TECHNICAL MANUAL

Maxwell® RSC Rapid ccfDNA Kit

Instructions for Use of Product
AS1590

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

Note: To use the Maxwell® RSC Rapid ccfDNA Kit, the Rapid ccfDNA method must be loaded on the Maxwell® Instrument

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Maxwell® RSC Rapid ccfDNA 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 Rapid ccfDNA Kit is used with the Maxwell® 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 of 1–4ml. Maxwell® Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the RSC Rapid ccfDNA Kit can process from one to the maximum sample number in less than 30 minutes. The purified DNA can be used directly in a variety of downstream applications such as digital PCR and next-generation sequencing (NGS).

Table 1. Supported Instruments.

Instrument	Cat.#	Operating Manual
Maxwell® RSC	AS4500	TM411
Maxwell® RSC 48	AS8500	TM510
Maxwell® CSC RUO Mode	AS6000	TM573
Maxwell® CSC 48 RUO Mode	AS8000	TM628
Maxwell® FSC	AS4600	TM462

Method Principle

The Maxwell® RSC Rapid ccfDNA Kit purifies ccfDNA using paramagnetic particles, which provides a mobile solid phase that optimizes sample capture, washing and purification of ccfDNA. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind ccfDNA to the paramagnetic particles in the first three wells of a prefilled cartridge. The samples are processed through a series of washes before the ccfDNA is eluted. This magnetic capture approach 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 Rapid ccfDNA Kit	**48 preps**	**AS1590**

For Research Use Only. Sufficient for 48 automated isolations from 1–4ml of plasma samples. Includes:

- 48 Maxwell® RSC Rapid ccfDNA Cartridges
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer (RCFD)
- 1ml Proteinase K (PK2) Solution

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

Safety Information: Refer to the Safety Data Sheet (SDS) for detailed safety information. Adhere to institutional guidelines for the handling and disposal of all chemical waste used with this system.

The Maxwell® RSC Rapid ccfDNA Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and safety glasses) when handling infectious substances. Adhere to your 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.

3. Product Intended Use

The Maxwell® RSC Rapid ccfDNA Kit is intended for use in combination with the Maxwell® RSC Instruments and the Maxwell® RSC Rapid ccfDNA purification method and is for research use only. This kit is intended for performing automated isolation of circulating cell-free DNA from plasma generated from blood samples collected in EDTA tubes and Streck Cell-Free DNA BCT® devices.

4. Product Use Limitations

The Maxwell® RSC Rapid ccfDNA Kit has been evaluated with plasma prepared from human whole blood samples collected in EDTA tubes and Streck Cell-Free DNA BCT® devices. The user is responsible for validating its use to extract circulating cell-free DNA from other sample types. The Maxwell® RSC Rapid ccfDNA Kit is not intended for use in diagnostic procedures.

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5. Preparing Plasma Samples

Materials to Be Supplied by the User

- whole blood or plasma
- benchtop centrifuge

For whole blood collected in EDTA tubes, the blood should be processed immediately after collection or stored at +2°C to +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. For Streck Cell-Free DNA BCT® devices, follow manufacturer's instructions. After either Streck Cell-Free DNA BCT® or EDTA blood collection tubes are first centrifuged, use a pipette to 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°C to +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. See Section 8.A for considerations when using frozen plasma.

6. Preparing Maxwell® RSC Rapid ccfDNA Cartridges

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 first of the largest wells 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.

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

2. Depending on total sample size, transfer plasma to only well #1 (first largest well), to only wells #1 and #3, or to wells #1, #2 and #3 (three largest wells) as indicated in Table 2. Ensure all plasma has been transferred and use the pipette tip to mix the plasma sample in well(s). Change pipette tips between samples.

Table 2. Plasma Sample Transfer to Different Wells of the Maxwell® RSC Cartridge Based on Sample Input Volume.

Sample Volume (ml)	Sample Transfer Instructions
1.0ml to 1.5ml plasma	Add plasma to well #1 only. See Figure 1, Panel A.
>1.5ml to ≤3ml of plasma	Add equal volumes of plasma to wells #1 and #3. See Figure 1, Panel B.
3ml to 4ml of plasma	Add equal volumes of plasma to wells #1, #2 and #3. See Figure 1, Panel C.

Notes:

- **Do not** dispense more than 1.5ml of plasma per well.
- **With plasma input volumes of 1.0–1.5ml, the entire plasma sample must be loaded in well #1**. Loading plasma in well #2 or #3 may negatively affect ccfDNA recovery.
- **With plasma input volumes of 1.5–3ml, plasma must be loaded in wells #1 and #3.** Loading plasma in any other well configuration (e.g., in wells #2 and #3 or wells #1 and #2) may negatively affect ccfDNA recovery.

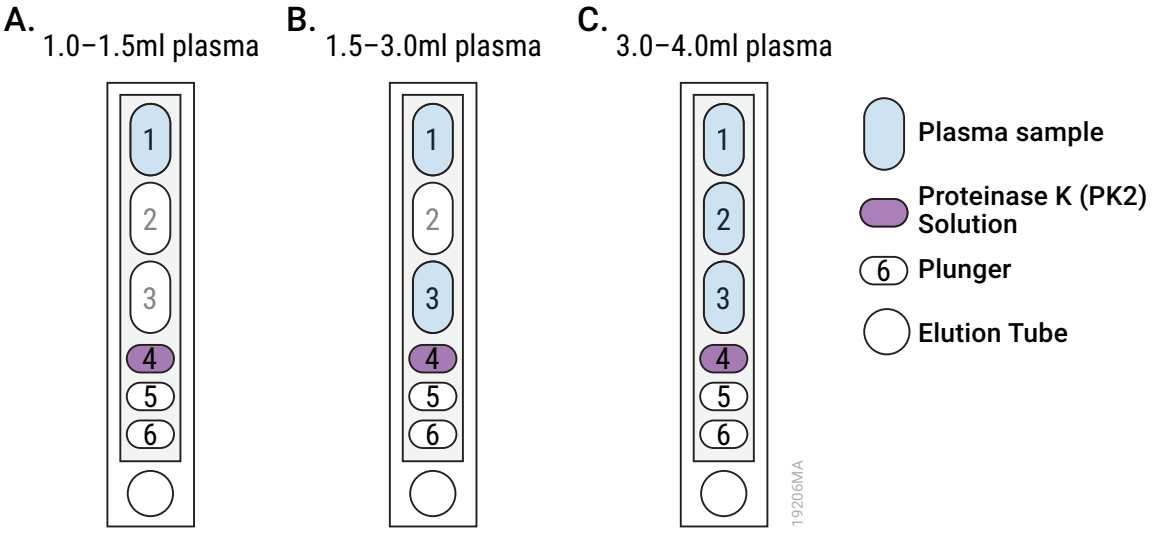


Figure 1. Plasma sample transfer to different wells of the Maxwell® RSC Cartridge is based on sample input volume. For plasma samples of 1–1.5ml, transfer the entire sample to well #1 (**Panel A**). For plasma samples of 1.5–3.0ml, transfer equal volumes of plasma to both wells #1 and #3 (**Panel B**). For plasma samples of 3.0–4.0ml, transfer equal volumes of plasma to wells #1, #2 and #3. Dispense 10µl of the Proteinase K (PK2) Solution into well #4. Add 40–50µl of Elution Buffer (RCFD) to the Elution Tube (represented by the circle at the bottom of each of the panels). Place a plunger into well #6.

3. Dispense 10µl of the Proteinase K (PK2) Solution into well #4 (the first of three smaller wells in the cartridge).
4. Place one plunger into well #6 of each cartridge.
5. Place an empty Elution Tube into the elution tube position for each cartridge in the deck tray. Add 40–50µl of Elution Buffer (RCFD) to the bottom of each Elution Tube.
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.

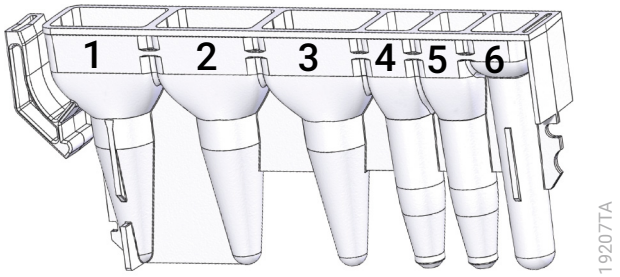
b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.

c. The supplied Elution Buffer (RCFD) is essential for efficient recovery of ccfDNA. We do not recommend substituting with alternative elution buffers. Using alternative elution buffers will negatively affect ccfDNA recovery.

d. Higher ccfDNA concentrations can be achieved with less than 50µl of Elution Buffer (RCFD), but total yield may be reduced.

e. The magnetic resin is not dried prior to elution, so final elution volumes will approximate the volume of Elution Buffer (RCFD) added to each elution tube.

6. Preparing Maxwell® RSC Rapid ccfDNA Cartridges (continued)



User Adds to Wells:

1.	Plasma sample (see Table 2 and Figure 1 for details)
	or
1.,3.	Plasma sample
	or
1.,2.,3.	Plasma sample
4.	Proteinase K (PK2) Solution
6.	RSC Plunger

Figure 2. Maxwell® RSC Cartridge. Plasma sample is added to well #1 (1.0–1.5ml of sample), wells #1 and #3 (1.5–3ml of sample), or wells #1, #2 and #3 (3–4ml of sample), depending on sample volume; 10µl of Proteinase K (PK2) Solution is dispensed into well #4 and a plunger is added to well #6.

Figure 3. Setup and configuration of the deck tray(s). Elution Buffer (RCFD) is added to the elution tubes as indicated. Plungers are in position #6 of the cartridge. Deck tray shown is from the Maxwell® RSC Instrument (Cat.# AS4500).

7. Maxwell® 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 proceed through a self-check and home all moving parts.
2. Select **Start** to begin running a method.
3. Depending on the 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, select **Continue** to use the sample tracking information for the deck tray(s) or select **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. Select the **Rapid ccfDNA** method.

   Note: Selecting the incorrect method will result in Maxwell® RSC Instrument damage and sample loss.
4. If applicable to your Maxwell® Instrument, verify that the Rapid ccfDNA method has been selected, and select 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), select the cartridge positions to select or deselect any positions to be used for this extraction run. Enter any required sample tracking information, and select 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 the appropriate well(s) of the cartridges, the cartridges are loaded on the instrument, uncapped elution tubes are present with Elution Buffer (RCFD) and plungers are in well #6. 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. Select 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 platform retracts. Any errors in deck tray setup (e.g., plungers not in well #6, 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.

7. Maxwell® Instrument Setup and Run (continued)

8. The Maxwell® Instrument will immediately begin the purification run. The screen will display the steps 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 #6 of the cartridge at the end of the run. If plungers have not been 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 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.

    Note: Following the automated purification procedure, the deck tray(s) will be warm. To remove a deck tray from the instrument platform, hold onto the deck tray by its sides.

    Ensure samples are removed from the instrument before running a UV sanitation protocol to avoid damage to the nucleic acid.

12. Remove the cartridges and plungers from the Maxwell® deck tray(s). Discard as hazardous waste according to your institution's procedures. Do not reuse Maxwell® RSC Cartridges, RSC Plungers or Elution Tubes.

8. Post-Purification

Determine that the purified ccfDNA sample meets the input requirements for the appropriate downstream assay prior to use in that assay. If the purified ccfDNA samples are not processed immediately, store the ccfDNA samples at 4°C for up to 7 days. For longer term storage, freeze at –20°C or –70°C and below. Consult the instructions for downstream applications for specific ccfDNA sample storage and handling recommendations.

9. Considerations When Working with ccfDNA

9.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 Rapid ccfDNA Kit, it can affect pipetting of plasma. To pellet the cryoprecipitate, centrifuge the plasma sample at ≥1,000 × *g* for ≥5 minutes prior to processing.

9.B. Recommendations for ccfDNA Quantitation

The low concentration and fragmented nature of ccfDNA provide unique challenges for researchers. In normal plasma, yields of 5–30ng of ccfDNA per milliliter of plasma are typical. The majority of ccfDNA fragments are approximately 160–200bp, with additional fragments at approximately 340bp and 510bp.

UV Quantitation

It is impossible to accurately determine ccfDNA concentration using 260nm absorbances due to the low concentration. Some available products use a carrier RNA to enhance ccfDNA purification. The carrier RNA is in much higher abundance than the ccfDNA and copurifies. This can give a false A_{260} value and drastically higher apparent ccfDNA concentrations. For accurate quantitation, use fluorescent dyes or PCR.

Fluorescence Quantitation

The sensitivity dsDNA-specific dyes makes these dyes 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.

PCR Quantitation

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

10. 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
Instrument unable to pick up plungers	Make sure you are using a Maxwell® 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 the plungers supplied with the Maxwell® RSC Plunger Pack.
Low yield	The Maxwell® RSC Rapid ccfDNA Plasma Kit can accept a maximum of 4ml of plasma sample. Repeat extraction using up to 4ml of plasma.
	If less than 1ml of plasma is used, ccfDNA recovery may be negatively affected.
	Confirm the correct volume of prepared Proteinase K (PK2) Solution was added well #4 of the cartridge. Repeat extraction after dispensing 10µl of Proteinase K (PK2) Solution into well #4.
	Yields can be reduced if eluting in less than 50µl. Repeat extraction using 50µl of Elution Buffer (RCFD).
	The supplied Elution Buffer (RCFD) is essential for efficient ccfDNA recovery. Do not substitute alternative elution buffers. Repeat extraction using Elution Buffer (RCFD).
	Confirm that the plasma was transferred to the correct wells of the Maxwell® Cartridge (see Table 2 in Section 5). • For plasma input volumes of 1.0–1.5ml, transfer all of plasma to well #1. • For plasma input volumes of 1.5–3ml, divide the plasma volume equally between wells #1 and #3. • For plasma input volumes of 3ml–4ml, add equal volumes of plasma to wells #1, #2 and #3.
Genomic DNA contamination	Plasma preparation contains lysed white blood cells.

11. Related Products

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® CSC 48 Instrument	1 each	AS8000
Maxwell® RSC/RSC Deck Tray	1 each	SP6019
Maxwell® RSC/RSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/RSC 48 Back Deck Tray	1 each	AS8402
Maxwell® RSC Plunger Pack	1 each	AS1670
Elution Tubes (0.5ml)	50/pack	AS6201
Elution Magnet, 16 Position	1 each	AS4017
Elution Magnet, 24 Position	1 each	AS4018

Maxwell® RSC Reagent Kits

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