

TECHNICAL MANUAL

# Maxwell<sup>®</sup> RSC ccfDNA Plasma Kit

Instructions for Use of Product  
**AS1480**

**Note:** To use the Maxwell<sup>®</sup> RSC ccfDNA Plasma Kit, you must have the “ccfDNA Plasma” method loaded on the Maxwell<sup>®</sup> Instrument.

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

# Maxwell<sup>®</sup> RSC ccfDNA Plasma Kit

All technical literature is available at: [www.promega.com/protocols/](http://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](mailto:techserv@promega.com)

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## 1. Description

The Maxwell® RSC ccfDNA Plasma Kit<sup>(a)</sup> is used with the Maxwell® and Maxprep® Instruments specified below to provide an easy method for efficient, automated sample preparation and purification of circulating cell-free DNA (ccfDNA) from human plasma samples. Maxwell® Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the RSC ccfDNA Plasma Kit can process from one to the maximum sample number in approximately 70 minutes. The purified DNA can be used directly in a variety of downstream applications such as PCR.

### 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 RUO Mode	AS8000	TM628
Maxprep® Liquid Handler	AS9100, AS9101, AS9105, AS9200, AS9201, AS9205	TM509
Maxwell® CSC 48 RUO Mode	AS8000	TM628

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. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind circulating DNA to the paramagnetic particle in the first well of a prefilled cartridge and mix during processing.

Prior to extraction, samples can be preprocessed manually or using the Maxprep® Liquid Handler. The Maxprep® Liquid Handler will transfer samples from primary tubes to Maxwell® RSC ccfDNA Plasma cartridges, transfer plungers to Maxwell® RSC ccfDNA Plasma 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. #
<b>Maxwell® RSC ccfDNA Plasma Kit</b>	<b>48 preps</b>	<b>AS1480</b>

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

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

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

**Safety Information:** The Maxwell® RSC Cartridges contain ethanol, isopropanol, guanidine thiocyanate and guanidine hydrochloride. Ethanol and isopropanol should be considered flammable, harmful and irritants. Guanidine thiocyanate and guanidine hydrochloride should be considered toxic, harmful and irritants. 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 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.

### For Preprocessing with the Maxprep® Liquid Handler

PRODUCT	SIZE	CAT. #
<b>Maxprep® 1000µl Conductive Disposable Tips, Filtered</b>	<b>40/box</b>	<b>AS9303</b>
<b>Maxprep® 300µl Conductive Disposable Tips, Filtered</b>	<b>60/box</b>	<b>AS9302</b>
<b>Maxprep® Reagent Reservoir, 50ml</b>	<b>28/pack</b>	<b>AS9304</b>
<b>Maxwell® RSC Plunger Pack</b>	<b>1 each</b>	<b>AS1670</b>
<b>Maxprep® Plunger Holder</b>	<b>1 each</b>	<b>AS9408</b>



### 3. Usage Information

#### 3.A. Intended Use

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

#### 3.B. 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 nonwhole 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.

### 4. Preparation of Plasma Samples

#### Materials to Be Supplied by the User

- whole blood or plasma
- benchtop centrifuge

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 –30°C to –10°C (or below –65°C). Avoid exposing plasma to freeze-thaw cycles.

### 5. Manual Preprocessing

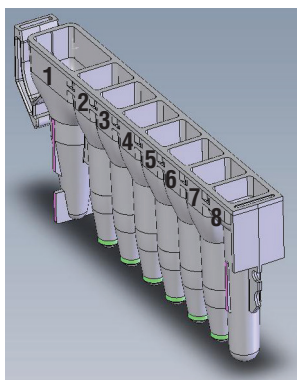
#### Maxwell® RSC ccfDNA Plasma Cartridge Preparation

1. Change gloves before handling Maxwell® RSC Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used 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.
2. Transfer 0.2–1.0ml of each plasma sample to well #1 of each cartridge (well #1 is the largest well).

3. Tip-mix the plasma sample in well #1 to ensure all plasma has been transferred. Change pipette tips between samples.
4. Place one plunger into well #8 of each cartridge.
5. 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.
6. Proceed to Section 7, Maxwell® Instrument Setup and Run.

**Notes:**

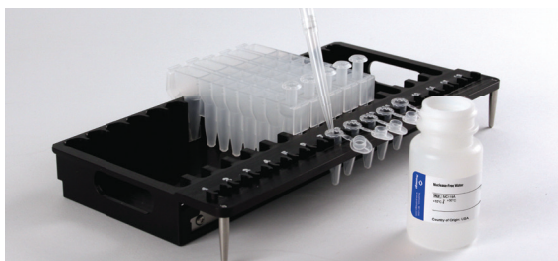
- a. Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bacteriocidal spray or wipe, and then water. Do not use bleach on any instrument parts.
- b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.
- c. 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 10µl less than the starting volume.



**User Adds to Wells**

1. 0.2–1.0ml of Plasma
8. RSC Plunger

**Figure 1. Maxwell® RSC Cartridge.**



**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.



## 6. Maxprep® Preprocessing

### 6.A. Maxprep® Cartridge Preparation

1. 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.
2. Touch **Start** to access the 'Methods' screen.
3. On the 'Methods' screen, select a method using one of the two options below:
  - a. Touch the ccfDNA Plasma preprocessing method or laboratory-specific variant of the ccfDNA Plasma 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 variant of the ccfDNA Plasma preprocessing method if desired.
4. 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.
5. Enter any method-specific variables (Sample Number, Sample Volume, Elution Volume).

**Note:** 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 10µl less than the starting volume.
6. Before 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:

- 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, and 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.

7. 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:

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

**6.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 plasma from each sample tube to its corresponding Maxwell® RSC cartridge.
3. Method is complete. Open the instrument door and move the deck tray(s) to the Maxwell® Instruments 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 used with this system.







## 7. Maxwell® Instrument Setup and Run

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

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. Sign 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 test and home all moving parts.
2. Touch **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 codes(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 **ccfDNA Plasma** method.
4. If applicable to your Maxwell® Instrument model, verify that the ccfDNA Plasma method has been selected, and press the **Proceed** button. If requested by the software, scan or 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/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 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.

**Note:** 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.



**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:**

- a. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.
  - b. 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 above) 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 ccfDNA, 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.

## 7. Maxwell® Instrument Setup and Run (continued)

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.

## 8. Considerations When Working with ccfDNA

### 8.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.

### 8.B. Recommendations for Quantitation

#### 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. The 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.

## 9. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: [www.promega.com](http://www.promega.com). Email: [techserv@promega.com](mailto: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 supported Maxwell® Instruments (See Section 1).  Make sure to use plungers from the Maxwell® RSC Plunger Rack.
Low yield	In normal plasma, yields of 10–30ng of ccfDNA per milliliter of plasma are typical. The Maxwell® RSC ccfDNA Plasma kit can accept up to 1ml of plasma sample.
Wrong method run on the Maxwell® Instrument	Check that the ccfDNA Plasma method was run.

## 10. Related Products

### Instrument and Accessories

Product	Size	Cat. #
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® RSC Plunger Pack	1 each	AS1670
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® FSC Deck Tray	1 each	AS4016
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
Nunc™ 2.0ml Deep Well Plates	60/pack	AS9307



## 10. Related Products (continued)

Product	Size	Cat. #
Maxprep® 1000µl Conductive Disposable Tips, Filtered	40/box	AS9303
Maxprep® 300µl Conductive Disposable Tips, Filtered	60/box	AS9302
Maxprep® Reagent Reservoir, 60ml	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
Maxprep® Tube Rack Stabilizer	1 each	AS1910
Elution Tubes	50/pk	AS6201

### Maxwell® RSC Reagent Kits

For a list of available Maxwell® RSC purification kits, visit: [www.promega.com](http://www.promega.com)

## 11. Summary of Changes

The following changes were made to the 2/25 revision of this document:

1. Added Cat. # AS9105, AS9205 to Sections 1 and 10. Cat. # AS1910 was also added to Section 10.
2. Updated Maxprep to a registered trademark.
3. Updated cover image and fonts. Made miscellaneous text edits, including changing Notes to alphabetical lists.
4. Updated the patent statement.

©U.S. Pat. No. 6,855,499 and other patents.

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All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.