TECHNICAL MANUAL

Maxwell® RSC Cell DNA Purification Kit

Instructions for Use of Product
AS1370

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

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

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Promega

Maxwell® RSC Cell DNA Purification 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 Cell DNA Purification Kit[a] is used with the Maxwell® Instruments listed in Table 1, and is specifically designed for automated purification of genomic DNA from less than 10,000 (1×10^4) cells. The binding capacity of the system is limited to a few hundred nanograms of highly pure DNA suitable for amplification applications. If sensitive qPCR methods are used for detection, DNA can be purified and amplified from as few as 10 cells.

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® Instruments are supplied with preprogrammed purification procedures and are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the RSC Cell DNA Kit can process from one to the maximum sample number in about 30 minutes. The Maxwell® RSC Cell DNA Purification Kit purifies samples using paramagnetic particles (PMPs), which provide a mobile solid phase that optimizes capture, washing and elution of the target nucleic acid.

1. Description (continued)

The Maxwell® Instruments are magnetic particle-handlers that efficiently preprocess liquid and solid samples, transport the PMPs through purification reagents in the prefilled cartridges (Figure 1) and mix during processing. The magnetic particle-based methodology avoids common problems such as clogged tips or partial reagent transfers that result in suboptimal purification processing by other automated systems.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Cell DNA Purification Kit	**48 preps**	**AS1370**

For Research Use. Sufficient for 48 automated isolations. Includes:

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

Storage Conditions: Store the Maxwell® RSC Cell DNA Purification Kit at 15–30°C.

Safety Information: Maxwell® RSC Cartridges contain ethanol, isopropanol and guanidine thiocyanate. These substances should be considered flammable, harmful and irritants. Refer to the SDS for detailed safety information.

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

3. Sample Preprocessing

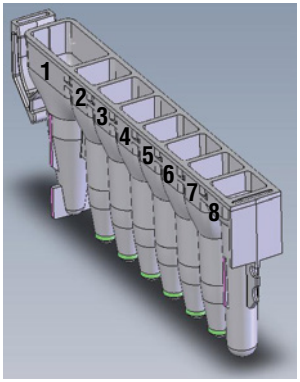
Materials to Be Supplied by the User

- Microtubes, 1.5ml (Cat.# V1231) or larger
- PBS (10.1mM Na_2HPO_4, 1.8mM KH_2PO_4, 140mM NaCl, 2.7mM KCl [pH 7.3])

1. Pellet cells from the sample by centrifugation. For volumes less than 1.5ml, centrifuge at 13,000–16,000 × *g* for 1–2 minutes at room temperature. For larger volumes, centrifuge at 2,000 × *g* for 10 minutes.
2. Remove the supernatant, being careful not to disturb the cell pellet. If no pellet is visible, avoid the position in the tube where a pellet would be expected. Small cell numbers may result in a pellet that is not visible by eye.
3. Resuspend the pellet in up to 100µl of PBS.

4. Maxwell® Automated DNA Purification

4.A. Maxwell® RSC Cell DNA Cartridge Preparation



User Adds to Wells
1. Preprocessed samples
8. RSC Plunger

Figure 1. Maxwell® RSC Cartridge.

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(s). Place each cartridge in the deck tray with well #1 (the largest well) 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 a cartridge in the instrument.

   Note: Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by 70% ethanol, then water. Do not use bleach on any instrument parts.

2. Place a plunger in well #8 of each cartridge. Well #8 is the well closest to the elution tube.

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

3. Place elution tubes in the front of the deck tray(s). Add 50µl of Elution Buffer to the bottom of each elution tube.

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.

Notes:

1. Ensure that the Elution Buffer is in the bottom of the tube. If Elution Buffer is on the side of the tube, the elution may be suboptimal.
2. Use only the Elution Tubes (0.5ml) provided with the kit; other tubes may not work with the supported Maxwell® Instruments.

4. Transfer the cell suspension to well #1 (the largest well) of the cartridge.

Note: The total volume of the sample should not exceed 100µl. Adding more than 100µl of cell suspension may result in poor yields.

4.B. Maxwell® Instrument Setup and Run

For more 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 on the Tablet PC. The instrument will proceed through a self-check and home all moving parts.
2. Press **Start** to run the 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 software, 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 on the kit box to automatically select the appropriate method.
   c. Touch the Cell DNA method.
4. If applicable to your Maxwell® Instrument, verify that the Cell DNA method has been 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 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. 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 the 24-position Maxwell® deck trays to determine whether they should be placed in the front or back of the instrument.

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

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.

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. 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.
2. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.
3. If a 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, perform **Clean Up** when requested. If plungers are not present on the plunger bar, you can choose to skip **Clean Up**. The samples will be lost.

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 1) 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 close 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.

Notes:

1. Small amounts of paramagnetic particles may be present in the elution tube. This will not affect downstream applications. Residual particles may be removed by centrifuging the elution tube and transferring the supernatant to a clean tube (not provided).
2. To prevent evaporation of eluted DNA, cap elution tubes within 15 minutes after completing the purification run.

Warning: Hot Surface. Burn Hazard.

10. Remove cartridges and plungers from the deck tray(s), and discard as hazardous waste following your institution's recommended guidelines. Do not reuse cartridges, plungers or elution tubes.

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

5. 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
Low DNA concentration	The Maxwell® Instrument was set for the wrong method. Ensure that the correct method is chosen.
	Insufficient sample was processed: • Process a larger number of cells (up to 10^4 cells) to increase yield. • Take care not to disturb the cell pellet when removing the sample supernatant.
Poor PCR results	Too much starting material. Reduce the amount of sample used for purification.
	Wrong elution buffer was added. Use only the Elution Buffer supplied with the Maxwell® RSC Cell DNA Purification Kit.
Step Loss error	Verify nothing is physically blocking the movement of the platform, plunger bar or magnetic rod assembly.
	Perform a **Self Test** from the Settings menu. The instrument will rehome itself. If the error occurs again, please contact Promega for service.
	The cartridges are not completely seated on the deck tray. Ensure the cartridges are pressed firmly into place.
	Incorrect elution tube used with the system. To prevent a Z-axis collision, only use the Elution Tube (0.5ml) provided with the Maxwell® RSC Cell DNA Purification Kit. Other tubes may have different dimensions.
Resin carryover during elution	A small amount of resin is visible in elution tube. The presence of resin particles in the elution tube will not affect the final DNA concentration or downstream applications. If desired, an additional resin capture step may be performed using the 0.5ml MagneSphere® Stand (Cat.# Z5341).
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 Table 1).

6. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC Plunger Pack	1 each	AS1670
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® FSC Instrument	1 each	AS4600
Maxwell® FSC Deck Tray	1 each	AS4016
Maxwell® CSC Instrument	1 each	AS6000

Maxwell® RSC Reagent Kits

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

7. Summary of Changes

The following changes were made to the 8/20 revision of this document:

1. Genericized all references to Maxwell® Instruments.
2. Edited Section 3.
3. Updated Sections 4.B and 6.

[a]U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Japanese Pat. No. 3253638, European Pat. No. 1 204 741 and other patents.