{260}/A_{230}) ratio	The MagnaCel™ particles may co-isolate plant compounds that can affect the absorbance ratio. Use an amplification-based assay to better assess the quality and suitability of the isolated DNA for downstream amplification analysis.
	Too much plant debris in cartridge. Ensure that no solid materials are pipetted into the cartridge, and do not pipette out lysate from too close to the pellet. Centrifuge the lysate at higher speeds. Do not use a tissue homogenizer. Reduce the amount of starting plant material used per sample.

6. Related Products

Product	Size	Cat.#
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
ClickFit Microtube, 1.5ml	1,000/pack	V4741

7. Summary of Changes

The following changes were made to the 10/21 revision of this document:

1. Updated Section 6.
2. Updated cover page.

[a]U.S. Pat. No. 6,855,499, European Pat. Nos. 1368629, 2090655 and 2363476, Japanese Pat. No. 4399164 and other patents.