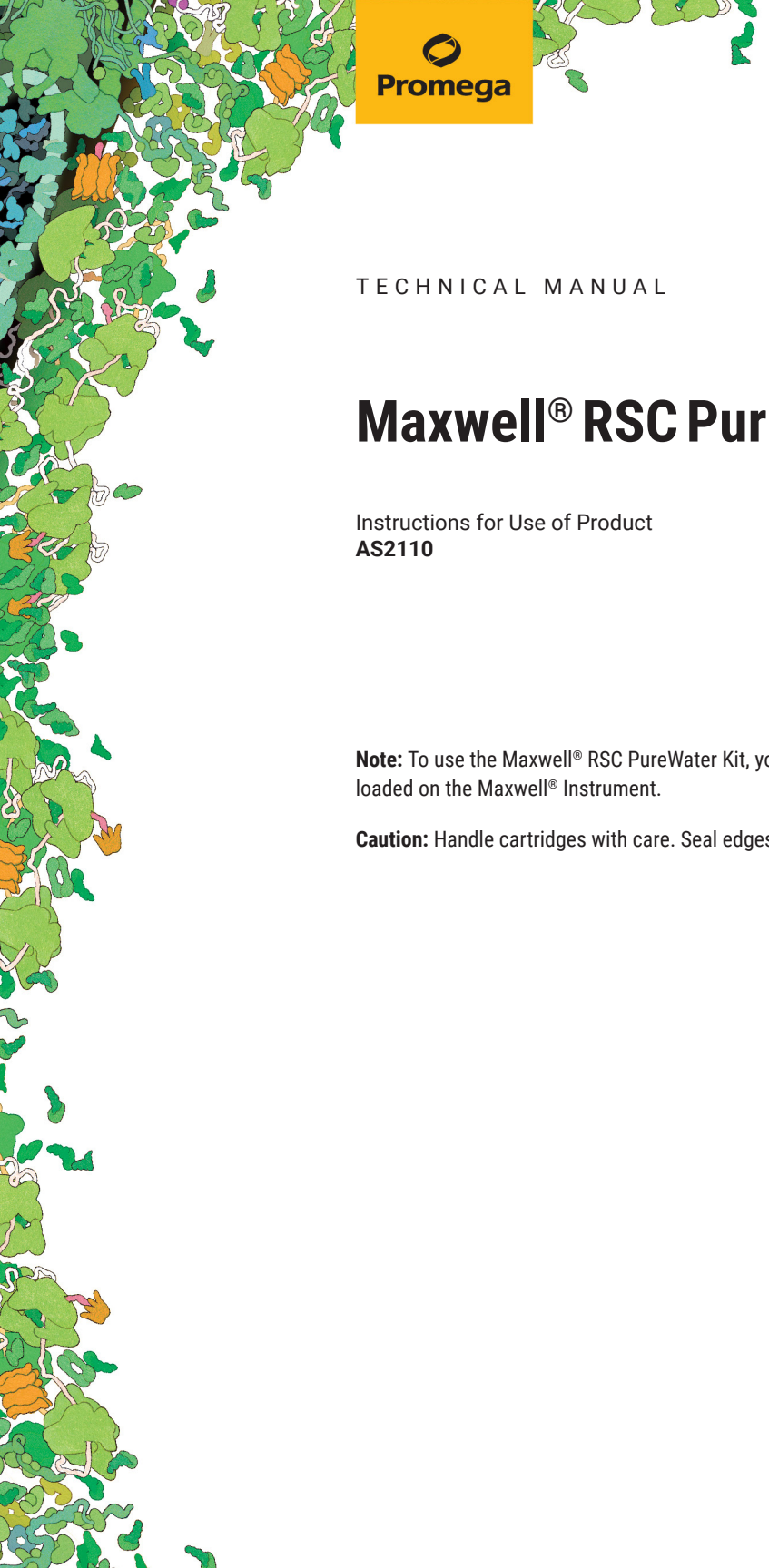

TECHNICAL MANUAL

Maxwell® RSC PureWater Kit

Instructions for Use of Product
AS2110

Note: To use the Maxwell® RSC PureWater Kit, you must have the "PureWater" method loaded on the Maxwell® Instrument.

Caution: Handle cartridges with care. Seal edges may be sharp.

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Maxwell® RSC PureWater Kit

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

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1. Description

Molecular tests, and in particular PCR-based assays, are increasingly prevalent in water safety testing. The Maxwell® RSC PureWater Kit represents a significant advancement in the field of molecular water testing, addressing the limitations inherent in traditional manual methodologies for extracting bacterial DNA from aqueous samples. Current approaches often suffer from inconsistencies due to method variability and user-to-user differences, which can lead to unreliable results in downstream applications such as PCR, next-generation sequencing (NGS) and microbial source tracking. In contrast, the Maxwell® RSC PureWater Kit, used with the Maxwell® Instruments (see Table 1), employs an automated purification process that ensures a high level of precision and reproducibility, drastically reducing the likelihood of human error. The high-quality DNA obtained through this system is optimal for a range of molecular biology techniques, ensuring accurate identification and quantification of bacterial pathogens. By streamlining the DNA isolation process, this kit not only enhances the efficiency of water testing laboratories but also plays a crucial role in safeguarding human health by enabling the rapid and accurate detection of waterborne diseases.

Maxwell® Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. The methods for the Maxwell® RSC PureWater Kit can process from one to the maximum number of water samples in approximately 40 minutes. The purification process is flexible, allowing for both the purification of DNA from unconcentrated suspension samples and concentrated samples collected on a filter (Figure 1).

Table 1. Supported Instruments.

Instrument	Cat.#	Technical Manual	Maximum Sample Number
Maxwell® RSC	AS4500	TM411	16
Maxwell® RSC 48	AS8500	TM510	48
Maxwell® FSC	AS4600	TM462	16
Maxwell® CSC RUO Mode	AS6000	TM573	16
Maxwell® CSC 48 RUO Mode	AS8000	TM628	48

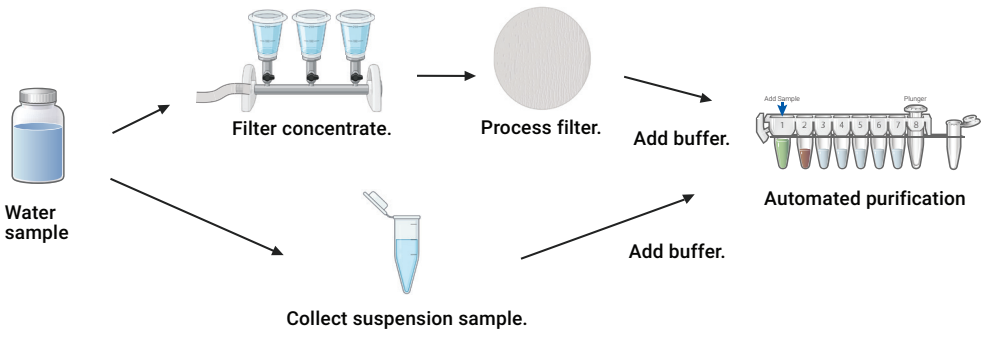

Figure 1. Maxwell® RSC PureWater Kit purification workflow.

Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind DNA to the paramagnetic particles in the first well of a prefilled cartridge and move the sample through the wells of the cartridge, mixing during processing. This magnetic capture approach avoids common problems experienced with other automated systems such as clogged tips or partial reagent transfers that result in suboptimal purification processing.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC PureWater Kit	**48 preps**	**AS2110**

For Laboratory use. Not for Human diagnostic use. Contains sufficient reagents for 48 automated nucleic acid isolations from water samples. Includes:

- 100ml CTAB Buffer
- 2 × 1ml Proteinase K (PK) Solution
- 2 × 1ml RNase A Solution
- 20ml Lysis Buffer
- 48 Maxwell® RSC Cartridges (RSCJ)
- 1 pack Maxwell ® RSC Plunger Pack (48 plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® RSC PureWater Kit at +15°C to +30°C.

Safety Information: The Maxwell® RSC Cartridges contain ethanol and isopropanol, which are flammable. Guanidine hydrochloride (a component of the Lysis Buffer) should be considered harmful and an irritant. 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.

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3. Suspension Sample Pretreatment and Processing

Use this protocol if test samples have already been collected and/or filter concentrated using externally validated bacterial collection methods (CDC-9260-J, etc.). The protocol outlined below requires 200µl of sample input.

Note: If necessary, resuspend bacteria in an isotonic buffer such as 1X PBS (phosphate-buffered saline) instead of nuclease-free water to minimize osmotic shock that could affect the viability of collected microorganisms.

Materials to Be Supplied By User

- microcentrifuge tubes, 1.5ml or 2.0ml
- pipettors and sterile, aerosol-resistant pipette tips
- heating block set to 56°C
- vortex mixer

1. Add 200µl of CTAB Buffer and 40µl of Proteinase K to 200µl of liquid sample in a microcentrifuge tube.
2. Vortex for 5 seconds to mix.
3. Place in a heating block at 56°C for 10 minutes.
4. Proceed to Section 5 for purification on the Maxwell® Instrument. The entire sample volume (approximately 450µl) from Step 3 should be transferred to well #1 (the largest well) of the prepared Maxwell® reagent cartridge.

4. Filtering, Concentrating and Purifying Nucleic Acid from Bulk Water Samples

Use this protocol to filter concentrate bacteria from bulk water or liquid samples up to 200ml. Processing of larger sample volumes is possible; however, larger volumes will require optimization. Suggested filter material and filter apparatus setup is described below. Other filter collection methods may be feasible; the suitability of alternative 25mm or other filter and filter collection methods should be verified.

Materials to Be Supplied By User

- filter concentration apparatus, including: vacuum manifold (e.g., Cytiva Cat.# 4889); standard adaptor (e.g., Cytiva Cat.# 4892); 25mm filter funnel (e.g., Cole Parmer Cat.# EW-35200-55); rubber stoppers (VWR Cat.# 59581-367)
- 0.2µm polycarbonate membrane filters, 25mm diameter (e.g., Millipore Cat.# GTTP02500)
- vacuum pump or other vacuum source
- 1X PBS (phosphate-buffered saline)
- sterile scalpel
- 1.5ml microcentrifuge tubes
- pipettors and sterile, aerosol-resistant pipette tips
- sterile petri dishes (10cm or 15cm diameter)
- heating block capable of 70°C and 95°C
- vortex mixer
- **optional:** sterile forceps (for transferring filter)

4.A. Filter Collection and Concentration

1. Collect water or liquid sample to be tested.
2. Filter concentrate the samples onto 0.2µm polycarbonate filters.
3. Aseptically transfer the filter to a sterile petri dish.
4. Using the sterile scalpel, cut the filter into 2–4 small fragments.
5. Aseptically transfer the fragmented filter into a 1.5ml microcentrifuge tube containing 700µl of 1X PBS.

4.B. Sample Pretreatment and Processing

1. Place tubes with fragmented filters into a microcentrifuge and centrifuge at room temperature for 1 minute at 10,000rpm.
2. Carefully remove and discard the supernatant from the filters, taking care to pipet from the center of the tube. Do not pipet near the bottom of the tube to avoid disturbing the pelleted bacteria
3. Add 700µl of CTAB Buffer to the sample tube containing the filter and pelleted bacteria.
4. Vortex for 30 seconds.
5. Incubate at 95°C for 5 minutes.
6. Allow the sample tube to cool at room temperature for 2 minutes.
7. Vortex for 1 minute.
8. Add 40µl of Proteinase K and 20 µl of RNase A, then briefly vortex to mix thoroughly.
9. Incubate at 70°C for 10 minutes.
10. Proceed to Section 5 for purification on the Maxwell® Instrument. Up to 700µl of liquid sample from Step 9 should be transferred into well #1 (the largest well) of the prepared Maxwell® reagent cartridge. Leave the filter fragments in the 1.5ml tube; do not transfer them to the Maxwell® reagent cartridge.

5. Preparing the Maxwell® RSC PureWater Kit Cartridge (RSCJ)

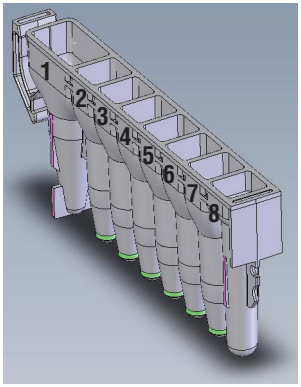
1. Change gloves before handling cartridges, plungers and Elution Tubes. Place the required number of cartridges in the deck tray(s). Place each cartridge in the deck tray with well #1 (the largest well) facing away from the Elution Tube position. Press down on the cartridge to snap it into position. 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 the deck tray should be cleaned with a detergent-water solution, followed by a bactericidal spray or wipe, then water. Do not use bleach on any instrument parts.

   Caution: Handle cartridges with care. Seal edges may be sharp.

2. Place a Maxwell® RSC Plunger into well #8 of each cartridge. Well #8 is the well closest to the Elution Tube position. See Figures 2 and 3.

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



User Adds to Wells:

1. Preprocessed lysate + Lysis Buffer

8. RSC Plunger

Figure 2. Maxwell® RSCJ Cartridge.

3. Place empty Elution Tubes into the front of the deck tray. Add 80µl of Elution Buffer to the bottom of each Elution Tube. See Figure 3.

   Notes:

   a. If Elution Buffer 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 be incompatible with the Maxwell® Instrument.

Figure 3. 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 Lysis Buffer to well #1 (the largest well) of each cartridge.
5. Add up to 700µl of liquid sample processed as instructed in Section 3, Step 4, or Section 4.B, Step 10.

6. Maxwell® Instrument Setup and Run

For detailed information, refer to the Technical Manual specific to your instrument (see Table 1).

1. Turn on the Maxwell® Instrument and Tablet PC. Log 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 begin the process of running a method.
3. Depending on your Maxwell® Instrument model, use one of the following options to select a method:
   a. Scan or enter the 2D bar code information on the kit box to automatically select the appropriate method.
   b. Touch the **PureWater** method.
4. If applicable to your Maxwell® Instrument model, verify that the **PureWater** method has been selected, and touch the **Proceed** button. If requested by the software, scan or enter any kit lot information that has been required by the Administrator.
5. On the 'Cartridge Setup' screen, touch the cartridge positions to select/deselect the positions to be used for this extraction run. Enter any required sample tracking information and touch the **Proceed** button to continue.

   Note: When using 48-position Maxwell® Instruments, press the Front and Back buttons to select/deselect cartridge positions on each deck tray.

6. Maxwell® Instrument Setup and Run (continued)

6. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that cartridges are loaded on the instrument, preprocessed samples are added to well #1 of the cartridges, uncapped elution tubes are present with 80µl of Elution Buffer and plungers are present 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 it 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: Check the identifier on the 24-position Maxwell® deck trays to determine whether they should be placed in the front or back of the instrument. Deck trays are keyed and will only fit in their intended positions.

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

Warning: Pinch point hazard.

8. 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:

   a. When using a 48-position Maxwell® 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 and 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.

   b. Touching **Abort** will abandon the run.

   c. 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.

9. When the run is complete, the user interface will display a message that the method has ended.

End of Run

10. 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 Technical Manual appropriate to your Maxwell® Instrument (see Table 1) to perform a Clean Up process to attempt to unload the plungers.
11. Remove the deck tray(s) from the instrument immediately following the run to prevent evaporation of the eluates. Remove elution tubes containing DNA, and cap the tubes.

    Note: Following the automated purification procedure, the deck tray(s) will be warm. To remove a deck tray from the instrument platform, hold onto the deck tray by its sides.
12. Ensure samples are removed from the instrument before running a UV sanitation protocol to avoid damage to the nucleic acid.

13. Remove the cartridges and plungers from the Maxwell® deck tray(s). Discard as hazardous waste according to your institution's procedures. Do not reuse Maxwell® RSC Cartridges, RSC Plungers or Elution Tubes.

7. Troubleshooting

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

Symptom	Causes and Comments
Resin fines are present in the eluate	Resin fines should not affect qPCR. However, if you prefer to remove the fines, briefly centrifuge and transfer the eluate to a clean tube.
Precipitate in CTAB or Lysis Buffer bottle	Precipitate may form at lower temperatures. Resuspend the precipitated solution by warming the bottle at room temperature, and shake.
Lower than expected absorbance (A_{260}/A_{280} or A_{260}/A_{230})	The Maxwell® cartridge paramagnetic particles may co-isolate 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.

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8. Related Products

Instruments and Accessories

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
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® FSC Deck Tray	1 each	AS4016

Legionella Testing Kits and Reagents

Product	Size	Cat.#
GoTaq® *Legionella pneumophila* qPCR Kit	1 each	AM2201
GoTaq® *Legionella spp/pneumophila*/SG1 qPCR Kit	1 each	AM2202
GoTaq® *Legionella pneumophilia* Viability qPCR Kit	1 each	AM2205
GoTaq® *Legionella spp/pneumophila*/SG1 Viability qPCR Kit	1 each	AM2206
Viability PCR Reagent System	1 kit	A8881
Viability PCR Reagent System, High Concentration	1 kit	A8883
Wizard® PureWater Kit	50 preps	A3130

Solutions and Buffers

Product	Size	Cat.#
Nuclease-Free Water	500ml	P1197
RNase A Solution (4mg/ml)*	1ml	A7973
Proteinase K (PK) Solution (20mg/ml)*	4ml	MC5005
CTAB Buffer	100ml	MC1411

*Additional sizes are available.