TECHNICAL MANUAL

Maxwell® CSC Rapid ccfDNA Kit

Instructions for Use of Product
AS1580

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

Note: To use the Maxwell® CSC Rapid ccfDNA Kit, the Maxwell® CSC Rapid ccfDNA method must be loaded onto the Maxwell® CSC or Maxwell® CSC 48 Instrument.

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INSTRUCTIONS FOR
USE OF PRODUCT
AS1580

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Maxwell® CSC 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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The Maxwell® CSC Rapid ccfDNA Kit is only available in certain countries.

1. Description

The Maxwell® CSC Rapid ccfDNA Kit is used with the Maxwell® Instruments specified in Table 1 to provide an easy method for efficient, automated extraction and purification of circulating cell-free DNA (ccfDNA) from 1–4ml of human plasma samples. Maxwell® CSC Instruments are designed for use with predispensed reagent cartridges and preprogrammed extraction methods, maximizing simplicity and convenience. The Maxwell® CSC Rapid ccfDNA method can process from one to the maximum sample number supported by the Maxwell® CSC instruments in less than 30 minutes. The extracted ccfDNA 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.#	Technical Manual	Maximum Sample Number
Maxwell® CSC	AS6000	TM457	16
Maxwell® CSC 48	AS8000	TM623	48

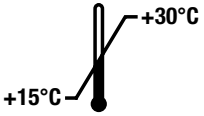
Method Principle

The Maxwell® CSC Rapid ccfDNA Kit purifies ccfDNA from plasma samples using paramagnetic particles, which provide 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, Storage Conditions and Symbols Key

PRODUCT	SIZE	CAT.#
Maxwell® CSC Rapid ccfDNA Kit	**48 preps**	**AS1580**

For In Vitro Diagnostic Use. Professional use only. Contains sufficient reagents for 48 automated ccfDNA isolations from plasma samples. Cartridges are for single use only.



Includes:

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

Storage Conditions: Store the Maxwell® CSC 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® CSC Rapid ccfDNA Cartridges (CSCS) 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.

Additional Information: The Maxwell® CSC Rapid ccfDNA Kit components are qualified and quality-control tested to work together. Do not mix kit components between different kit lots. Use only the components provided in the kit. For additional safety information, see the Safety Data Sheet, available at: **www.promega.com**

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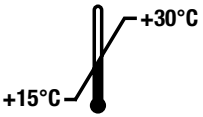
Symbols Key

Symbol	Explanation	Symbol	Explanation
	In Vitro Diagnostic Medical Device		Authorized Representative
	Store at +15°C to +30°C.		Manufacturer
	Caution		Irritant
	Health hazard		Contains sufficient for “n” tests
	Conformité Européenne		Warning. Biohazard.
	Warning. Pinch point hazard.		Catalog number
	Lot number		Do not reuse

3. Product Intended Purpose/Intended Use

The Maxwell® CSC Rapid ccfDNA Kit is intended for use, in combination with the Maxwell® CSC Instruments and the Maxwell® CSC Rapid ccfDNA method, as an in vitro diagnostic (IVD) medical device to perform automated isolation of circulating cell-free DNA (ccfDNA) from human plasma specimens. The purified ccfDNA is suitable for use in amplification-based in vitro diagnostic assays, including digital PCR.

The Maxwell® CSC Rapid ccfDNA Kit is intended to be used at a temperature between 15°C and 30°C. Use outside of this temperature range may result in suboptimal results.

The Maxwell® CSC Rapid ccfDNA Kit is intended for professional use only. Diagnostic results obtained using ccfDNA purified with this system must be interpreted in conjunction with other clinical or laboratory data.

4. Product Use Limitations

The performance of the Maxwell® CSC Rapid ccfDNA Kit was evaluated with human plasma specimens prepared from whole blood collected in Streck Cell-Free DNA BCT and blood collection tubes with K_2EDTA anticoagulant. The user is responsible for validating the use of the Maxwell® CSC Rapid ccfDNA Kit to purify ccfDNA from plasma collected in other blood collection tubes.

Suitability of the nucleic acid purified using the Maxwell® CSC Rapid ccfDNA Kit for use in next generation sequencing (NGS) was demonstrated during product development but has not been validated.

Appropriate controls must be included in any downstream diagnostic applications using ccfDNA purified using the Maxwell® CSC Rapid ccfDNA Kit. The user is responsible for establishing performance characteristics necessary for downstream diagnostic applications.

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 and transfer into a new tube. 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 1 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 11.A for considerations when using frozen plasma.

6. Preparing Maxwell® CSC Rapid ccfDNA Cartridges

1. Change gloves before handling Maxwell® CSC cartridges, CSC/RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the Maxwell® CSC/RSC 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 the entire seal is removed from 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 plasma sample volume, transfer plasma to only well #1 (first largest well), to only wells #1 and #3 (first and third largest wells), or to wells #1, #2 and #3 (three largest wells) of the Maxwell® CSC Rapid ccfDNA cartridge as indicated in Table 2. Change pipette tips between different plasma samples to avoid cross-contamination.

Table 2. Plasma Sample Transfer to Different Wells of the Maxwell® CSC 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 ≤3.0ml of plasma	Add equal volumes of plasma to wells #1 and #3. See Figure 1, Panel B.
3.0ml to 4.0ml 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 only in well #1**. Loading plasma in well #2 or #3 will negatively affect ccfDNA recovery.
- **With plasma input volumes of 1.5–3.0ml, plasma must be loaded only in wells #1 and #3.** Loading plasma in any other well configuration (e.g., in wells #2 and #3 or wells #1 and #2) will negatively affect ccfDNA recovery.

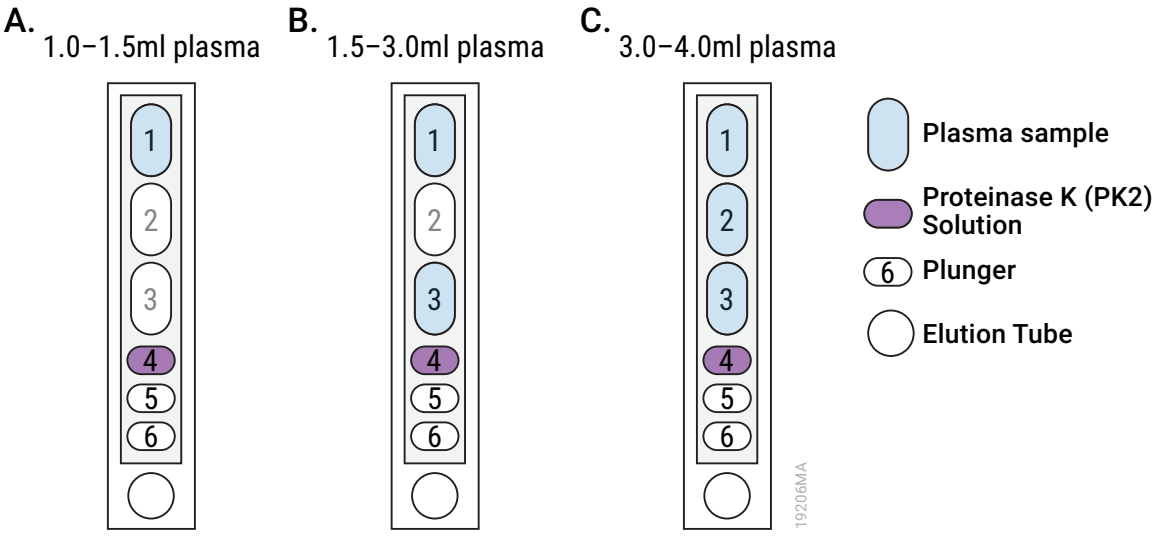


Figure 1. Plasma sample transfer to different wells of the Maxwell® CSC Rapid ccfDNA Cartridge is based on sample input volume. For 1.0–1.5ml plasma samples, transfer the entire sample to well #1 (**Panel A**). For 1.5–3.0ml plasma samples, transfer equal volumes of plasma to both wells #1 and #3 (**Panel B**). For 3.0–4.0ml plasma samples, transfer equal volumes of plasma to wells #1, #2 and #3 (**Panel C**). Dispense 10µl of the Proteinase K (PK2) Solution into well #4. Add 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.
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 50µl of Elution Buffer (RCFD) to the bottom of each Elution Tube.
6. Proceed to Section 7, Maxwell® Instrument Setup and Run.

   Notes:

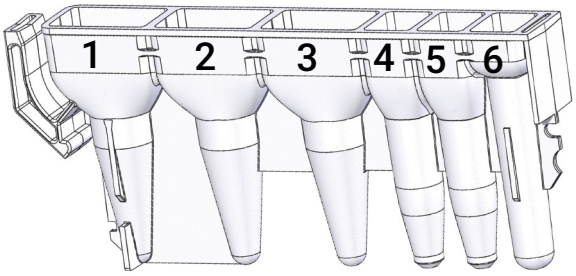
   a. Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bacteriocidal spray or wipe, and then water. Do not use bleach on any Maxwell® Instrument parts.

   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 ccfDNA recovery. Do not replace Elution Buffer (RCFD) 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.

6. Preparing Maxwell® CSC 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.	CSC/RSC Plunger

Figure 2. Maxwell® CSC Cartridge. Plasma sample is added to well #1 (1.0–1.5ml of sample), wells #1 and #3 (1.5–3.0ml of sample), or wells #1, #2 and #3 (3.0–4.0ml 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® CSC Instrument (Cat.# AS6000).

7. Maxwell® Instrument Setup and Run

For detailed information, refer to the Operating Manual specific to your Maxwell® CSC Instrument. See Table 1.

1. Turn on the Maxwell® Instrument and Tablet PC. Log in to the Tablet PC, and start the Maxwell® CSC IVD-mode 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** on the 'Home' screen.
3. Scan or enter the bar code in the upper right corner of the Maxwell® CSC Rapid ccfDNA Kit label and select **OK** to automatically select the method to be run (Figure 4).

   Note: The Maxwell® CSC Rapid ccfDNA Kit method bar code is required for ccfDNA purification on the Maxwell® CSC Instruments. The kit label contains two bar codes. The method bar code is indicated in Figure 4. If the bar code cannot be scanned, contact Promega Technical Services.

Figure 4. Kit label indicating the bar code to scan. The bar code to scan for starting a purification run is shown in the blue box, in the upper right of the kit label.

4. On the 'Cartridge Setup' screen, confirm that the Maxwell® CSC Rapid ccfDNA method is displayed at the top of the screen. 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 the Maxwell® CSC 48 Instrument, select or deselect cartridge positions on each deck tray using the **Front** and **Back** buttons.
5. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that plasma samples were added to the appropriate wells of the cartridges, Proteinase K (PK2) solution was added to well #4 of the cartridge, plungers are in well #6, the cartridges are loaded on the instrument and uncapped elution tubes are present with Elution Buffer (RCFD). 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 leveled 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. Maxwell® Instrument Setup and Run (continued)

6. 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. 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 Maxwell® Instrument 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** when requested. The samples will be lost.

8. When the run is complete, the user interface will display a message that the method has ended.

End of Run

9. 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 Technical Manual appropriate to your Maxwell® Instrument (see Table 1) to perform a **Clean Up** process to attempt to unload the plungers.

10. Remove the deck tray(s) from the instrument immediately following the run to prevent evaporation of the eluates. Remove elution tubes containing ccfDNA, and cap the tubes.

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

11. 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® CSC Cartridges, CSC/RSC Plungers or Elution Tubes.

8. Post-Purification

Determine that the purified ccfDNA sample meets the input requirements for the appropriate downstream diagnostic 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. Analytical Performance Evaluation

The analytical performance of the Maxwell® CSC Rapid ccfDNA Kit was evaluated using human plasma samples processed on the Maxwell® CSC and Maxwell® CSC 48 Instruments. Key metrics such as ccfDNA quantity, quality, amplifiability, reproducibility and cross-contamination were assessed to confirm the reliability and suitability of ccfDNA extracted using the Maxwell® CSC Rapid ccfDNA Kit for downstream applications.

9.A. ccfDNA Quantity and Quality

Plasma samples were isolated from the blood of six individuals collected in either K_2EDTA tubes or Streck Cell-Free DNA BCT® devices. ccfDNA was extracted from 1–4ml of plasma samples with the Maxwell® CSC Rapid ccfDNA Kit. ccfDNA quantity and quality were assessed using TapeStation analysis (Agilent Technologies, Inc.) of one biological replicate per condition, focusing on the percent cell-free DNA (cfDNA) and the size of the largest DNA peak, which reflects fragment quality. Percent cfDNA refers to the ratio of DNA fragments within the 50–700bp range relative to the total DNA content in the purified sample as determined by TapeStation analysis. This value provides a measure of sample enrichment for ccfDNA, which is typically smaller in size due to its origin from fragmented DNA released after cell death.

The TapeStation analysis indicated that the percent cfDNA range was 76–93%, with the largest peak sizes between 177bp and 203bp across all purified ccfDNA samples. There were no significant differences in proportion or quality of ccfDNA extracted from plasma collected in different anticoagulant tubes or different plasma sample input volumes.

Table 3. TapeStation Analysis of Purified ccfDNA Showing Percent Cell-Free DNA and Largest Peak Size Across Various Plasma Sample Input Volumes and Anticoagulants.

Sample ID (Anticoagulant)	Plasma Sample Input Volume (ml)	Percent Cell-Free DNA	Largest Peak Size (bp)
1 (K_2EDTA)	1.0	81	186
	1.5	79	181
	3.0	80	183
	4.0	79	183
2 (K_2EDTA)	1.0	81	177
	1.5	88	178
	3.0	84	179
	4.0	91	175
3 (K_2EDTA)	1.0	90	184
	1.5	90	187
	3.0	89	183
	4.0	88	185

Sample ID (Anticoagulant)	Plasma Sample Input Volume (ml)	Percent Cell-Free DNA	Largest Peak Size (bp)
4 (Streck)	1.0	93	195
	1.5	76	200
	3.0	89	193
	4.0	84	192
5 (Streck)	1.0	92	195
	1.5	92	197
	3.0	90	195
	4.0	89	194
6 (Streck)	1.0	89	183
	1.5	79	182
	3.0	86	203
	4.0	86	203

9.B. Amplifiability and Inhibition (Interfering Substances)

Purified ccfDNA amplifiability and lack of inhibition were evaluated using a qPCR assay for amplifying a 75bp DNA target and analyzing the qPCR data for any inhibition. ccfDNA was extracted in quadruplicate from each of the 4ml of human plasma samples collected from six individuals in either K_2EDTA tubes or Streck Cell-Free DNA BCT® devices. Each extracted ccfDNA sample was analyzed by the qPCR assay. Mean internal positive control (IPC) $|\Delta C_q|$ values were calculated relative to the qPCR standard whose concentration was closest to that of the qPCR-amplified ccfDNA sample to determine the extent of inhibition.

qPCR results (Table 4) indicated minimal or no amplification inhibition, as evident from the mean IPC $|\Delta C_q|$ values ≤0.5 for all purified ccfDNA samples.

Table 4. Inhibition Assessment by Amplification of ccfDNA Extracted from 4ml Human Plasma Samples.

Sample ID (Anticoagulant)	Mean IPC $\lvert\Delta C_q\rvert$
1 (K_2EDTA)	0.2
2 (K_2EDTA)	0.2
3 (K_2EDTA)	0.4
4 (Streck)	0.5
5 (Streck)	0.3
6 (Streck)	0.3

9.C. Quantitating ccfDNA by Fluorescent Dye and qPCR

The consistency of ccfDNA quantitation was assessed by comparing ccfDNA concentrations using a double-stranded DNA-specific fluorescent dye-based method and a qPCR assay that amplified a 75bp DNA target. ccfDNA samples extracted in quadruplicate from 4ml of human plasma samples that were collected from six individuals in either K_2EDTA tubes or Streck Cell-Free DNA BCT® device were used for analyses. Ratios of fluorescence-based ccfDNA quantitation to qPCR-based ccfDNA quantitation were calculated.

The results demonstrated strong correlation between the two quantitation methods, with ccfDNA concentration ratios ranging from 0.7 to 1.4 across all samples.

Table 5. Comparing ccfDNA Concentration Using Fluorescence-Based and qPCR-Based Quantitation Methods.

Sample ID (Anticoagulant)	Fluorescence-to-qPCR Concentration Ratio
1 (K_2EDTA)	0.7
2 (K_2EDTA)	1.3
3 (K_2EDTA)	0.9
4 (Streck)	1.3
5 (Streck)	0.9
6 (Streck)	1.4

9.D. Reproducibility

ccfDNA extraction reproducibility when using the Maxwell® CSC Rapid ccfDNA Kit was evaluated by analyzing intra- and inter-run percent coefficient of variation (CV) for ccfDNA yield. Using 4ml of plasma collected in K_2EDTA anticoagulant, ccfDNA was extracted using three consecutive extraction runs with both the Maxwell® CSC and Maxwell® CSC 48 Instruments. Each extraction run yielded 24 ccfDNA eluates. ccfDNA yield was quantified using a qPCR assay that amplified a 75bp DNA target.

The results showed that intra-run percent CV values were 11% and 14% for the Maxwell® CSC Instrument and the Maxwell® CSC 48 Instrument, respectively. The inter-run percent CV values were 5% for both the instruments.

Table 6. Intra- and Inter-Run Variability in ccfDNA Yield.

Instrument	Anticoagulant	Plasma Sample Input Volume (ml)	Intra-Run Percent Coefficient of Variation	Inter-Run Percent Coefficient of Variation
Maxwell® CSC	K_2EDTA	4	11%	5%
Maxwell® CSC 48	K_2EDTA	4	14%	5%

9.E. Cross-Contamination

The potential for cross-contamination during ccfDNA extraction using the Maxwell® CSC Rapid ccfDNA Kit was evaluated by processing human and bovine plasma samples placed at alternating deck positions of the Maxwell® CSC and Maxwell® CSC 48 instruments. ccfDNA eluates from bovine plasma were analyzed for presence of any contaminating human DNA using a qPCR assay that amplified a human-specific 75bp DNA target to determine any cross-contamination.

When human plasma samples were processed in Maxwell® Instrument deck positions adjacent to bovine plasma samples, none of the bovine ccfDNA eluates exhibited quantifiable amounts of human DNA in the qPCR assay, confirming the absence of any detectable cross-contamination.

9.F. Compatibility with Next-Generation Sequencing

ccfDNA eluate compatibility with next-generation sequencing (NGS) workflows was evaluated through ccfDNA library preparation followed by TapeStation analysis. ccfDNA was extracted from plasma samples collected in either K_2EDTA tubes or Streck Cell-Free DNA BCT® devices using the Maxwell® CSC Rapid ccfDNA Kit with the Maxwell® CSC or the Maxwell® CSC 48 Instrument. Successful library preparation was determined by observing a characteristic adapter base pair shift in TapeStation analysis.

All evaluated ccfDNA libraries demonstrated the expected adapter base pair shift, confirming successful NGS library preparation.

9.G. Compatibility with Digital PCR

The compatibility of ccfDNA eluates with digital PCR (dPCR) workflow was evaluated using a droplet digital PCR assay. The study included ccfDNA samples extracted from human plasma collected in both K_2EDTA tubes and Streck Cell-Free DNA BCT® devices, with 3ml and 4ml plasma sample input volumes. ccfDNA eluates were assessed using a Copy Number Variation Assay (PIK3CA gene) and droplet counts were recorded.

The results demonstrated successful amplification across all tested ccfDNA samples, with droplet counts exceeding 15,000 per reaction.

10. Clinical Performance Evaluation

Clinical performance of the Maxwell® CSC Rapid ccfDNA Kit was evaluated by an external clinical laboratory using human plasma samples processed with the Maxwell® CSC 48 Instrument.

In the first study, two testers independently purified ccfDNA from 1.5ml human plasma samples (n = 10) using both the Maxwell® CSC Rapid ccfDNA Kit and the standard purification method used by the laboratory as reference. The resulting ccfDNA eluates were analyzed for Rhesus factor using an amplification-based assay. ccfDNA extracted from human plasma samples using the Maxwell® CSC Rapid ccfDNA Kit exhibited expected results that were concordant with the results obtained with ccfDNA extracted using the laboratory reference method.

In the second study, two testers independently purified ccfDNA from 1.5ml human plasma samples (n = 10) using the Maxwell® CSC Rapid ccfDNA Kit. The purified ccfDNA eluates were analyzed for the Rhesus factor using an amplification-based assay, and concordant results were obtained between the two testers.

11. Considerations When Working with ccfDNA

11.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® CSC 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.

11.B. Recommendations for ccfDNA Quantitation

The low concentration and fragmented nature of ccfDNA provide unique challenges. In plasma from healthy human individuals, 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

Accurately determining ccfDNA concentration using 260nm absorbance is difficult due to the low concentration. Some products use a carrier RNA to enhance ccfDNA purification. The carrier RNA is in much greater 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-Based Quantitation

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

A second factor is that the standards used with fluorescent dyes are typically high-molecular-weight genomic or Lambda DNA. ccfDNA is highly fragmented and has a relatively low molecular weight. Therefore, it 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.

Amplification-Based 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.

12. 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 that a Maxwell® CSC-specific chemistry kit is used; the plungers for the Maxwell® CSC kits are specific for the supported Maxwell® Instruments (see Table 1).
Low ccfDNA yield	The Maxwell® CSC Rapid ccfDNA 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 that the correct volume of Proteinase K (PK2) Solution was added to 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 of Elution Buffer (RCFD). Repeat extraction using 50µl of Elution Buffer (RCFD).
	The supplied Elution Buffer (RCFD) is essential for efficient ccfDNA recovery. Do not replace Elution Buffer (RCFD) with alternative elution buffers. Repeat extraction using Elution Buffer (RCFD).
	Confirm that the plasma was transferred to the correct wells of the Maxwell® CSC Rapid ccfDNA Cartridge (see Table 2 and Figure 1 in Section 6). • For plasma input volumes of 1.0–1.5ml, transfer all of plasma to well #1. • For plasma input volumes of 1.5–3.0ml, divide the plasma volume equally between wells #1 and #3. • For plasma input volumes of 3.0ml–4.0ml, add equal volumes of plasma to wells #1, #2 and #3.
Genomic DNA contamination	Plasma preparation contains lysed white blood cells. See Sections 5 and 11.A for recommendations on preparing plasma samples from whole blood.

Any serious incident that occurred in relation to the device that led to, or might lead to, death or serious injury of a user or patient should be immediately reported to the manufacturer. Users based in the European Union should also report any serious incidents to the Competent Authority of the Member State in which the user and/or the patient is established.

13. Related Products

Product	Size	Cat.#
Maxwell® CSC Instrument*	1 each	AS6000
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® CSC 48 Instrument*	1 each	AS8000
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
RSC/CSC Plungers	50/pack	AS1331
Elution Tubes (0.5ml)	50/pack	AS6201
Elution Magnet, 16 Position	1 each	AS4017
Elution Magnet, 24 Position	1 each	AS4018

*For In Vitro Diagnostic Use. This product is only available in certain countries.

Maxwell® CSC Reagent Kits

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

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