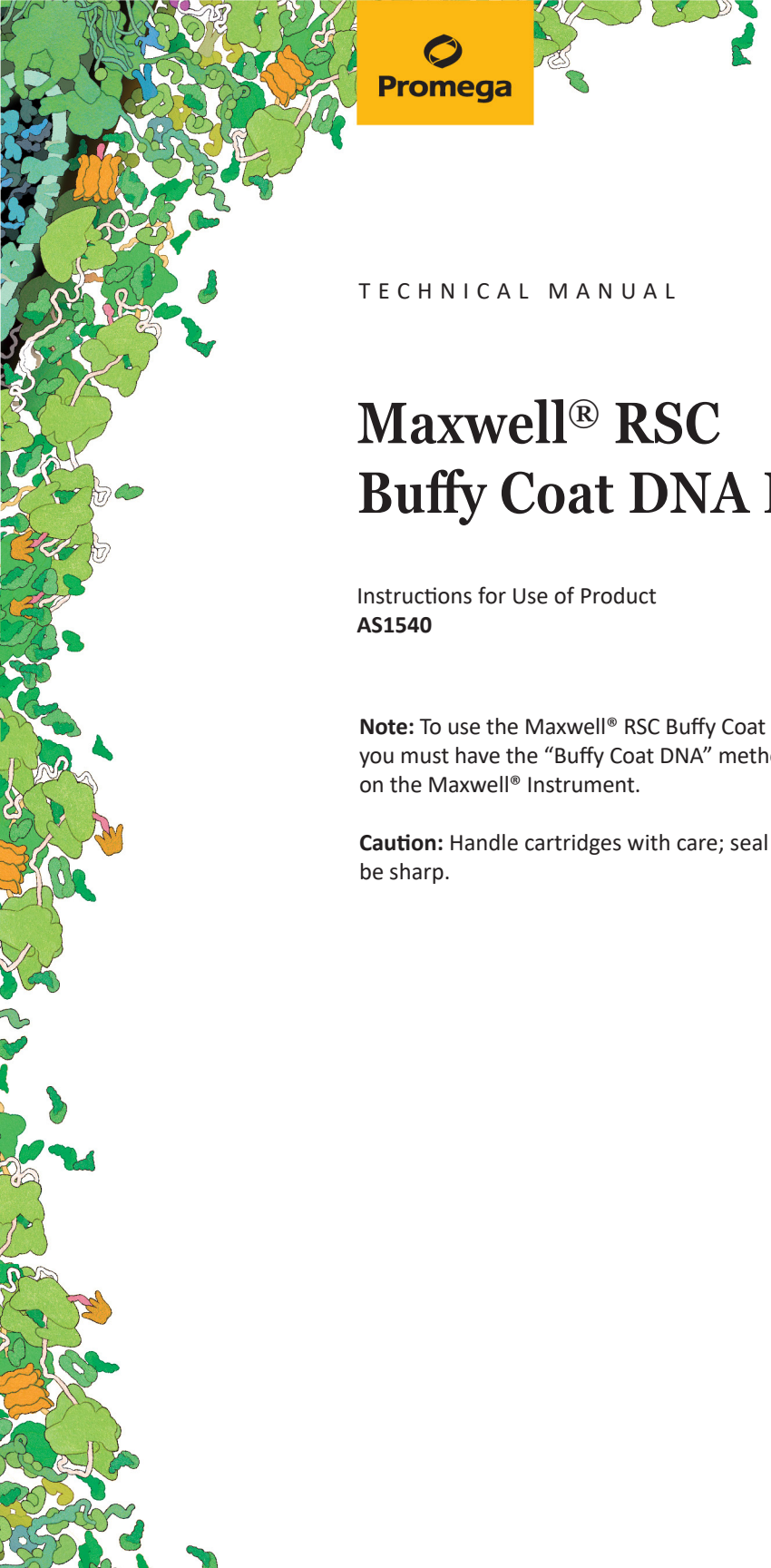


TECHNICAL MANUAL

Maxwell® RSC Buffy Coat DNA Kit

Instructions for Use of Product
AS1540

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

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

Revised 3/21
TM464

Maxwell® RSC Buffy Coat 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 Buffy Coat DNA Kit[a] (Cat.# AS1540) is used with the Maxwell® Instruments specified below to provide a simple method for efficient, automated purification of genomic DNA (gDNA) from buffy coat samples. Maxwell® Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the RSC Buffy Coat DNA Kit can process from one to the maximum sample number in about 50 minutes. The purified DNA can be used directly in a variety of downstream applications, including PCR and agarose gel electrophoresis.

Table 1. Supported Instruments.

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

1. Description (continued)

The Maxwell® RSC Buffy Coat DNA Kit purifies samples using a silica-based paramagnetic particle, called the MagneSil® particle, which provides a mobile solid phase that optimizes sample capture, washing and purification of gDNA. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind gDNA to the paramagnetic particle in the first well of a prefilled cartridge and mix during processing. 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.

Prior to extraction, samples can be preprocessed manually or using the Maxprep™ Liquid Handler. The Maxprep™ Liquid Handler will transfer buffy coat samples to Maxwell® RSC Buffy Coat DNA cartridges, transfer plungers to Maxwell® RSC Buffy Coat DNA cartridges, and dispense elution buffer to elution tubes. Follow the instruction set specific to the preprocessing option used.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Buffy Coat DNA Kit	**48 preps**	**AS1540**

For Research Use Only. Not for use in diagnostic procedures. Sufficient for 48 automated isolations from 50µl to 250µl of buffy coat samples. Cartridges are for single use only. Includes:

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

Storage Conditions: Store the Maxwell® RSC Buffy Coat DNA Kit at ambient temperature (15–30°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.

Maxwell® RSC Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) 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	**28/pack**	**AS9304**
Maxprep™ Plunger Holder	**1 each**	**AS9408**
Maxwell® RSC Plunger Pack	**1 each**	**AS1670**

3. Sample Preparation

Materials to Be Supplied by the User

- pipettors and pipette tips for sample transfer into prefilled reagent cartridges

The total yield of genomic DNA from buffy coat samples depends on the sample volume and number of white blood cells present in the sample. Each Maxwell® RSC Cartridge supplied in the Maxwell® RSC Buffy Coat DNA Kit is designed to purify genomic DNA from 50µl to 250µl of buffy coat sample. This assumes the initial whole blood sample used to prepare the buffy coat had an average number of white blood cells in the range of 4×10^6 to 1.1×10^7 cells/ml whole blood (values for a normal healthy adult; 1). Generally the volume of buffy coat prepared from a sample is approximately 1/10 the volume of the original blood sample (e.g., 250µl of buffy coat would be prepared from a 2.5ml blood sample). Ensure that the buffy coat is well mixed before processing.

Note: Buffy coats may be prepared from whole blood samples collected in EDTA, ACD or heparin tubes. These samples may be either fresh or frozen. Frozen samples should be thawed before processing.

4. Manual Preprocessing

4.A. Maxwell® RSC Buffy Coat DNA Cartridge Preparation

1. Change gloves before handling Maxwell® RSC Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray. Place each cartridge in the deck tray(s) with well #1 (the largest well in the cartridge) 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 50–250µl of each buffy coat sample to well #1 (the largest well) of each cartridge and tip-mix the samples into the lysis buffer in the well. Change pipette tips between samples.

! Tip-mix the buffy coat sample in well #1 at least 10 times to ensure all sample has been transferred.

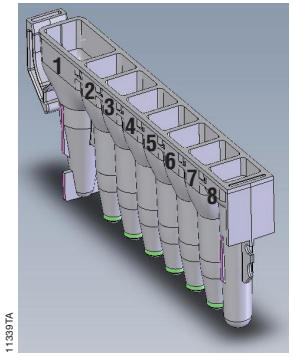
3. Place one plunger into well #8 of each cartridge.
4. Place an empty elution tube into the elution tube position for each cartridge in the deck tray. Add 200µl of Elution Buffer to the bottom of each elution tube.
5. Proceed to Section 6, Maxwell® Instrument Setup and Run.

4.A. Maxwell® RSC Buffy Coat DNA Cartridge Preparation (continued)

The starting volume of Elution Buffer will not be the same as the eluted volume after running the method. Typically, the final eluted volume will be approximately 50–150µl less than the starting volume.

Notes:

1. 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. Do not use bleach on any instrument parts.
2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.
3. Significant amounts of resin carryover on the sides of the Elution Tube is typical. Thoroughly tip-mix the buffy coat sample into the first well, or use buffy coat that has been frozen previously, to reduce the appearance of resin carryover on the sides of the Elution Tube.



User Adds to Wells

1. Buffy coat sample (50–250µl)
8. RSC Plunger

Figure 1. Maxwell® RSC Cartridge.

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

5. Maxprep™ Preprocessing

5.A. Maxprep™ Cartridge Preparation

1. Mix all buffy coat samples for at least 5 minutes at room temperature.
2. 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.
3. Press **Start** to access the 'Methods' screen.
4. On the 'Methods' screen, select a method using one of the two options below:
    1. Touch the Buffy Coat DNA pre-processing method or laboratory-specific variant of the Buffy Coat DNA preprocessing method.
    2. 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 Buffy Coat DNA preprocessing method, if desired.
5. 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.
6. Enter any method-specific variables (Sample Number, Sample Volume, Elution Volume).

    Notes:

    1. The starting volume of Elution Buffer will not result in the same elution volume after running the method. Typically, the resulting elution volume will be approximately 50–150µl less than the starting volume.
    2. Significant amounts of resin carryover into the Elution Tube is typical. Thoroughly tip-mix the buffy coat sample into the first well, or use buffy coat that as been frozen previously, to reduce the appearance of resin carryover on the sides of the Elution Tube. Using a larger starting volume of Elution Buffer will reduce carryover.
7. Prior to placing Maxwell® deck tray(s) on the instrument, prepare the deck tray(s) with cartridges and elution tubes. Change gloves before handling Maxwell® RSC Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) 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. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s).

    Notes:

    1. Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bacteriocidal spray or wipe and then water. Do not use bleach on any instrument parts.
    2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.
8. 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:

5.A. Maxprep™ Cartridge Preparation (continued)

Labware Type

- 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 sample tubes. All tubes within a carrier must be of the same type.
- Maxprep™ 1000µl Conductive Disposable Tips, Filtered (2; one may be partially full)
- Maxprep™ 300µl Conductive Disposable Tips, Filtered (racks may be partial or full)

9. Close the instrument door, and touch the **Next** button to start the automated preprocessing of samples.

5.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). The specified volume of Elution Buffer is transferred to the elution tubes for each position in the Maxwell® deck tray(s).
2. The system transfers the specified volume of buffy coat from each sample tube to its corresponding Maxwell® RSC cartridge.
3. 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 goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

6. Maxwell® Instrument Setup and Run

For detailed information, refer to the Technical Manual specific to your Maxwell® Instrument.

Table 2. Maxwell® Instrument Technical Manuals.

Instrument	Technical Manual
Maxwell® RSC	TM411
Maxwell® RSC 48	TM510
Maxwell® FSC	TM462
Maxwell® CSC RUO Mode	TM573
Maxwell® CSC 48 RUO Mode	TM628

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 proceed through a self-check and home all moving parts.
2. Press **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. When running in **Portal** mode, scan the bar code(s) on the deck tray(s). After data has been returned from the Portal database, press **Continue** to use the sample tracking information for the deck tray(s) or press **New** to start a run and enter new sample tracking information.
   b. Scan or enter the 2D bar code information on the kit box to automatically select the appropriate method.
   c. Touch the **Buffy Coat DNA** method.
4. If applicable to your Maxwell® Instrument model, verify that the Buffy Coat DNA method is selected, and press 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 (if shown), touch the cartridge positions to select/deselect the positions that will be used for this extraction run. Selecting or deselecting any cartridge position is only used for reporting purposes and does not affect the way the instrument processes samples. Enter any required sample tracking information, and press 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. After the door has been opened, confirm that all checklist items have been performed. Verify that samples were tip-mixed thoroughly into well #1 of the cartridges, the cartridges are loaded on the instrument, the Elution Tubes are uncapped with 200µl of Elution Buffer, and the 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.

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.

(continued)

6. Maxwell® Instrument Setup and Run (continued)

Notes:

1. 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.
2. Pressing the **Abort** button will abandon the run. The samples will be lost for all aborted runs.
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, you should 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.

8. 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 Technical Manual appropriate to your Maxwell® Instrument (see Table 2) 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. After the run has been completed, the extraction run report will be displayed. From the report 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.

Note: Following the automated purification procedure, the deck tray 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. Remove the cartridges and plungers from the deck tray 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.

7. Reference

1. Henry, J.B. (2001) *Clinical Diagnosis and Management by Laboratory Methods*, 20th ed., W.B. Saunders Company, 509.

8. 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} (lower than expected yield)	Buffy coat has been stored unfrozen for an extended period of time or has undergone multiple freeze-thaw cycles. Avoid these storage conditions.
	Buffy coat sample contained low white blood cell count. The yield of genomic DNA from buffy coat samples depends on the number of white blood cells present in the sample.
Lower than expected purity ratios (low A_{260}/A_{280} or A_{260}/A_{230} ratios)	Buffy coat 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, total RNA can be copurified with the genomic DNA. To remove copurified RNA, an RNase treatment can be performed. Add 5µl of RNase A (Cat.# A7973) per milliliter of Elution Buffer.
Instrument unable to pick up plungers	Make sure you are using an RSC-specific kit; the plungers for the Maxwell® RSC reagent kits are specific for the supported Maxwell® Instruments.
Resin carryover on the sides of the Elution Tubes	Thoroughly tip-mix the buffy coat sample into the first well, or use buffy coat that has previously been frozen to reduce the appearance of resin carryover on the sides of the Elution Tube.

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9. Related Products

Instrument 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/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® CSC Instrument	1 each	AS6000
Maxwell® CSC 48 Instrument	1 each	AS8000
Maxwell® FSC Deck Tray	1 each	AS4016
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	AS9100
Maxprep™ Liquid Handler, RSC Carriers w/ UV light	1 each	AS9101
Maxprep™ Liquid Handler, RSC 48 Carriers	1 each	AS9200
Maxprep™ Liquid Handler, RSC 48 Carriers w/ UV light	1 each	AS9201
Nunc™ 2.0ml Deep Well Plates	60/pack	AS9307
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
RNase A Solution, 4mg/ml	1ml	A7973

Maxwell® RSC Reagent Kits

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

10. Summary of Changes

The following changes were made to the 3/21 revision of this document:

1. Updates were made to Section 9, Related Products.
2. Updates were made to Tables 1 and 2.
3. The cover was updated.

[a]U.S. Pat. Nos. 6,027,945 and 6,368,800 and other patents pending.