TECHNICAL MANUAL

Maxwell® RSC PureFood Pathogen Kit

Instructions for Use of Product
AS1660

Note: To use the Maxwell® RSC PureFood Pathogen Kit, you must have the "PureFood Pathogen" 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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Maxwell® RSC PureFood Pathogen 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

Molecular tests, and in particular real-time PCR-based assays, continue to gain widespread use in food-safety testing. PCR-based assays are significantly faster and more reliable than traditional methods and also can detect more specific pathogenic targets. However, using a suboptimal DNA purification method for food samples containing high amounts of fats, oils or polysaccharides can lead to the inhibition of PCR-based pathogen testing. Therefore, the selection of a DNA purification method that provides amplifiable DNA of high quality is critical for accurate results.

The Maxwell® RSC PureFood Pathogen Kit (Cat.# AS1660) is designed to provide an easy and automated method for efficient purification of DNA used in PCR-based testing for food pathogen identification. Used with the Maxwell® RSC (Cat.# AS4500) or Maxwell® RSC 48 (Cat.# AS8500) Instruments, the kit can effectively purify DNA from food matrix samples in 40 minutes. The Maxwell® RSC Instrument can process up to 16 samples and the Maxwell® RSC 48 Instrument can process up to 48 samples. This method has successfully isolated pathogen DNA from a number of different food samples, including *E. coli 0157:H7* from uncooked beef, *Salmonella enterica* from uncooked chicken, and *Listeria monocytogenes* from whole milk.

1. Description (continued)

The Maxwell® RSC and Maxwell® RSC 48 are magnetic particle handling instruments that allow efficient binding of DNA to the paramagnetic particles in the first well of a prefilled cartridge and moves the sample through the wells of the cartridge, mixing during processing. This approach to magnetic capture avoids common problems with other automated systems, such as clogged tips or partial reagent transfers that result in suboptimal purification processing. The instrument is supplied with a preprogrammed purification method and is designed for use with predispensed reagent cartridges, maximizing simplicity and convenience.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC PureFood Pathogen Kit	**48 preps**	**AS1660**

Not for Medical Diagnostic Use. Sufficient for 48 automated isolations from food lysate samples. Includes:

- 20ml Lysis Buffer A
- 25ml Lysis Buffer
- 48 Maxwell® RSC Cartridges (RSCJ)
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

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

Safety Information: The reagent cartridges contain ethanol and isopropanol, which are flammable. Guanidine thiocyanate (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 SDS for detailed safety information.

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

3. Sample Preprocessing Protocols

Before using the Maxwell® RSC PureFood Pathogen Kit for the first time, the PureFood Pathogen method must be installed on your instrument. The methods are 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.A. Sample Processing Notes

The Maxwell® RSC PureFood Pathogen Kit can process up to 800µl of food matrix samples per DNA isolation with the standard protocol.

The total yield and quality of genomic DNA from the food matrix depends on the type of food material processed. Each cartridge supplied in the Maxwell® RSC PureFood Pathogen Kit is designed to purify genomic DNA from 800µl of lysate. All reagents needed to lyse samples and purify DNA are included in the kit.

This method is designed for food samples that are mixed in a Stomacher® lab paddle prior to incubation for bacterial enrichment.

Materials to Be Supplied by the User

- microcentrifuge tubes, 1.5ml or 2.0ml
- sterile, aerosol-resistant pipette tips
- ThermoMixer® heat block
- Stomacher® lab paddle or vortexer
- Stomacher® bags

3.B. Preparation of Samples with Stomacher® Lab Paddle

This preprocessing protocol requires a mechanical mixing device such as a Stomacher® lab paddle or vortexer for homogenization of food samples.

Follow the manufacturer's recommendation for processing the sample.

1. Weigh and add 25g of food sample into a filtered Stomacher® bag.
2. Add 225ml of pre-warmed select enrichment broth to the sample bag.
3. If necessary, add select bacteria to bag.
4. Place bag in Stomacher® lab paddle and select to mix at 230rpm for 30–60 seconds.
5. Incubate at 37°C for 20–24 hours.
6. Proceed to Section 4.

4. Bacterial Sample Lysis from Food Matrix

1. Add 800µl of food/bacterial sample to a microcentrifuge tube.
2. Add 200µl of Lysis Buffer A to each tube.
3. Incubate at 56°C for 4 minutes in a ThermoMixer® Device, shaking at 500–1,000rpm.
4. Add 300µl of Lysis Buffer to each tube.
5. Mix by vortexing for 5–10 seconds.
6. Add the entire sample to well #1 of the Maxwell® RSC PureFood Pathogen Cartridge.
7. Proceed to Section 5 for purification on the Maxwell® Instruments.

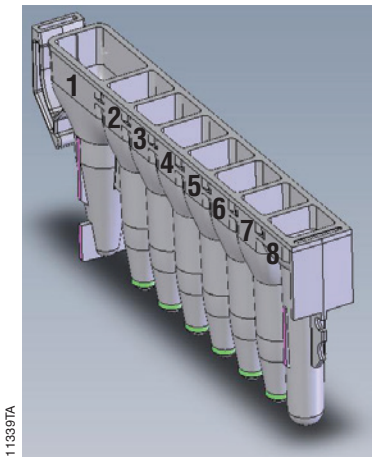
5. Purifying DNA on the Maxwell® Instruments

5.A. Maxwell® RSC PureFood Pathogen Cartridge Preparation

1. Change gloves before handling cartridges, Maxwell® RSC Plungers and Elution Tubes. Place the required number of cartridges in the deck tray(s). Place each cartridge in the deck tray with the labeled side 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 on 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 and then water. Do not use bleach on any instrument parts.

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 1 and 2.



User Adds to Wells:

1. Approximately 1300µl of preprocessed lysate
8. RSC Plunger

Figure 1. Maxwell® RSC Cartridge.

3. Place empty Elution Tubes into the front of the deck tray. 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 of the deck tray(s). Elution Buffer is added to the Elution Tubes as shown. Plungers are in well #8 of the cartridge.

4. Add the entire amount of sample lysate processed as instructed in Section 4, Step 6, to well #1 (the largest well) of each cartridge.

5.B. Maxwell® Instrument Setup and Run

Note: 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. Press **Start** to access the extraction 'Methods' selection screen.
3. On the 'Methods' screen, select a method using one of the following two options:
   a. Touch the **PureFood Pathogen** method.
   b. Use a bar code reader to scan the 2D bar code on the kit box (Figure 3) to automatically select the appropriate method.

5.B. Maxwell® Instrument Setup and Run (continued)

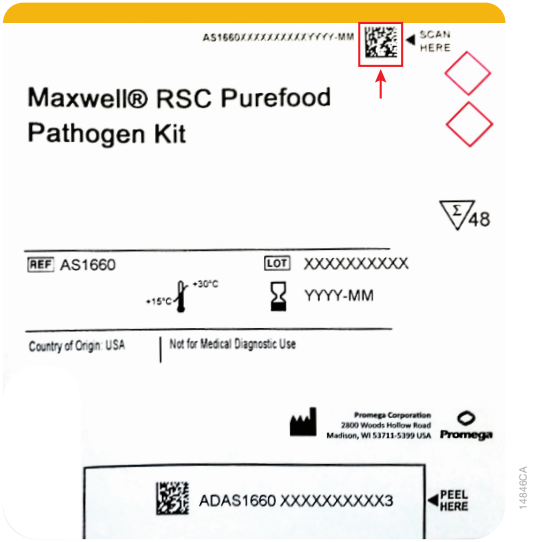


Figure 3. Kit label indicating the method bar code. Scan the bar code indicated by the red arrow to automatically select the method for a purification run.

4. Verify that the PureFood Pathogen 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 cartridges are loaded on the instrument, samples with Lysis Buffer are added to well #1 of the cartridges, uncapped elution tubes are present with 50µl of Elution Buffer and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® RSC Instrument platform.

   Inserting the Maxwell deck trays: 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 **Start** 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 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.
2. Touching the **Abort** button 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, 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 on to the sides of the deck tray.

11. Remove the cartridges and plungers from the deck tray(s), and discard 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.

6. 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**

Symptoms	Causes and Comments
Lower than expected A_{260} (yield)	Insufficient lysis. Consider optimization of the extraction protocol. Consider increasing the amount of pre-processing time. Increasing the pre-processing time up to 10 minutes can be beneficial with certain food matrices. Adding up to 400µl of Lysis Buffer A or up to 500µl of Lysis Buffer can improve lysis conditions.
	Sample is relatively low in DNA content. Consider transferring less of the food material and more of the liquid portion of the food/bacteria matrix. Excess food particles could interfere with resin particle transfer and result in lower DNA yields. Allowing the food matrix to settle several minutes prior to transferring to the microcentrifuge tube should result in less food particle transfer.
Resin fines 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.
Lower than expected absorbance (A_{260}/A_{280} or A_{260}/A_{230})	The MagnaCel™ 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.

7. Composition of Buffer

Elution Buffer

10mM Tris (pH 8.0)
0.1mM EDTA (pH 8.0)

8. Related Products

Instruments and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC 48 Back Deck Tray	1 each	AS8402
Maxwell® RSC Plunger Pack (48 Plungers)	1 each	AS1670
ClickFit Microtube, 1.5ml	1,000/pack	V4741

Maxwell® RSC Reagent Kits

Visit **www.promega.com** for a list of available Maxwell® RSC purification kits.

9. Summary of Changes

The following changes were made to the 8/18 revision of this document:

1. References to the Maxwell® RSC 48 Instrument were added to this Technical Manual.
2. Additional details were added to the protocol steps in Section 5.B.