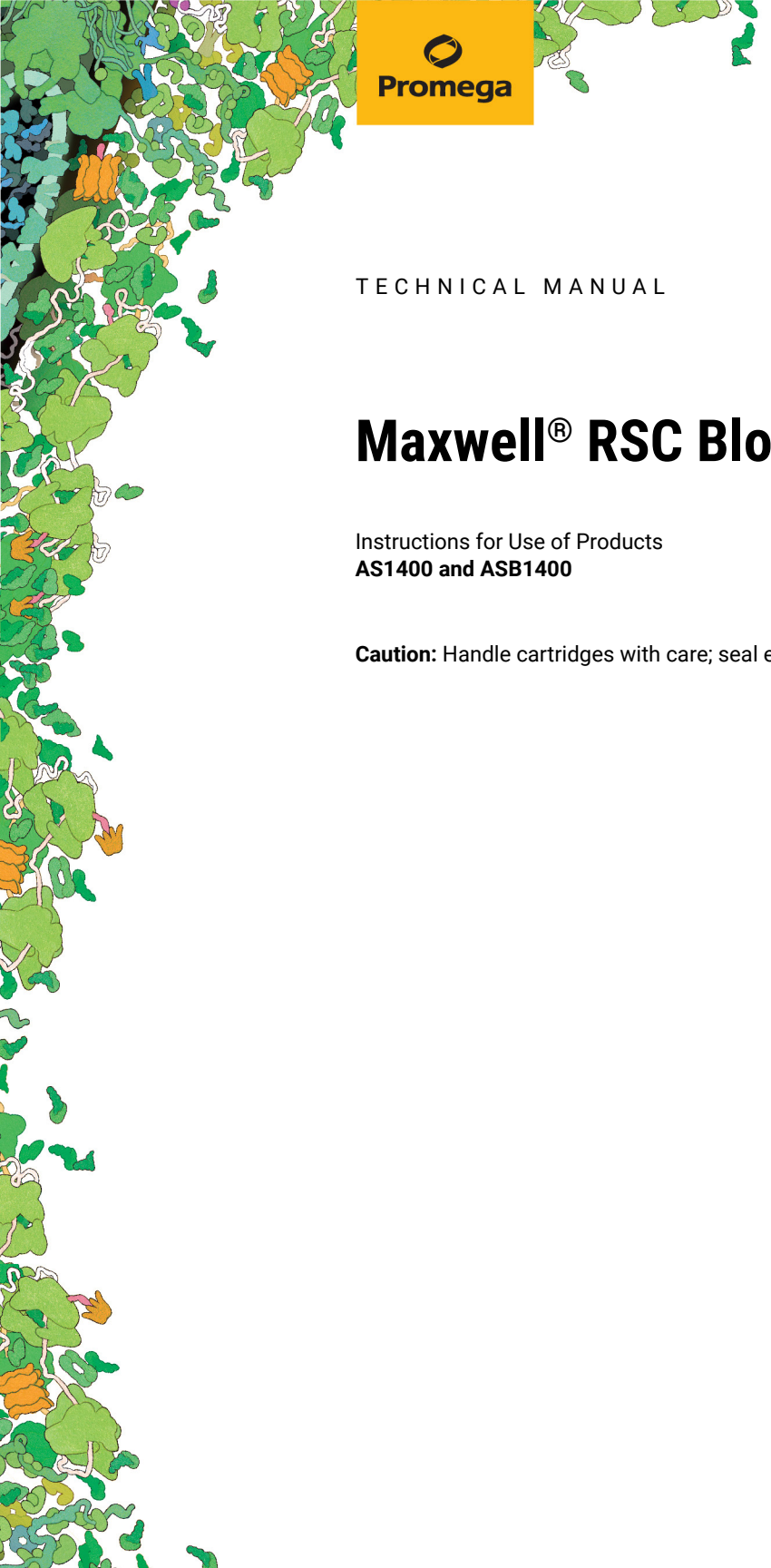

TECHNICAL MANUAL

Maxwell® RSC Blood DNA Kit

Instructions for Use of Products
AS1400 and ASB1400

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

Revised 1/25
TM419

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Maxwell® RSC Blood 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 Blood DNA Kit[a] is used with the Maxwell® Instruments specified below to provide an easy method for efficient, automated purification of genomic DNA (gDNA) from 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® methods for the RSC Blood DNA Kit can process from one to the maximum sample number in about 40 minutes. The purified DNA can be used directly in a variety of downstream applications.

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
Maxprep® Liquid Handler	AS9100, AS9101, AS9105, AS9200, AS9201, AS9205	TM509

The Maxwell® RSC Blood DNA Kit purifies samples using a novel paramagnetic particle, called the MagnaCel™ particle, which provides a mobile solid phase that optimizes sample capture, washing and purification of gDNA. This particle utilizes cellulose-based binding of nucleic acids and provides a higher bind capacity and cleaner eluate than traditional DNA purification. The Maxwell® RSC and Maxwell® RSC 48 are magnetic particle-handling instruments that efficiently bind gDNA to the paramagnetic particle in the first well of a prefilled cartridge. The samples are processed through a series of washes before the gDNA is eluted.

Prior to extraction, samples can be preprocessed manually or using the Maxprep® Liquid Handler. The Maxprep® Liquid Handler will scan bar codes and transfer samples from primary blood tubes, perform sample lysis prior to extraction, transfer lysed samples to Maxwell® RSC Blood DNA cartridges, add plungers to Maxwell® RSC Blood DNA cartridges, and add Elution Buffer to elution tubes. Instructions are provided for preprocessing samples manually or with the Maxprep® Liquid Handler.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Blood DNA Kit	**48 preps**	**AS1400**

For Research Use. Sufficient for 48 automated isolations from up to 300µl of whole blood samples. Includes:

- 2 × 1ml Proteinase K (PK) Solution
- 20ml Lysis Buffer
- 48 Maxwell® RSC Cartridges
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

PRODUCT	SIZE	CAT.#
Maxwell® RSC Blood DNA Multi-Pack Kit	**144 preps**	**ASB1400**

For Research Use. Sufficient for 144 automated isolations from up to 300µl of whole blood samples.
Note: ASB1400 is not recommended for use with the Maxprep® Liquid Handler. Includes:

- 6 × 1ml Proteinase K (PK) Solution
- 3 × 20ml Lysis Buffer
- 144 Maxwell® RSC Cartridges
- 3 × 50/pk Maxwell® CSC/RSC Plungers
- 3 × 50 Elution Tubes (0.5ml)
- 3 × 20ml Elution Buffer

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

Safety Information: The Maxwell® RSC Cartridges contain ethanol and isopropanol. These substances should be considered flammable, harmful and irritants. Lysis Buffer contains guanidine hydrochloride and urea. These substances should be considered toxic, harmful and irritants.

Maxwell® RSC Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) when handling potentially infectious substances. Adhere to your institutional guidelines for the handling and disposal of all potentially infectious substances used with this system.

Available Separately (recommended for sample extraction)

For Manual Preprocessing

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

2. Product Components and Storage Conditions (continued)

For Preprocessing with the Maxprep® Liquid Handler

PRODUCT	SIZE	CAT.#
2.0ml Deep Well Plates (Non-Sterile)	60/pack	AS9309
Nunc 2.0ml Deep Well Plates (Sterile)	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® Plunger Holder	1 each	AS9408
Maxwell® RSC Plunger Pack	48/each	AS1670
Maxprep® 3-Position Reagent Tube Holder	1 each	AS9409

3. Manual Peparation of Blood Samples

Materials to Be Supplied by the User

- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for incubation of samples (e.g., ClickFit Microtube, 1.5ml [Cat.# V4741]; recommended to prevent the cap from opening during heating)
- heating block set at 56°C
- **optional:** rotating tube mixer for liquid blood samples

The total yield of genomic DNA from whole blood samples depends on the sample volume and number of white blood cells/ml. Each Maxwell® RSC Cartridge supplied in the Maxwell® RSC Blood DNA Kit is designed to purify genomic DNA from up to 300µl of whole blood, assuming 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).

Note: Whole blood samples collected in EDTA, ACD or heparin tubes can be used. These samples may be either fresh or frozen. Frozen samples must be thawed before processing.

3.A. Preparation of Whole Blood Samples

1. Mix all blood samples for at least 5 minutes at room temperature.
2. Prepare and label incubation tubes compatible with a heating block.
3. Add 30µl of Proteinase K (PK) Solution to each incubation tube.
4. Add liquid blood (up to 300µl) to each incubation tube.
5. Add 300µl of Lysis Buffer to each incubation tube.
6. Vortex each tube for 10 seconds.
7. Incubate each tube in the heating block (set to 56°C) for 20 minutes. During this incubation, prepare cartridges as described in Section 3.B.
8. Transfer each blood lysate sample from the incubation tube to well #1 of each cartridge. (Well #1 is the well closest to the printed side and furthest from the elution tube.)

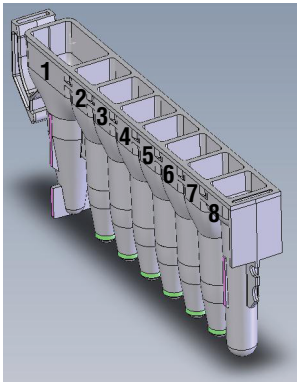
3.B. Maxwell® RSC Blood DNA Cartridge Preparation

1. Change gloves before handling Maxwell® RSC Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place each cartridge in the deck tray with the printed side facing away from the elution tubes. Press down on both the front and back of 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. Place one plunger into well #8 of each cartridge.
3. Place an empty elution tube into the elution tube position for each cartridge in the deck tray. Add 50µl of Elution Buffer to the bottom of each elution tube.
4. Proceed to Section 5 for Maxwell® Instrument setup and run.

Notes:

a. 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, 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.



User Adds to Wells
1. Sample lysate
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.

4. Maxprep® Preprocessing

Materials to Be Supplied by the User

- **optional:** rotating tube mixer for liquid blood samples

The total yield of genomic DNA from whole blood samples depends on the sample volume and number of white blood cells/ml. Each Maxwell® RSC Cartridge supplied in the Maxwell® RSC Blood DNA Kit is designed to purify genomic DNA from up to 300µl of whole blood, assuming 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).

Note: Whole blood samples collected in EDTA, ACD or heparin tubes can be used. These samples may be either fresh or frozen. Frozen samples must be thawed before processing.

4.A. Maxprep® Cartridge Preparation

1. Mix all blood 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:
   a. Touch the Blood DNA preprocessing method or laboratory-specific variant of the Blood 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 user variant of the Blood DNA preprocessing method if desired.
5. Verify that the appropriate preprocessing method or user-variant method has been selected, and press the **Proceed** button. 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).
7. Prior to placing Maxwell® deck tray(s) on the Maxprep® Liquid Handler, 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 each cartridge in the deck tray(s) with the printed side facing away from the elution tubes. Press down on both the front and back of 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 Maxprep® Liquid Handler. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s).

   Notes:

   a. 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, 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® Instruments.

4.A. Maxprep® Cartridge Preparation (continued)

8. Follow instrument setup instructions displayed in the method. The Maxprep® software will direct you where to place the following items on the instrument:

Labware Type

- Maxprep® Plunger Holders and Maxwell® RSC Plunger Packs (2; one may be partially full)
- Maxwell® RSC 48 Front deck tray or Maxwell® RSC deck tray containing Maxwell® RSC cartridges with seals removed and open elution tubes
- Maxwell® RSC 48 Back deck tray or Maxwell® RSC deck tray containing Maxwell® RSC cartridges with seals removed and open elution tubes (if you are running greater than 24 samples in a run)
- Maxprep® 3-Position Reagent Tube Holder with up to 3 Proteinase K tubes
- Maxprep® Reagent Reservoir, 50ml, with Lysis Buffer
- Maxprep® Reagent Reservoir, 50ml, with Elution Buffer
- Nunc 2.0ml Deep-well Plate (empty)
- Maxprep® 1000µl Conductive Disposable Tips, Filtered (2; one rack may be partially full)
- Maxprep® 300µl Conductive Disposable Tips, Filtered (rack may be partially full)

9. Close the Maxprep® Liquid Handler door, and touch the **Next** button to start the automated preprocessing of your samples.

4.B. Maxprep® Liquid Handler Preprocessing Protocol

The Maxprep® Liquid Handler will use the following steps to prepare samples prior to extraction with the Maxwell® RSC or Maxwell® RSC 48 Instrument.

1. The system prepares a lysis reaction in the Nunc 2.0ml Processing Plate consisting of the following components:
    a. The specified volume of whole blood
    b. 30µl of Proteinase K Solution
    c. 300µl of Lysis Buffer
2. The Processing Plate incubates for 20 minutes.
3. During the lysis incubation, plungers are transferred to each of the cartridges in the Maxwell® RSC or Maxwell® RSC 48 deck trays. The specified volume of Elution Buffer is transferred to the elution tubes for each position in the Maxwell® RSC or Maxwell® RSC 48 deck tray.
4. After lysis incubation is complete, each sample is transferred from the Processing Plate to its corresponding Maxwell® RSC cartridge.
5. Once the method is complete, open the Maxprep® Liqiud Handler door, and transfer the deck trays to your Maxwell® RSC or Maxwell® RSC 48 Instrument for extraction. Remove primary sample tubes and Processing Plate, and discard the used tips from the waste bin as hazardous waste following your institution's recommended guidelines. Either discard or tightly cap and store remaining reagents.

    Consumables used by the Maxprep® Instrument for 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.

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5. Maxwell® RSC Instrument Setup and Run

For detailed information, refer to the Operating 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 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 'Methods' screen.

   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.
3. On the 'Methods' screen, if a method has not been chosen by scanning the bar code on the deck trays, select a method using one of the two options below:
   a. Touch the RSC Blood 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. If applicable to your Maxwell® Instrument, verify that the RSC Blood DNA method has been selected, and press 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 or deselect any positions to be used for this extraction run. Enter any required sample tracking information, and press the **Proceed** button to continue.

   Note: With 48-position Maxwell® Instruments, select or deselect cartridge positions on each deck tray using the **Front** and **Back** buttons.
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: 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: If using a 48-position Maxwell® Instrument and 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. Select the exclamation point for a description of the error and resolve all error states. Select 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. Selecting the **Abort** button will abandon the run. All samples from an aborted run will be lost.

   b. If the run is abandoned before completion, you may 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.

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.

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.

12. 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.

6. Reference

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

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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**

Symptoms	Causes and Comments
Lower than expected A_{260} (lower than expected yield)	Blood that has undergone multiple freeze-thaw cycles may have degraded DNA. Use fresh samples whenever possible, or avoid multiple freeze-thaw cycles.
	Proteinase K Solution was not added. The lysis and yield are dependent upon complete extraction with Proteinase K. If Proteinase K was not added in Section 3.A, Step 3, or set up on the Maxprep® Liquid Handler, the resulting blood sample will be red. Proteinase K-treated samples turn greenish brown. This can be used as a quick diagnostic method of determining whether or not Proteinase K was added.
	Whole blood sample contained low white blood cell count. The yield of genomic DNA from blood samples depends on the number of white blood cells present in the sample
	Whole blood sample was not mixed before processing. Be sure to mix whole blood samples before processing to ensure that the white blood cells are in suspension.
	The Maxwell® Instrument was set for the wrong method. Ensure that the Blood DNA method is chosen.
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 chemistry kit; the plungers for the Maxwell® RSC reagent kits are specific for supported Maxwell® Instruments (see Section 1).

8. Related Products

Instrument 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
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 w/UV light	1 each	AS9105
Maxprep® Liquid Handler, RSC 48 Carriers w/UV light	1 each	AS9205
2.0ml Deep Well Plates (Non-Sterile)	60/pack	AS9309
Nunc 2.0ml Deep Well Plates (Sterile)	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® Plunger Holder	1 each	AS9408
Maxprep® 3-Position Reagent Tube Holder	1 each	AS9409
Maxprep® Waste Bags, Clear	100/box	AS9305
Maxwell® RSC Plunger Pack	48/pack	AS1670
Maxwell® RSC/CSC Plungers	50/pack	AS1331
RNase A Solution, 4mg/ml	1ml	A7973
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 1/25 revision of this document:

1. Updated Section 1, including Table 1, Supported Instruments.
2. Updated Maxprep to a registered trademark.
3. Edited Section 6 for consistency with other Maxwell® RSC manuals.
4. Edited Section 8 to include Cat.# AS9105 and AS9105.
5. Updated the document font and made minor text edits.