TECHNICAL MANUAL

Maxwell® RSC Blood DNA Kit

Instructions for Use of Product
AS1400

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

Revised 1/15
TM419

Maxwell® RSC Blood 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 Blood DNA Kit[a] is used with the Maxwell® RSC Instrument to provide an easy method for efficient, automated purification of genomic DNA (gDNA) from samples. The Maxwell® RSC Instrument is supplied with preprogrammed purification procedures and is designed for use with predispensed reagent cartridges, maximizing simplicity and convenience. The instrument can process up to 16 samples in 40 minutes. The purified DNA can be used directly in a variety of downstream applications, including PCR and agarose gel electrophoresis.

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 Instrument is a magnetic particle-handling instrument that efficiently binds gDNA to the paramagnetic particle in the first well of a prefilled cartridge and mixes 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.

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 300µl of whole blood samples. Includes:

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

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

Available Separately (recommended for sample extraction)

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

Safety Information: The Maxwell® RSC Cartridges contain ethanol and isopropanol. These substances should be considered flammable, 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 infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

3. Before You Begin

Materials to Be Supplied by the User

- optional, rotating tube mixer for liquid blood samples
- 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

3.A. Preparation of Whole 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 should be thawed before processing. We recommend mixing all blood samples before use. EDTA blood collection tubes are preferred if the purified DNA will be used in downstream amplification assays.

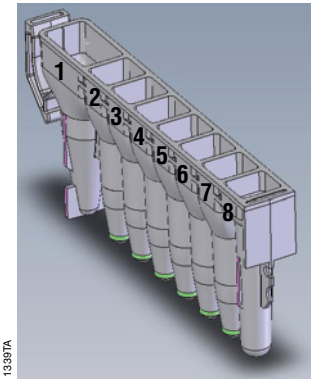
1. Mix all blood samples for at least 5 minutes at room temperature.
2. Prepare and label incubation tubes compatible with heating block.
3. Add 30µl of Proteinase K (PK) Solution to each incubation tube.
4. Add liquid blood (up to 300µl) to each incubation tube.
5. Add 300µl of Lysis Buffer to each 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.C.
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, CSC/RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray. Place each cartridge in the deck tray with the printed side 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.
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.

Notes:

1. If you are processing fewer than 16 samples, center the cartridges on the deck tray.
2. 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.



User Adds to Wells
1. Sample lysate
8. CSC/RSC Plunger

Figure 1. Maxwell® RSC Cartridge.

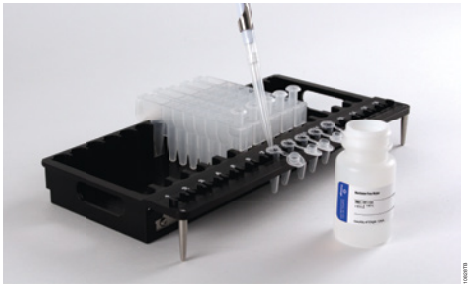


Figure 2. Setup and configuration of the deck tray. Elution Buffer is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

4. Maxwell® RSC Instrument Setup and Run

Refer to the *Maxwell® RSC Instrument Operating Manual* #TM411 for detailed information.

1. Turn on the Maxwell® RSC Instrument and Tablet PC. Log in to the Tablet PC, and start the RSC software on the Tablet PC. The instrument will power up, proceed through a self-check and home all moving parts.
2. Press **Start** to access the extraction method selection screen.
3. On the extraction method selection screen, select a method using one of the two options below:
   a. Manually 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. 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, touch the cartridge positions to deselect any positions that will not 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.
6. After the door has been opened, confirm that all checklist items have been performed. Transfer the deck tray containing the prepared cartridges onto the Maxwell® RSC Instrument platform. Ensure that the deck tray is placed in the Maxwell® RSC Instrument with the elution tubes closest to the door. 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: Hold the deck tray by the sides to avoid dislodging cartridges from the deck tray.
7. Verify that samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with 50µl of Elution Buffer and plungers are in well #8.
8. Touch the **Start** button to begin the extraction run. The platform will retract, and the door will close.

Warning: Pinch point hazard.

The Maxwell® RSC 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. Pressing the **Abort** button will abandon the run.
2. 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.

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

9. 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 *Maxwell® RSC Instrument Operating Manual* to perform a Clean Up process to attempt to unload the plungers.

10. Remove the deck tray 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.

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.

11. 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.

5. Reference

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

6. 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)	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, 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® RSC Instrument was set for the wrong method. Ensure that the correct 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 the Maxwell® RSC Instrument.

7. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
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.

8. Summary of Changes

The following change was made to the 1/15 revision of this document:

The name of the CSC Plungers was changed to CSC/RSC Plungers.

[a]U.S. Pat. No. 6,855,499, European Pat. No. 1368629, Japanese Pat. No. 4399164 and other patents.