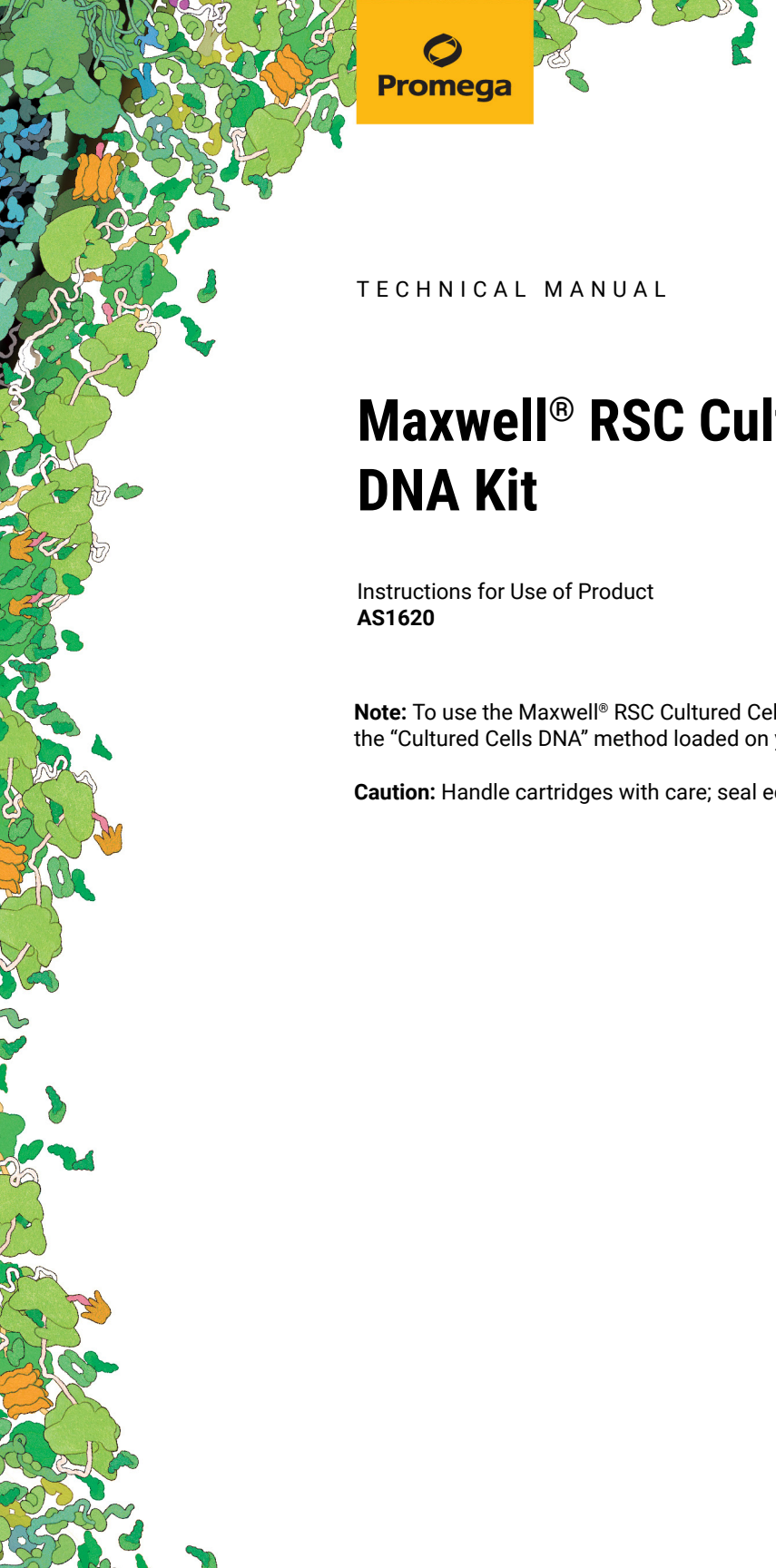

TECHNICAL MANUAL

Maxwell® RSC Cultured Cells DNA Kit

Instructions for Use of Product
AS1620

Note: To use the Maxwell® RSC Cultured Cells DNA Kit, you must have the "Cultured Cells DNA" method loaded on your Maxwell® Instrument.

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

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Maxwell® RSC Cultured Cells DNA 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

The Maxwell® RSC Cultured Cells DNA Kit (Cat.# AS1620) is used with the Maxwell® and Maxprep® Instruments specified in Table 1 to provide a simple method for efficient, automated purification of genomic DNA (gDNA) from tissue culture cell and cultured bacterial cell samples. The Maxwell® Instruments are supplied with preprogrammed purification procedures and are designed for use with predispensed reagent cartridges, maximizing simplicity and convenience. The Maxwell® Instruments can process from one to the maximum sample number in about 45 minutes. The purified DNA can be used directly in a variety of downstream applications, including amplification and agarose gel electrophoresis.

The Maxwell® RSC Cultured Cells DNA Kit purifies samples using a silica-based paramagnetic particle, called the MagneSil® particle, which provides a mobile solid phase that optimizes capture, washing and purification of sample gDNA. The Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind gDNA to the paramagnetic particle in the first well of a prefilled cartridge. This approach to magnetic capture avoids common liquid-handling problems such as clogged tips or partial reagent transfers that result in suboptimal purification processing by other automated systems.

1. Description (continued)

Prior to extraction, samples can be preprocessed manually or using the Maxprep® Liquid Handler. The Maxprep® Liquid Handler can transfer tissue culture or Gram-negative bacterial cells from sample tubes to Maxwell® RSC Cultured Cells DNA cartridges, transfer plungers to Maxwell® RSC Cultured Cells DNA cartridges, and dispense Elution Buffer to elution tubes.

Table 1. Supported Instruments.

Instrument	Cat.#	Technical Manual
Maxwell® RSC	AS4500	TM411
Maxwell® RSC 48	AS8500	TM510
Maxwell® CSC RUO Mode	AS6000	TM573
Maxwell® CSC 48 RUO Mode	AS8000	TM628
Maxprep® Liquid Handler	AS9100, AS9101, AS9105, AS9200, AS9201, AS9205	TM509

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Cultured Cells DNA Kit	**48 preps**	**AS1620**

For Research Use Only. Not for use in diagnostic procedures. Sufficient for 48 automated isolations from samples containing up to 5×10^6 tissue culture cells or up to 2×10^9 bacterial cells. Cartridges are for single use only. Includes:

- 48 Maxwell® RSC Cartridge (RSCI)
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

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

Safety Information: The Maxwell® RSC Cartridges contain ethanol, isopropanol and guanidine thiocyanate. Ethanol and isopropanol should be considered flammable, harmful and irritants. Guanidine thiocyanate should be considered toxic, harmful and an irritant. Refer to the SDS for detailed safety information.

Samples used with the Maxwell® RSC Cartridges may contain potentially infectious substances. Wear appropriate protection (e.g., gloves and safety glasses) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

Caution: Handle cartridges with care; seal edges may be sharp. Bleach reacts with guanidine thiocyanate and should not be added to any sample waste from these cartridges.

Available Separately For Preprocessing with the Maxprep® Liquid Handler

PRODUCT	SIZE	CAT.#
Maxprep® 1000µl Conductive Disposable Tips, Filtered	40/box	AS9303
Maxprep® 300µl Conductive Disposable Tips, Filtered	60/box	AS9302
Maxprep® Reagent Reservoir, 50ml	8/pack	AS9304
Maxprep® Plunger Holder	1 each	AS9408
Maxwell® RSC Plunger Pack	1 each	AS1670

3. Before You Begin

Materials to Be Supplied by the User

- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- lysozyme reconstituted as a 25mg/ml stock in water (for Gram-positive bacteria; Sigma Aldrich Cat.# L6876-5G)
- **optional:** RNase A Solution, 4mg/ml (Cat.# A7973)

Before using the Maxwell® RSC Cultured Cells DNA Kit for the first time, the Cultured Cells DNA method must be installed on your instrument.

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4. Preparing Cultured Cell Samples

The total yield of genomic DNA from cultured cell samples depends on the cell type and the number of cells being processed. Typical genomic DNA yields that may be expected from DNA extraction from various cultured cell samples is presented in Table 2 for general reference purposes. Each Maxwell® RSC Cartridge supplied in the Maxwell® RSC Cultured Cells DNA Kit is designed to purify genomic DNA from samples containing up to 5×10^6 tissue culture cells or up to 2×10^9 bacterial cells.

When using the maximum number of cells with this kit, it is normal to see some residual resin in well #1 after processing.

Table 2. Typical Genomic DNA Yields.

Sample	Sample Size	Typical Yield
Tissue culture cells		
HEK293	5×10^6 cells	18.0µg
K562 cells	5×10^6 cells	17.9µg
Gram-negative bacteria		
E. coli (Migula) Castellani and Chalmers	2×10^9 cells	9.4µg
Gram-positive bacteria		
B. cereus	2×10^9 cells	9.1µg
L. innocua	2×10^9 cells	7.8µg

Note: Cell samples were measured using QuantiFluor® ONE fluorescent dye to determine yield.

Preparation of Tissue Culture Cells

1. Up to 5×10^6 tissue culture cells suspended in a volume of up to 400µl culture medium may be added to well #1 of the predispensed cartridge.
2. No additional preprocessing steps are required beyond standard methods of releasing adherent cells.

Preparation of Gram-Negative Bacterial Cells

1. Up to 2×10^9 cells may be added to well #1 of the predispensed cartridge suspended in up to 400µl of culture medium.
2. No additional preprocessing steps are required beyond standard centrifugation required to concentrate cells.

Preparation of Gram-Positive Bacterial Cells

1. Harvest up to 2×10^9 cells by centrifugation.
2. Resuspend cell pellet in 300µl of TE Buffer.

3. Add 100µl of lysozyme (25mg/ml).
4. Incubate for 30 minutes at 37°C.

Optional RNase Treatment: In some cases, RNA may copurify with genomic DNA from cell samples. To remove copurified RNA, an RNase treatment can be performed. Add 10µl of RNase A (Cat.# A7973) to each sample culture prior to running on the instrument. Incubate at room temperature for 10 minutes before adding to the cartridge.

Note: Preparing Gram-positive bacterial cells is not compatible with Maxprep® preprocessing protocol.

5. Manual Preparation of Maxwell® RSC Cultured Cells DNA Cartridge

1. Change gloves before handling Maxwell® RSC Cartridges, Plungers and Elution Tubes. Place each cartridge in the deck tray(s) with the printed side facing away from the elution position, which is the numbered side of the tray. 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.
2. Transfer each cultured cell sample to well #1 (the largest well) of each cartridge. Mix the cultured cell sample into the lysis buffer by pipetting 10 times. Change pipette tips between samples.

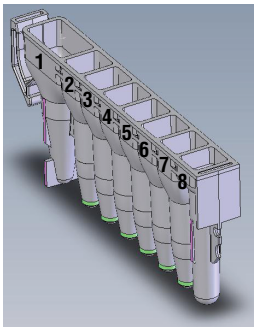
   Note: Pipet the cultured cell sample into the lysis buffer in well #1 to ensure that all of the sample has been transferred.
3. Place one plunger into well #8 of each cartridge.
4. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s). Add 100µl of Elution Buffer to the bottom of each elution tube. The starting volume of Elution Buffer will not be the same as the eluted volume after running the method.

Notes:

a. Specimen 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, then water. Do not use bleach on any instrument parts.

b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.

c. Typically, the final eluted volume will be approximately 30–50µl less than the starting volume.



User Adds to Wells

1. Cultured Cell sample (up to 5 × 10^6 tissue culture cells; up to 2 × 10^9 bacterial cells)
8. RSC Plunger

Figure 1. Maxwell® RSC Cartridge. Cultured cell sample is added to well #1, and a plunger is added to well #8.

5. Manual Preparation of Maxwell® RSC Cultured Cells DNA Cartridge (continued)

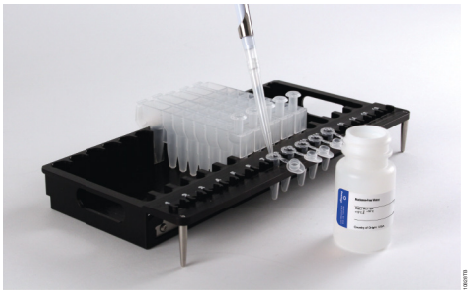


Figure 2. Setup and configuration of the deck tray(s). Elution Buffer is added to the elution tubes as shown.

6. Maxprep® Liquid Handler Preprocessing

The Maxprep® Liquid Handler can preprocess either tissue culture or Gram-negative bacterial cells that have been prepared as described in Section 4.

6.A. Maxprep® Cartridge Preparation

1. Turn on the Maxprep® Liquid Handler and PC. Log in to the PC, and start the Maxprep® software on the PC by double-clicking the desktop icon.
2. Touch **Start** to access the 'Methods' screen.
3. On the 'Methods' screen, select a method using one of the two options below:
   a. Touch the Cultured Cells DNA preprocessing method or laboratory-specific variant of the Cultured Cells DNA preprocessing method.
   b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate base method. Touch the laboratory-specific variant of the Cultured Cells DNA preprocessing method, if desired.
4. Verify that the appropriate preprocessing method or variant method has been selected, and touch the **Proceed** button. Close the instrument door and touch the **Run** button on the method run screen to start the run.
5. Enter any method-specific variables (Sample Number, Elution Volume).

6. Follow instrument setup instructions displayed in the method. You will be directed by the Maxprep® software where to place the following items on the instrument:
   - Maxprep® Plunger Holders with Maxwell® RSC Plunger Packs (2; one may be partially full)
   - 24-position Maxwell® Front deck tray or 16-position Maxwell® deck tray containing Maxwell® RSC cartridges with seals removed and open elution tubes
   - 24-position Maxwell® Back deck tray or 16-position Maxwell® deck tray containing Maxwell® RSC cartridges with seals removed and open elution tubes (depending on sample number)
   - Maxprep® Reagent Reservoir, 50ml, with Elution Buffer
   - Tube racks with tissue culture or Gram-negative bacterial cells resuspended in up to 400µl of culture medium in sample tubes. All tubes within a carrier must be of the same type.
   - Maxprep® 1000µl Conductive Disposable Tips, Filtered (2; one rack may be partially full)
   - Maxprep® 300µl Conductive Disposable Tips, Filtered (rack may be partial or full)
7. Close the instrument door, and touch the **Next** button to start the automated preprocessing setup of samples.

6.B. Maxprep® Liquid Handler Preprocessing Protocol

The Maxprep® Liquid Handler will prepare samples prior to extraction using Maxwell® Instruments. The following steps are performed by the Maxprep® Liquid Handler:

1. Plungers are transferred to each of the cartridges in the Maxwell® deck tray(s).
2. The specified volume of Elution Buffer is transferred to the elution tubes for each position in the Maxwell® deck tray(s).
3. The system transfers the specified volume of cultured cells from each sample tube to its corresponding Maxwell® RSC cartridge.
4. Method is complete. Open instrument door and move the deck tray(s) to the Maxwell® Instrument for extraction. Remove primary sample tubes and used tips from the waste bin, and discard as hazardous waste following your institution's recommended guidelines. Either discard or tightly cap and store remaining reagents.

Consumables for Maxprep® preprocessing methods are designed to be used with potentially infectious substances. Use appropriate protective equipment (e.g., gloves and safety glasses) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

7. Maxwell® Instrument Setup and Run

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

1. Turn on the Maxwell® Instrument and Tablet PC. Log in to the Tablet PC, and start the Maxwell® software on the Tablet PC. The instrument will power up, proceed through a self-check and home all moving parts.
2. Touch **Start** to access the 'Methods' screen.
3. On the extraction 'Methods' screen, select a method using one of the two options below:
   a. Touch the **Cultured Cells DNA** method.
   b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate method.
4. Verify that the Cultured Cells DNA method is 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' (if shown) screen, touch the cartridge positions to select or 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 48-position Maxwell® Instruments, touch the **Front** and **Back** buttons to select or 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 well mixed by pipetting into 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: Check the identifier on 24-position Maxwell® deck trays to determine whether they 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.

Note: 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. Touch the exclamation point for a description of the error and resolve all error states. Touch the **Start** button again to repeat deck tray scanning and begin the extraction run.

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. Touching the **Abort** button will abandon the run. The samples will be lost for all aborted runs.

   b. 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. The samples will be lost for all abandoned runs.

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

End of Run

10. Follow 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 Operating 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. Remove elution tubes containing DNA, 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. After purification, the elution tubes may have resin that adheres to the side of the tube. This is normal and will not affect downstream assay performance. Residual particles can be removed by centrifuging the elution tube and transferring the supernatant to a clean tube (not provided).

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

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

12. 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.

8. 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} (lower than expected yield)	Cultured cell sample has been stored unfrozen for an extended period of time or has undergone multiple freeze-thaw cycles. Avoid these storage conditions.
	Sample type contains low amount of DNA per cell, or a small number of cells was used. The yield of genomic DNA from cultured cell samples depends on the cell type and the number of cells processed.
	The preprocessing method for Gram-positive bacteria is not sufficient to release DNA from sporulated samples. A more aggressive disruption method will be necessary and should be evaluated.
Lower than expected purity ratios (low A_{260}/A_{280} or A_{260}/A_{230} ratios)	Cultured cell sample has been stored unfrozen for an extended period of time or has undergone multiple freeze-thaw cycles. Avoid these storage conditions.
RNA contamination in DNA eluates	In some cases, RNA can be copurified with the genomic DNA. To remove copurified RNA, perform the optional addition of RNase A to the sample (Section 4).
Instrument unable to pick up plungers	Make sure you are using a Maxwell® RSC reagent kit; the plungers for the Maxwell® RSC reagent kits are specific for the Maxwell® Instruments.
Residual resin left in well #1 after processing the cartridge in the Maxwell® RSC Instrument	When running the maximum amount of cells, it is normal to experience resin loss in well #1. To eliminate this, process fewer cells.
Resin carryover on the sides of the Elution tubes	Loading the maximum amount of cells into the cartridge is likely to result in carryover of resin on the side of the elution tube. This will not affect the performance of eluates in downstream reactions. Loading fewer cells will reduce the amount of resin on the tube. Alternatively, transfer the clear eluate to a fresh storage tube before further use.

9. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® CSC 48 Instrument	1 each	AS8000
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
RNase A Solution, 4mg/ml	1ml	A7973
Maxprep® Carrier, Maxwell® RSC	1 each	AS9402
Maxprep® Carrier, Maxwell® RSC 48 Front	1 each	AS9403
Maxprep® Carrier, Maxwell® RSC 48 Back	1 each	AS9404
Maxprep® Liquid Handler, RSC Carriers	1 each	AS9105
Maxprep® Liquid Handler, RSC 48 Carriers	1 each	AS9205
Maxprep® 1000µl Conductive Disposable Tips, Filtered	40/box	AS9303
Maxprep® 300µl Conductive Disposable Tips, Filtered	60/box	AS9302
Maxprep® Reagent Reservoir, 50ml	28/pack	AS9304
Maxprep® Waste Bags, Clear	100/box	AS9305
Maxprep® Plunger Holder	1 each	AS9408
Maxprep® 3-Position Reagent Tube Holder	1 each	AS9409
Maxprep® Tube Rack Stabilizer	1 each	AS1910
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.

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10. Summary of Changes

The following changes were made to the 4/25 revision of this document:

1. Updated Table 1 and Section 9, Related Products.
2. Updated Maxprep to a registered trademark.
3. Moved Table 2 to Section 4 and added a sentence that refers to Table 2.
4. Edited Section 7 for consistency with other Maxwell® RSC manuals.
5. Updated the document font.
6. Removed the disclaimer.