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.

1. Description

The Maxwell® CSC Blood DNA Kit[a,b] is used, in combination with the Maxwell® Instruments specified in Table 1, to provide an easy method for efficient, automated purification of genomic DNA (gDNA) from human blood samples. The Maxwell® CSC Instruments are designed for use with the predispensed reagent cartridges and additional reagents supplied in the kit with preprogrammed purification methods, maximizing simplicity and convenience. The Maxwell® CSC Instruments can process from one to the maximum number of samples allowed in approximately 40 minutes, and the purified DNA can be used directly in a variety of downstream applications such as PCR.

Table 1. Supported Instruments.

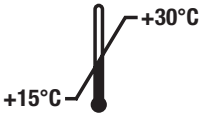
Instrument	Cat.#	Technical Manual
Maxwell® CSC	AS6000	TM457
Maxwell® CSC 48	AS8000	TM623

Principle of the Method: The Maxwell® CSC Blood DNA Kit purifies nucleic acid using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of gDNA. The Maxwell® CSC Instruments are magnetic particle-handling instruments that efficiently bind gDNA to paramagnetic particles in the first well of a prefilled cartridge and move the sample through the wells of the cartridge. 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 to +30°C.

Safety Information: The cartridges contain ethanol and isopropanol. These substances should be considered flammable, harmful and irritants. Lysis Buffer contains guanidine hydrochloride and urea. These substances should be considered toxic, harmful and irritants. Refer to the SDS for detailed safety information.

The Maxwell® CSC Blood DNA Kit components are designed to be used with potentially infectious substances. Wear appropriate personal protective equipment (e.g., gloves and safety glasses) 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. Do not use cartridges if the seal on the cartridge is not intact on receipt.

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2. Product Components, Storage Conditions and Symbols Key (continued)

Symbols Key

Symbol	Explanation	Symbol	Explanation
IVD	In Vitro Diagnostic Medical Device	EC REP	Authorized Representative
+30°C +15°C	Store at +15 to +30°C.	PROMEGA 2800 Woods Hollow Rd. Madison, WI USA	Manufacturer
	Caution		Irritant
	Health hazard.		Contains sufficient for "n" tests.
CE	Conformité Européenne		Warning. Biohazard.
	Warning. Pinch point hazard.	REF	Catalog number
LOT	Lot number		Do not reuse.

3. Product Intended Purpose/Intended Use

The Maxwell® CSC Blood DNA Kit is intended for use, in combination with the Maxwell® CSC Instruments and the Maxwell® CSC Blood DNA purification method, as an in vitro diagnostic (IVD) medical device to perform automated isolation of genomic DNA from whole human 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°C and 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. The table below 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 below.

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 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 10×10^6 wbc/ml.

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.

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5. Before You Begin

Materials to be Supplied by the User

- 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
- **optional:** rotating tube mixer for liquid blood samples

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 10×10^6 wbc/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 change 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.

5.B. Maxwell® CSC Blood DNA Cartridge Preparation

1. Change gloves before handling Cartridges, CSC/RSC Plungers and Elution Tubes. Cartridges are set up in the deck tray(s) outside of the instrument and the deck tray(s) containing the cartridges and samples are then transferred to the instrument for purification. Place each cartridge in the deck tray with well #1 (the largest well in the cartridge) farthest away from the Elution Tubes (Figure 2). 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 from the cartridge.

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 deck tray(s).

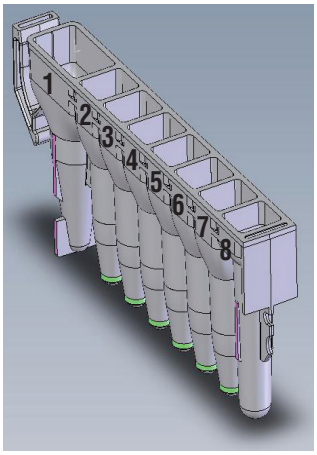
   Note: Use only the elution tubes provided in the Maxwell® CSC Blood DNA Kit. Other elution tubes may not be compatible with the Maxwell® CSC Instruments and may affect DNA purification performance.
4. Add 50–100µl of Elution Buffer to the bottom of each Elution Tube.

   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 Blood DNA Cartridge Preparation Notes

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

5.B. Maxwell® CSC Blood DNA Cartridge Preparation (continued)



Well Content User Adds:
1. Lysed whole blood sample
8. CSC/RSC Plunger

Figure 1. Maxwell® CSC 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 deck tray. Elution Buffer is added to the Elution Tubes as indicated.

6. Instrument Run

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

1. Turn on the Maxwell® Instrument and Tablet PC. Log into the Tablet PC and start the Maxwell® 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 Blood DNA Kit label and press **OK** 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 Instruments. The kit label contains two bar codes. The method bar code is indicated in Figure 3. If the bar code cannot be scanned, contact Promega Technical Services.

Figure 3. Kit label indicating the bar code to scan. Scan the bar code shown in the red box, upper right of the kit label, to start a purification run.

4. On the 'Cartridge Setup' screen, touch the cartridge positions to select or deselect any positions to be used for this extraction run. Enter any required sample tracking information and press the **Proceed** button to continue.

   Note: When using the Maxwell® CSC 48 Instrument, press the **Front** or **Back** button to select or deselect cartridge positions on each deck tray.
5. After the door has opened, confirm that all extraction checklist items have been performed. Verify that preprocessed 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 to the Maxwell® instrument platform.

   Inserting the Maxwell® deck tray: 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.

6. Instrument Run (continued)

6. 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.

Warning: Pinch point hazard.

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

   Notes:

   1. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.
   2. 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.

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 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 Operating 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 DNA, and close the tubes.

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

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

7. Post-Purification

Determine whether the purified DNA sample concentration and purity meet the input requirements for the appropriate downstream diagnostic assay prior to use in that assay.

8. Analytical Performance Evaluation

Analytical performance evaluation for the Maxwell® CSC Blood DNA Kit was performed using whole blood samples on a Maxwell® CSC Instrument. Equivalent performance of the Maxwell® CSC Blood DNA Kit with the Maxwell® CSC 48 Instrument was demonstrated as part of development of that instrument.

8.A. DNA Quantity, Quality and Amplifiability

Table 2. DNA Yield and Purity from Whole Blood Specimens with Differing WBC Counts. DNA extraction from eight replicates was tested for each condition listed to assess DNA quantity, quality and amplifiability. DNA was extracted from 300µl of whole blood with white blood cell (WBC) counts ranging from 4×10^6–10×10^6 WBC/ml and eluted in 50µl. Absorbance of purified DNA was measured at 230nm, 260nm, 280nm and 340nm. DNA concentration was determined using absorbance at 260nm after subtracting absorbance of the blank and correcting for instrument noise (absorbance at 340nm). All absorbance calculations described in this performance evaluation were performed this way. DNA concentration was multiplied by eluted DNA volume to determine yield, and A_{260}/A_{280} and A_{260}/A_{230} ratios were calculated to assess DNA quality. The Maxwell® CSC Blood DNA Kit yielded acceptable quantities of DNA (≥3.00pg/WBC) for all whole blood samples tested, regardless of white blood cell count. Average yields of purified DNA were ≥4.18pg/WBC, with A_{260}/A_{280} ratios ≥1.89 and A_{260}/A_{230} ratios ≥1.68.

Donor	Average Total DNA Yield (µg) (n = 8)	White Blood Cell Count (WBC/ml)	Average DNA Yield Per White Blood Cell (pg)	Average A_{260}/A_{280} Ratio	Average A_{260}/A_{230} Ratio
1	6.0	4.5×10^6	4.45	1.90	1.68
2	8.6	6.9×10^6	4.18	1.89	1.91
3	12.4	9.4×10^6	4.45	1.91	2.01

8.A. DNA Quantity, Quality and Amplifiability (continued)

Table 3. DNA Yield and Purity from Whole Blood Collected with Different Anticoagulants. Quantity and quality were assessed for purified DNA eluates prepared from whole blood collected in commonly used blood collection tubes containing EDTA, sodium citrate or heparin as the anticoagulant. The blood samples were refrigerated or frozen and equilibrated to room temperature prior to DNA extraction. DNA was purified from eight 300µl replicates for each anticoagulant and eluted in 50µl. DNA concentration, DNA yield and A_{260}/A_{280} and A_{260}/A_{230} ratios were calculated and are shown in the table below. Consistent yields and purity ratios were observed for DNA extracted from all samples using the Maxwell® CSC Blood DNA Kit.

Blood Collection Tube Type	Average DNA Total Yield (µg) (n = 8)	Average DNA Concentration (ng/µl)	Average A_{260}/A_{280} Ratio	Average A_{260}/A_{230} Ratio
EDTA	10.97	281.28	1.91	1.94
Sodium Citrate	11.29	283.57	1.93	2.02
Heparin	11.99	307.48	1.94	2.08

Table 4. Amplifiability of DNA Extracted from Whole Blood. Amplifiability was assessed by qPCR for DNA extracted from whole blood using the Maxwell® CSC Blood DNA Kit and the Maxwell® CSC Instrument. Each purified DNA sample was amplified using a 71bp CAPZA3 target sequence. C_q values that were less than the lowest concentration on the qPCR standard curve were observed for all of the sample types and were within the linear range of the assay.

Whole Blood Volume	Sample	C_q (Cycles)
50µl	1	27.17
	2	26.86
	3	26.95
	4	26.92
	5	27.23
	6	27.12
	7	27.21
	8	26.98
300µl	1	26.90
	2	26.78
	3	26.84
	4	26.87
	5	26.66
	6	26.66
	7	26.84
	8	26.75

8.B. Reproducibility

Table 5. Reproducibility Across Users and Days. Reproducibility of DNA purification was determined across different users and days, using eight replicates from a single blood specimen as follows:

- Three users purified DNA from replicate samples of the same whole blood specimen using the same Maxwell® CSC Instrument on the same day, with one purification run per user to characterize reproducibility across different users.
- One user purified DNA from replicate samples of the same blood specimen using the same Maxwell® CSC Instrument, with one run per day for a total of 5 days to characterize reproducibility across different days.

All purification runs included eight replicates of 300µl of whole blood. Average yield, A_{260}/A_{280} and A_{260}/A_{230} ratios and percent coefficient of variation (% CV) were calculated within and between users and days. Yields and purity ratios of DNA extracted using the Maxwell® CSC Blood DNA Kit were reproducible across all variables tested.

	Variable	Average DNA Total Yield (µg) (n = 8)	% CV	Average A_{260}/A_{280} Ratio	% CV	Average A_{260}/A_{230} Ratio	% CV
User	1	11.18	5.7	1.93	0.2	2.00	3.4
	2	10.02	8.8	1.91	0.2	1.92	5.2
	3	12.27	4.2	1.92	0.3	1.92	4.4
Average of three different users		**11.16**	**10.3**	**1.92**	**0.4**	**1.95**	**4.6**
Day	1	11.88	5.3	1.93	0.4	1.93	5.1
	2	12.27	4.2	1.92	0.3	1.92	4.4
	3	12.78	6.9	1.93	0.3	1.93	3.6
	4	11.31	8.6	1.91	0.3	1.89	4.6
	5	12.92	5.5	1.93	0.3	1.92	3.0
Average of five different days		**12.23**	**7.7**	**1.92**	**0.4**	**1.92**	**4.1**

8.C. Interfering Substances (Inhibition)

Table 6. Inhibition in Amplification of DNA Extracted from Whole Blood Containing Different Anticoagulants. Inhibition in DNA amplification was assessed for DNA extracted from whole blood using the Maxwell® CSC Blood DNA Kit and the Maxwell® CSC Instrument. DNA was purified from 300µl samples of whole blood specimens collected in EDTA, heparin and sodium citrate collection tubes, with eight replicates per tube type. The effect of interfering substances that may be present in the extracted DNA was characterized using a commercially available exogenous positive control DNA. The exogenous positive control DNA was amplified in the presence and absence of DNA eluate from each whole blood replicate and the results were compared to determine if there were inhibiting factors in the DNA eluates. The difference in C_q value (ΔC_q) for the two amplifications was calculated by subtracting the C_q value of the control DNA amplification from the C_q value of amplification containing the DNA extracted using the Maxwell® CSC Blood DNA Kit. A ΔC_q value of less than 2 cycles indicated that any carryover of inhibitors from the blood collection tubes, whole blood or DNA purification reagents had limited effect on amplification. The mean ΔC_q values for all DNA eluates were ≤1.87 cycles, demonstrating that the DNA extracted using the Maxwell® CSC Blood DNA Kit had no detectable inhibitors of DNA amplification.

	Mean C_q Value (Amplification Cycles; n = 8)	Mean ΔC_q Value (Amplification Cycles; n = 8)
No Eluate	30.49	NA
EDTA	32.36	1.87
Heparin	31.85	1.36
Sodium Citrate	32.19	1.70

8.D. Cross Contamination

To assess whether cross contamination occurred during DNA extraction, samples of male and female whole blood specimens were run in alternating positions on the Maxwell® CSC Instrument cartridge deck. The purified DNA was amplified using a Y-chromosome-specific qPCR assay to detect male DNA and verify that no contaminating Y-chromosome DNA was present in the eluates from the female samples. Any concentration result below the lowest concentration on the standard curve designates no contaminating DNA present. There was no detectable cross contamination when DNA was purified using the Maxwell® CSC Blood DNA Kit and the Maxwell® CSC Instrument.

9. Clinical Performance Evaluation

Clinical performance evaluation of the Maxwell® CSC Blood DNA Kit was performed by an external clinical laboratory using human blood samples and the Maxwell® CSC Instrument.

9.A. DNA Amplifiability

Table 7. Amplifiability of DNA Extracted from Whole Blood using the Maxwell® CSC Blood DNA Kit and the Laboratory Reference Method. To evaluate DNA amplifiability, DNA was purified from 16 different blood specimens using 50µl of sample input volume and 100µl elution volume. DNA was also purified from the same blood specimens using 300µl of sample input volume and a 50µl elution volume to cover the range of input and elution volumes for the Maxwell® CSC Blood DNA Kit. DNA from the same specimens was purified using the laboratory's standard nucleic acid purification method (laboratory reference method) for comparison. Eluted DNA was used as a template in the clinical laboratory's molecular diagnostic test for JAK2 V617F to demonstrate that the extracted DNA could be amplified using a qPCR assay. This qPCR test uses two sets of primers, one specific for the wild-type gene and the other specific for the V617F mutant. Since the intent of the clinical performance evaluation was to demonstrate that DNA extracted using the Maxwell® CSC Blood DNA Kit could be amplified in an amplification-based diagnostic test and not to test sensitivity and specificity of the diagnostic assay, only the wild-type qPCR data were used for this study.

Yield and qPCR results for DNA obtained from the same specimens using the Maxwell® CSC Blood DNA Kit and the laboratory reference method were compared. Specimens extracted with the Maxwell® CSC Blood DNA Kit used the combination of lowest input blood volume (50µl) and highest elution volume (100µl) to represent the minimum Maxwell® CSC Blood DNA Kit yield, whereas the laboratory reference method used its standard input and elution volumes of 400µl each. DNA concentration, measured as ng/µl, was determined for 16 separate blood specimens by absorbance at 260nm. The Maxwell® CSC Blood DNA Kit showed similar DNA yield for all specimens as compared to the laboratory reference method; the ratio of DNA yield from the Maxwell® CSC Blood DNA Kit to the laboratory reference method was less than 2. All specimens produced a C_q value within the valid range of 10–35.

Number of Samples Tested	Maxwell® CSC Input Sample Volume (µl)	Maxwell® CSC Elution Volume (µl)	Maxwell® CSC Meets Acceptance Criteria (C_q Value within Linear Range)	Maxwell® CSC Concordance with Laboratory Reference Method for DNA Yield
16	50	100	16 of 16	16 of 16
16	300	50	16 of 16	Laboratory Reference Method not tested at this input/elution volume

9.B. Reproducibility

Table 8. Reproducibility Across Testers. DNA was purified from 8 different whole blood specimens by 2 separate testers using the Maxwell® CSC Instrument and Maxwell® CSC Blood DNA Kit. Eluates from each sample were tested in duplicate by qPCR to determine yield, following the clinical laboratory's JAK2 V617F amplification procedure, using the wild type data from the assay for this analysis. Yield and qPCR results for DNA obtained from the same specimens by two different testers using the Maxwell® CSC system were compared for two different input and elution volume combinations, using 8 different specimens for each combination. The DNA from all specimens obtained by both testers produced a C_q value within the valid range of 10–35. DNA concentration, measured as ng/µl of DNA in the eluate, was determined by absorbance at 260nm and the ratio of DNA yield obtained from the same specimen by Tester 1 to that obtained by Tester 2 was between 0.5 and 2.0 for all specimens.

Number of Specimens per Tester	Maxwell® CSC Input Sample Volume (µl)	Maxwell® CSC Elution Volume (µl)	Ct Value within Linear Range of Assay; 8 Samples per Tester	Concordance between Tester 1 and Tester 2
8	50	100	16 of 16	8 of 8
8	300	50	16 of 16	8 of 8

9.C. Cross Contamination

Cross contamination during DNA purification in the intended user environment was assessed for eluates prepared from whole blood using the Maxwell® CSC Blood DNA Kit and the Maxwell® CSC Instrument. Blood samples and negative controls (water blanks) were run in alternating positions of the Maxwell® CSC cartridge deck. DNA was purified from 8 different blood samples, with an input sample volume of 300µl and an elution volume of 50µl, and 8 negative control samples.

Eluates from each negative control sample were tested in duplicate by qPCR following the test laboratory's JAK2 V617F amplification procedure. All acceptance criteria were met. No contaminating DNA was detected in any of the negative control eluates.

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10. 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 5.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 5.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.

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.

11. Reference

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

12. Related Products

Instrument and Accessories

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
Microtube, 1.5ml	1,000/pack	V1231

*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 purification kits.

13. Summary of Changes

The following changes were made to the 10/22 revision of this document:

1. Section 3 was renamed Product Intended Purpose/Intended Use.
2. Sections 8 and 9 were added and subsequent sections renumbered.
3. Updated for compliance with Regulation (EU) 2017/746 on in vitro diagnostic medical devices.

[a]U.S. Pat. No. 6,855,499 and other patents.

[b]U.S. Pat. No. 7,329,488 and Korean Pat. No. 10-0483684.