TECHNICAL MANUAL

Maxwell® CSC Blood DNA Kit

Instructions for Use of Product
AS1321

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

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INSTRUCTIONS FOR USE OF PRODUCT
AS1321

IVD

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Maxwell® CSC Blood DNA Kit

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

The Maxwell® CSC Blood DNA Kit is only available in certain countries. This product meets the essential requirements of EU Directive 98/79/EC on in vitro diagnostic medical devices.

1. Description

The Maxwell® CSC Blood DNA Kit[a] is used, in combination with the Maxwell® CSC Instrument, to provide an easy method for efficient, automated purification of genomic DNA (gDNA) from human blood samples. The Maxwell® CSC Instrument is supplied with preprogrammed purification methods and is designed for use with the predispensed reagent cartridges and additional reagents supplied in the kit, maximizing simplicity and convenience. The instrument can process up to 16 samples in 40 minutes, and the purified DNA can be used directly in a variety of downstream applications such as PCR.

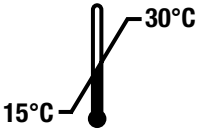
1. Description (continued)

The Maxwell® CSC Blood DNA Kit purifies nucleic acid using a novel paramagnetic particle, which provides a mobile solid phase that optimizes sample capture, washing and purification of gDNA. This particle utilizes cellulose-based binding of nucleic acids and provides a higher binding capacity and cleaner eluate than traditional silica-based DNA purification. The Maxwell® CSC Instrument is a magnetic particle-handling instrument that allows efficient binding of gDNA 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, Storage Conditions and Symbols Key

PRODUCT	SIZE	CAT.#
Maxwell® CSC Blood DNA Kit	**48 preps**	**AS1321**

For In Vitro Diagnostic Use. Professional use only. Sufficient for 48 automated isolations from 300µl of whole blood samples. The Maxwell® CSC Cartridges are for single use only.



Includes:

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

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

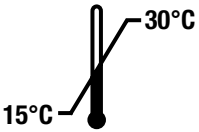
Safety Information: The reagent cartridges contain ethanol and isopropanol. These substances should be considered flammable, harmful and irritants.

The Maxwell® CSC Cartridges are designed to be used with potentially infectious substances. Wear appropriate personal protective equipment (e.g., gloves and goggles) when handling infectious substances. 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.

Additional Information: The Maxwell® CSC Blood DNA Kit components are qualified and quality control tested to work together. It is not recommended to mix kit components between different kit lots. Use only the components provided in the kit.

Symbols Key

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

3. Product Intended Use

The Maxwell® CSC Blood DNA Kit is intended for use, in combination with the Maxwell® CSC Instrument and the Maxwell® CSC Blood DNA purification method, as an in vitro diagnostic (IVD) medical device to perform automated isolation of genomic DNA from human whole blood samples. The purified DNA is suitable for use in amplification-based in vitro diagnostic assays.

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

Whole blood samples collected in blood collection tubes containing EDTA, heparin or sodium citrate anticoagulants can be used with the Maxwell® CSC Blood DNA Kit. Table 1 shows the acceptable time that samples can be stored under different conditions prior to use in the Maxwell® CSC Blood DNA Kit. The Maxwell® CSC Blood DNA Kit is not intended for use with samples that have been collected in other types of blood collection tubes or stored outside of the conditions listed in Table 1.

Sample Storage Temperature	Storage Time Before Purification
15–30°C	Up to 72 hours
2–10°C	Up to 7 days
–80°C or lower	Indefinitely

The Maxwell® CSC Blood DNA Kit is not intended for use as part of a specific diagnostic test.

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

4. Product Use Limitations

The Maxwell® CSC Blood DNA Kit is not intended for use with tissue samples or samples from body fluids other than human whole blood or with clotted human whole blood samples.

The Maxwell® CSC Blood DNA Kit is not intended for use with non-human samples, including bacterial and viral samples, or for the purification of RNA.

The Maxwell® CSC Blood DNA Kit performance has been evaluated by isolating DNA from 50–300µl whole blood samples with a white blood cell (wbc) count ranging from 4×10^6 to 10×10^6wbc/ml and eluting the DNA in 50–100µl. It is not intended for use with samples outside of this range.

The Maxwell® CSC Blood DNA Kit performance has been evaluated for compatibility with the following potential inhibiting factors for genomic DNA amplification: heme, alcohol, IgG and guanidine. Other compounds have not been evaluated.

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

5. Before You Begin

Materials to be Supplied by the User

- optional, rotating tube mixer for liquid blood samples
- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for incubation of samples (e.g., Microtubes, 1.5ml [Cat.# V1231])
- heating block set at 56°C (**Note:** The heat block should be set to 56°C. Actual heater block temperature should be measured as 56°C within the calibration specifications of the thermometer used for the measurement.)

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5.A. Preparation of Whole Blood Samples

Whole Blood Sample Processing Capacity

The total genomic DNA yield from whole blood samples depends on the sample volume and number of white blood cells/ml. Each cartridge supplied in the Maxwell® CSC Blood DNA Kit is designed to purify genomic DNA from 50–300µl of whole blood, with a white blood cell range of 4×10^6 to 10×10^6wbc/ml whole blood (values for a normal healthy adult; 1). We recommend performing a white blood cell count on each sample prior to purification of DNA to ensure the sample falls within this range. Samples outside of this range may not provide optimal results.

Note: This kit has been tested with human whole blood samples collected in EDTA, sodium citrate or heparin tubes. Performance of the chemistry cannot be guaranteed with other types of blood collection tubes. Blood samples may be fresh (stored at 15–30°C for up to 72 hours), refrigerated (stored at 2–10°C for up to seven days) or frozen (stored at –80°C or lower) prior to DNA purification. Frozen samples should be thawed before processing. All blood samples should be thoroughly mixed before use.

1. Mix all blood samples for at least 5 minutes at 15–30°C.
2. Prepare and label incubation tubes that will fit in the heating block set at 56°C.
3. Add 30µl of Proteinase K (PK) Solution to each incubation tube.
4. Add liquid blood (between 50µl and 300µl) to each incubation tube. Please take care to avoid clot material (if any) when transferring blood to the incubation tube. The system is not intended for use with clotted blood samples. Change tips between each blood sample transfer to prevent cross-contamination.
5. Add 300µl of Lysis Buffer to each incubation tube. Change tips between each Lysis Buffer transfer to prevent cross-contamination.
6. Vortex each tube at maximum speed for 10 seconds.
7. Incubate each tube in the heating block (set to 56°C) for 20 minutes. During this incubation, prepare cartridges as described in Section 5.B.
8. Inspect each lysate after incubation. Following proteinase K treatment, the sample changes color from red to greenish brown. If the samples do not change color after the proteinase K treatment, this indicates that the treatment was ineffective and post-purification DNA yield and purity will be affected. Do not process samples further if no color changes is observed by the end of the proteinase K incubation period.
9. Transfer each blood lysate sample from the incubation tube to well #1 of a separate cartridge. (Well #1 is the largest well in the cartridge). Change tips between each sample transfer to prevent sample cross-contamination.

   Note: Maxwell® CSC Cartridges should contain a visible black pellet of microparticle resin in the second well of the cartridge. If the resin is not visible shake down the particles in the cartridge to dislodge any resin that may have adhered to the cartridge seal before removing the seal.

5.B. Maxwell® CSC Cartridge Preparation

1. Change gloves before handling Maxwell® CSC Cartridges, CSC/RSC Plungers and Elution Tubes. Cartridges are set up on the Maxwell CSC Deck Tray outside of the instrument and the deck tray containing the cartridges and samples is then transferred to the instrument for purification. Place the cartridges to be used in the Maxwell® CSC Deck Tray (Figure 2). 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. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the deck tray.

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

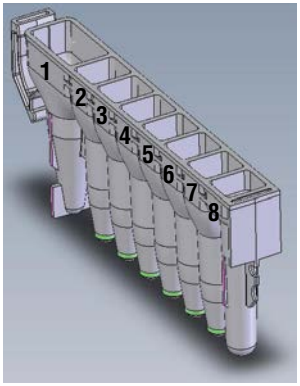
2. Place one plunger into well #8 of each cartridge.
3. Place an empty Elution Tube into the Elution Tube position for each cartridge in the Maxwell® CSC Deck Tray.

   Note: Use only the elution tubes provided in the Maxwell® CSC Blood DNA Kit. Other elution tubes may not be compatible with the Maxwell® CSC Instrument and may affect DNA purification performance.
4. Add 50–100µl of Elution Buffer to the bottom of each Elution Tube.

   Note: Only use the Elution Buffer provided in the Maxwell® CSC Blood DNA Kit. Use of other Elution Buffers may impact DNA purification performance.

Maxwell® CSC Cartridge Preparation Notes

1. If you are processing fewer than 16 samples, center the cartridges on the deck tray.
2. Clean specimen or reagent spills on any part of the Maxwell® CSC Deck Tray as indicated in the *Maxwell® CSC Instrument Manual*. Do not use bleach on any instrument parts.



1. Binding Buffer
2. Paramagnetic Cellulose Particles
   Note: If particles are not visible in Well 2, shake down the cartridge to dislodge particles that may have adhered to the seal material before removing the seal.
3. Wash Buffer
4. Wash Buffer
5. Wash
6. Wash
7. Empty
8. Empty

Well Content User Adds:

1. Lysed whole blood sample
8. CSC/RSC Plunger

Figure 1. Maxwell® CSC Cartridge. This figure shows the contents of a cartridge. Lysed whole blood sample is added to well #1, and a plunger is added to well #8.

Figure 2. Setup and configuration of the Maxwell® CSC Deck Tray. Elution Buffer is added to the Elution Tubes as indicated.

6. Instrument Run

Refer to the Maxwell® CSC Instrument Operating Manual for more detailed information.

1. Turn on the Maxwell® CSC Instrument and Tablet PC. The instrument user interface will start automatically, and 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 method bar code on the Maxwell® CSC Blood DNA Kit label to automatically select the method to be run (Figure 3).

   Note: The Maxwell® CSC Blood DNA Kit method bar code is required for DNA purification on the Maxwell® CSC Instrument. The kit label contains two bar codes. The method bar code is indicated in Figure 3.

Figure 3. Kit label indicating the method bar code to scan. Shown in the red box is the method bar code to scan on the kit label for starting a purification run.

4. Select cartridge positions to be run (see *Maxwell® CSC Instrument Operating Manual*), and scan in or manually enter the required sample tracking information.
5. Verify that the samples were added to well #1 of the cartridges, the cartridges are loaded on the instrument, the Elution Tubes containing 50–100µl of Elution Buffer are present and the Plungers are placed in well #8.

6. Instrument Run (continued)

6. Transfer the Maxwell® CSC Deck Tray containing the prepared cartridges to the Maxwell® CSC Instrument deck. Ensure that the deck tray is placed in the Maxwell® CSC Instrument with the Elution Tubes closest to the door. The deck tray will only fit in the instrument in this orientation. If you have difficulty fitting the deck tray on the platform, check that it is in the correct orientation. Ensure that the deck tray is level on the instrument deck.

   Note: Hold the Maxwell® CSC Deck Tray by the sides to avoid dislodging cartridges from the rack.

7. Confirm all indicated preprocessing has been performed, and touch "Start" to close the instrument door and start processing.

Warning: Pinch point hazard.

8. The Maxwell® CSC Instrument will immediately begin the purification run. The screen will display the steps performed and the approximate time remaining in the run.

   Note: If the run is aborted before completion, the instrument will wash the particles off the plungers and eject the plungers into well #8 of the cartridge. The samples will be lost. Do not attempt to repurify samples for which an instrument run was aborted.

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 door. Verify that plungers are located in well #8 of the cartridge at the end of the run. Remove the deck tray from the instrument and remove eluted samples from the deck tray. If plungers have not all been removed from the plunger bar: follow the on-screen prompts to perform the Clean Up method for an aborted run; or select the Clean Up method from the Settings Screen for a successfully completed run to remove the remaining plungers.

11. Remove the Maxwell® CSC Deck Tray from the instrument immediately following the run to prevent evaporation of the eluates. Remove Elution Tubes containing DNA, and close the tubes.

    Note: Following the automated purification procedure, the Maxwell® CSC Deck Tray may be warm. To remove the rack from the instrument platform, hold the rack by its sides.

    Ensure samples are removed from the instrument before running UV sanitization to avoid damage to the purified nucleic acid.

12. Remove the cartridges and plungers from the Maxwell® CSC Deck Tray. Discard as hazardous waste according to your institution's procedures. Do not reuse Maxwell® CSC Cartridges, CSC/RSC Plungers or Elution Tubes.

7. Post-Purification

Determine the purified DNA sample yield and purity meets the input requirements for the appropriate downstream diagnostic assay prior to use in that assays.

8. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Lower than expected concentration A 300µl whole blood sample containing 4×10^6 to 10×10^6 white blood cells/ml should yield >80ng/µl of genomic DNA in an elution volume of 50µl (as measured by absorbance at 260nm).	Blood that has undergone multiple freeze-thaw cycles may have degraded DNA. Use samples that have been collected and stored under the conditions listed in Section 3.
	Whole blood sample contained low white blood cell count. The yield of genomic DNA from blood samples depends on the number of white blood cells present in the sample.
	Proteinase K Solution was not added, an incomplete volume of Proteinase K Solution was added, or the Proteinase K was not effectively mixed with the blood sample prior to addition of lysis buffer. Lysis and yield are dependent upon complete extraction with Proteinase K. If Proteinase K was not added in Section 3.A, Step 3, the resulting blood sample will be red. Proteinase K-treated samples turn greenish brown, which can be used as a visual indicator that Proteinase K was added to the sample.
	Whole blood sample was not mixed before processing. Be sure to mix whole blood samples before processing to ensure that the white blood cells are in suspension
Lower than expected purity A 300µl whole blood sample containing 4×10^6 to 10×10^6 white blood cells/ml eluted in a volume of 50µl should produce gDNA with an A_{260}/A_{280} ratio (purity measured by absorbance at 260nm divided by absorbance at 280nm) of 1.7 or greater, and an A_{260}/A_{230} ratio (purity measured by absorbance at 260nm divided by absorbance at 230nm) of 1.5 or greater.	Proteinase K Solution was not added, an incomplete volume of Proteinase K Solution was added, or the Proteinase K was not effectively mixed with the blood sample prior to addition of lysis buffer. Lysis and purity are dependent upon complete extraction with Proteinase K. If Proteinase K was not added in Section 3.A, Step 3, the resulting blood sample will be red. Proteinase K-treated samples turn greenish brown, which can be used as a visual indicator check to determine that Proteinase K was added.

9. Reference

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

10. Summary of Change

The following change was made to the 5/18 revision of this document:

1. Updated to include EU IVD Directive (98/79/EC) requirements.

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