

TECHNICAL MANUAL

Maxwell[®] RSC ccfDNA Plasma Kit

Instructions for Use of Product
AS1480

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



Maxwell[®] RSC ccfDNA Plasma 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

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1. Description

The Maxwell[®] RSC ccfDNA Plasma Kit^(a) is used with the Maxwell[®] RSC Instrument to provide an easy method for efficient, automated purification of circulating cell-free DNA (ccfDNA) from human plasma samples. The Maxwell[®] RSC Instrument is supplied with preprogrammed purification methods and is designed for use with the predisposed reagent cartridges, maximizing simplicity and convenience. The instrument can process up to sixteen plasma samples ranging in volume from 0.2–1.0ml in approximately 70 minutes, and the purified DNA can be used directly in a variety of downstream applications such as PCR. The Maxwell[®] RSC ccfDNA Plasma Kit purifies circulating DNA using a novel paramagnetic particle, which provides a mobile solid phase that optimizes sample capture, washing and purification of circulating DNA.

The Maxwell[®] RSC Instrument is a magnetic particle-handling instrument that allows efficient binding of circulating DNA to the paramagnetic particle in the first well of a prefilled cartridge and moves the sample through the wells of the cartridge, mixing during processing. This approach to magnetic capture avoids common problems such as clogged tips or partial reagent transfers that result in suboptimal purification processing by other commonly used automated systems.



2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC ccfDNA Plasma Kit	48 preps	AS1480

For Research Use. Sufficient for 48 automated isolations from 0.2–1ml of plasma samples. Includes:

- 5ml Elution Buffer
- 48 Maxwell® RSC Cartridges (RSCG)
- 50 CSC/RSC Plungers
- 50 Elution Tubes (0.5ml)

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

3. Before You Begin

Materials to Be Supplied by the User

- whole blood or plasma
- table-top centrifuge

3.A. Safety Information



The reagent cartridges contain ethanol, isopropanol, guanidine thiocyanate and guanidine hydrochloride. These substances should be considered flammable, harmful and irritants. The Maxwell® RSC Cartridges are designed to be used with potentially infectious substances. Users should wear appropriate personal protective equipment (e.g., gloves and goggles) when handling infectious substances. Users should adhere to their 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. **Note:** Bleach reacts with guanidine thiocyanate to produce toxic fumes. Guanidine thiocyanate is present in the cartridge. Do not decontaminate waste from this kit using bleach.

3.B. Intended Use

The Maxwell® RSC ccfDNA Plasma Kit is intended for use in combination with the Maxwell® RSC Instrument and the Maxwell® ccfDNA Plasma purification method and is for research use only. The kit is intended for blood samples collected in EDTA tubes.

Limitations of Use

The Maxwell® RSC ccfDNA Plasma Kit is only intended for use with plasma prepared from human whole blood samples collected in EDTA tubes. It is not intended for use directly with whole blood samples or non-whole blood samples such as bone marrow or buffy coat, samples stored in other collection tubes or samples stored outside of the product claims. The Maxwell® RSC ccfDNA Plasma Kit is not intended for use in diagnostic procedures.

3.C. Preparing Plasma

Whole blood should be processed immediately after collection or stored at 2–10°C until plasma preparation.

Centrifuge whole blood from EDTA tubes for 10 minutes at 2,000 × *g* to pellet the red and white blood cells. Using a pipette, carefully remove as much plasma as possible without disturbing the buffy coat. To ensure that no white blood cells are transferred, centrifuge the plasma a second time for 10 minutes at 2,000 × *g*, and transfer the supernatant to a clean tube.

Store plasma at 2–10°C for up to one week. For longer storage times, store plasma at –10 to –30°C (or below –65°C). Avoid exposing plasma to freeze-thaw cycles.

3.D. Maxwell® RSC ccfDNA Plasma Cartridge Preparation

1. Change gloves before handling Maxwell® RSC Cartridges, CSC/RSC Plungers and Elution Tubes. Set up cartridges on the Maxwell® RSC Deck Tray outside of the instrument, and transfer the deck tray containing the cartridges and samples to the instrument for purification. Place the cartridges to be used in the Maxwell® RSC Deck Tray (Figure 1). Place each cartridge in the deck tray with well #1 (the largest well in the cartridge) farthest away from the Elution Tubes. Press down on the cartridge to snap it into position. Ensure both cartridge ends are fully seated in the deck tray.

Carefully peel back the seal so that the entire seal is removed from the top of the cartridge.

Caution: Handle cartridges with care. Seal edges may be sharp.



Figure 1. Setup and configuration of the deck tray. Elution Buffer is added to the elution tubes. Plungers are in well #8 of the cartridge. Plasma sample is added to well #1 of the cartridge.

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 60µl of Elution Buffer to the bottom of each elution tube. This will give a final elution volume after processing of approximately 50µl.

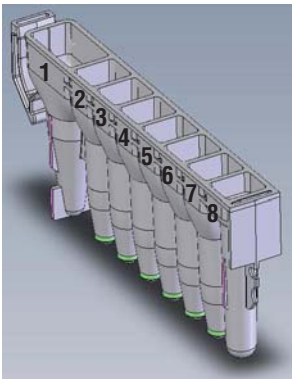
Note: Use only the elution tubes and plungers provided in the Maxwell® RSC ccfDNA Plasma Kit. Other elution tubes may not be compatible with the Maxwell® RSC Instrument and may affect DNA purification performance. Plungers from Maxwell® LEV kits are not compatible with the Maxwell® RSC Instrument.

3.D. Maxwell® RSC ccfDNA Plasma Cartridge Preparation (continued)

4. Add 0.2–1.0ml of plasma to the Binding Buffer in well #1 (Figure 2). Mixing is not required.

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.



Wells Contain:

1. Binding Buffer
2. Paramagnetic Particles
3. Wash Buffer
4. Wash Buffer
5. Wash Buffer
6. Wash Buffer
7. Wash Buffer

User Adds to Wells:

1. 0.2–1.0ml of Plasma
8. CSC/RSC Plunger

Figure 2. Maxwell® RSC Cartridge.

4. Maxwell® RSC Instrument Setup and Run

Refer to the *Maxwell® RSC Instrument Operating Manual #TM411* for detailed information. Refer to the *Maxwell® RSC Method Installation Technical Manual #TM435* for instructions for installation of Maxwell® RSC methods.

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 ccfDNA Plasma 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 ccfDNA Plasma 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. Verify that samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with 60µl of Elution Buffer and plungers are in well #8.
7. 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.

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, and the samples will be lost.
 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 the Clean Up method when requested. If plungers are not present on the plunger bar, you can choose to skip the Clean Up method when requested. The samples will be lost for all aborted runs.
9. When the automated purification run is complete, the Tablet PC screen 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 the plungers are still on the magnetic rods, follow the instructions in the *Maxwell® RSC Instrument Operating Manual* to perform a Clean Up process to unload the plungers.
11. Remove the deck tray from the instrument immediately following the run to prevent evaporation of the eluates. 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.
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.

5. Considerations When Working with ccfDNA

5.A. Preparing Plasma

One potential issue when purifying ccfDNA is the presence of contaminating genomic DNA from lysed white blood cells. Plasma is typically centrifuged twice; the first spin removes the red and white blood cells, and the second spin removes any residual white blood cells. If the blood sample was incubated for extended periods at room temperature, or was frozen and thawed prior to processing, some white blood cells may have lysed, releasing genomic DNA into the plasma.

If the plasma sample has been frozen, cryoprecipitate might be present after thawing. While cryoprecipitate has no effect on the purification of ccfDNA with the Maxwell[®] RSC ccfDNA Plasma Kit, it can affect pipetting of plasma. To pellet the cryoprecipitate, centrifuge the plasma sample prior to processing.

5.B. Quantitating ccfDNA

The low concentration and fragmented nature of ccfDNA provide unique challenges for researchers. In normal plasma, yields of 10–30ng of ccfDNA per milliliter of plasma are typical, at concentrations of less than 1ng/μl. The overwhelming majority of ccfDNA fragments are approximately 170bp, with additional fragments at approximately 340bp and 510bp.

Quantitating by UV

It is impossible to get an accurate determination of ccfDNA concentration using A_{260} absorbances given the low concentration. Some available products use a carrier RNA to facilitate purification of ccfDNA. The carrier RNA is in much higher abundance than the ccfDNA and co-purifies. This can give a false A_{260} value and drastically higher apparent ccfDNA concentrations.

Quantitating by Fluorescence

The sensitivity of dsDNA-specific dyes makes them a better choice for quantitating ccfDNA, but there are two concerns. The first involves carrier RNA. While dsDNA-specific dyes have a much higher specificity for DNA than RNA, the high levels of carrier RNA in other ccfDNA kits inflate the RFU values, making ccfDNA levels appear higher than actual concentrations.

A second factor is that the standards used in fluorescent dyes are typically high-molecular-weight genomic or Lambda DNA. ccfDNA is highly fragmented and does not bind fluorescent dyes as effectively as high-molecular-weight DNA, leading to lower apparent concentrations. If possible, use lower molecular weight DNA standards to get more accurate quantitation.

Quantitating by PCR

Quantitation by either qPCR or digital droplet PCR gives the most accurate measure of ccfDNA. In addition to sensitivity, amplification-based quantitation can indicate suitability of samples for amplification-based downstream applications.

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
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
Elution Tubes	50/pk	AS6201
Maxwell [®] CSC/RSC Plungers	50/pk	AS1331

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 2/16 revision of this document:

Text was revised to fix a minor typographical error.



^(a)U.S. Pat. No. 6,855,499, European Pat. No. 1368629, Japanese Pat. No. 4399164 and other patents.

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