TECHNICAL MANUAL

Maxwell® CSC RNA Blood Kit

Instructions for Use of Product
AS1410

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

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CE

INSTRUCTIONS FOR
USE OF PRODUCT
AS1410

IVD

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Promega

Maxwell® CSC RNA Blood 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 RNA Blood Kit is only available in certain countries.

1. Description

The Maxwell® CSC RNA Blood Kit[a] is used in combination with the Maxwell® Instruments specified in Table 1 to provide an easy method for efficient, automated purification of RNA from fresh (not frozen) human whole blood collected in EDTA tubes. The Maxwell® CSC Instruments are designed for use with predispensed reagent cartridges and additional reagents supplied in the kit with preprogrammed purification methods, thereby maximizing simplicity and convenience. The Maxwell® CSC Instruments can process from one to the maximum number of samples allowed in approximately 60 minutes, and the purified RNA can be used directly in a variety of amplification-based downstream applications, such as RT-PCR.

Table 1. Supported Instruments.

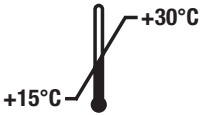
Instrument	Cat.#	Technical Manual
Maxwell® CSC	AS6000	TM457
Maxwell® CSC 48	AS8000	TM623

Principle of the Method: The Maxwell® CSC RNA Blood Kit purifies RNA using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of RNA. The Maxwell® CSC Instruments are magnetic particle-handling instruments. This system allows efficient binding of RNA to the paramagnetic particles in the first well of a prefilled cartridge and moves the sample through the wells of the cartridge. This approach to magnetic capture avoids common problems associated with liquid-handling systems such as clogged tips or partial reagent transfers, which result in suboptimal purification processing by other commonly used systems.

2. Product Components, Storage Conditions and Symbols Key

PRODUCT	SIZE	CAT.#
Maxwell® CSC RNA Blood Kit	**48 preps**	**AS1410**

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



Includes:

- 48 Maxwell® CSC RNA Blood Cartridges
- 4 × 100ml Solution A
- 30ml Solution B
- 20ml Lysis Buffer
- 2 vials DNase I (lyophilized)
- 900µl 1-Thioglycerol
- 100µl Blue Dye
- 2 × 1ml Proteinase K (PK) Solution
- 25ml Nuclease-Free Water
- 50 CSC/RSC Plungers
- 50 Elution Tubes (0.5ml)

Storage Conditions: Upon receipt, remove the 1-Thioglycerol and store at +2°C to +10°C. Store the remaining kit components at room temperature (+15 to +30°C). 1-Thioglycerol can be stored at room temperature (+15 to +30°C), where it is stable for up to 9 months. Store rehydrated DNase I at –30°C to –10°C. Do not exceed 10 freeze-thaw cycles.

Safety Information: The cartridges contain ethanol, which is flammable. 1-Thioglycerol is toxic. Guanidine thiocyanate and guanidine hydrochloride (components of Solution B and Lysis Buffer) are harmful and irritants. Wear gloves and follow standard safety procedures while working with these substances.

The Maxwell® CSC RNA Blood Kit components are designed to be used with potentially infectious substances. Users should wear appropriate personal protective equipment (e.g., gloves, a lab coat and goggles) when handling infectious substances. Users should adhere to their institutional guidelines for the handling and disposal of all infectious substances used with this system.

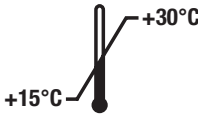
Note: Bleach reacts with ammonium chloride and guanidine thiocyanate to produce toxic fumes. Ammonium chloride and guanidine thiocyanate are present in Solution A and Solution B, respectively. Do not decontaminate waste from this kit using bleach.

Caution: Handle cartridges and open lyophilized DNase I vial with care; edges may be sharp.

Additional Information: The Maxwell® CSC RNA Blood 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. For additional safety information, see the Safety Data Sheet, available at: **www.promega.com**

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

Symbols Key

Symbol	Explanation	Symbol	Explanation
	In Vitro Diagnostic Medical Device		Authorized Representative
	Store at +15 to +30°C.		Manufacturer
	Caution		Irritant
	Health hazard.		Poison
	Corrosive		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 RNA Blood Kit is intended for use, in combination with the Maxwell® CSC Instruments and the Maxwell® CSC RNA Blood purification method, as an in vitro diagnostic (IVD) medical device to perform automated isolation of RNA from 2.5ml of whole human blood collected in EDTA collection tubes with a White Blood Cell (WBC) count in the range of 4×10^6 to 10×10^6 WBC per milliliter. The purified RNA is suitable for use in amplification-based in vitro diagnostic assays.

The Maxwell® CSC RNA Blood Kit is intended to be used with 2.5ml of human whole blood. The Maxwell® CSC RNA Blood 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 RNA Blood Kit is intended for professional use only. Diagnostic results obtained using the RNA purified with this system must be interpreted in conjunction with other clinical or laboratory data.

4. Product Use Limitations

The Maxwell® CSC RNA Blood Kit is only intended for use with whole human blood samples collected in EDTA tubes. It is not intended for use with non-whole blood samples, such as bone marrow or buffy coat, or samples stored in other collection tubes.

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

The Maxwell® CSC RNA Blood Kit performance has been evaluated for the isolation of RNA from 2.5ml of human whole blood in EDTA collection tubes.

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

5. Before You Begin: Preparation of Solutions

1-Thioglycerol/Solution B

Prepare a 1-Thioglycerol/Solution B mixture by one of the following methods:

Add 600µl of 1-Thioglycerol to the bottle of Solution B and mix thoroughly. 1-Thioglycerol is viscous, and careful pipetting is required for accurate measurement. Before use, chill the 1-Thioglycerol/Solution B on ice or at 2–10°C.

Alternatively, prepare smaller volumes by adding 20µl of 1-Thioglycerol per milliliter of Solution B. Prepare 200µl of chilled 1-Thioglycerol/Solution B per sample.

Note: Store prepared 1-Thioglycerol/Solution B at 2–10°C, where it is stable for up to 30 days.

DNase I

Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I. Invert to rinse DNase I from the underside of the cap and swirl gently to mix; do not vortex. Add 25µl of Blue Dye to the reconstituted DNase I as a visual aid for pipetting and cartridge preparation. Each purification requires 10µl of prepared DNase I solution. Store reconstituted DNase I at –30°C to –10°C. Do not freeze-thaw reconstituted DNase I more than ten times.

Caution: Open DNase I vial with care; vial edges may be sharp.

6. Purification of RNA from Fresh Whole Blood in EDTA Collection Tubes

Maintain an RNase-free environment during processing. Always use RNase-free and aerosol-resistant pipette tips. Change gloves frequently to reduce the chance of RNase contamination. See Section 12, Creating a Ribonuclease-Free Environment, for details.

Materials to Be Supplied by the User

- whole blood in EDTA collection tubes (not frozen); blood may be stored for up to 3 days at 2–10°C prior to purification
- microcentrifuge
- 10ml serological pipettes (sterile)
- pipettors and RNase-free, sterile, aerosol-resistant pipette tips
- 15ml tubes (sterile)
- centrifuge with swinging-bucket rotor

6.A. Preprocessing of Whole Blood Samples

1. Transfer 2.5ml of well mixed whole blood (not frozen) from the EDTA collection tube to a sterile 15ml tube.
2. Add 7.5ml of Solution A, and invert the tube 5–10 times to mix. This is a differential lysis step; the red blood cells are lysed, leaving the white blood cells intact.
3. Incubate lysates for 10 minutes at room temperature. Twice during the incubation, invert the samples as in Step 2 to mix.
4. Centrifuge the tube(s) at 3,000 × *g* for 10 minutes in a swinging-bucket rotor.
5. Remove the supernatant by decanting or pipetting. Briefly spin the tube to collect the residual liquid at the bottom of the tube. Using a pipette, remove and discard as much of the remaining supernatant as possible without disturbing the visible WBC pellet.
6. Add 200µl of chilled 1-Thioglycerol/Solution B to the pellet and vortex to resuspend the pellet.
7. Add 200µl of Lysis Buffer and 25µl of Proteinase K to the resuspended pellet. Mix by vortexing for 15–20 seconds.

   Note: If you need to stop during preprocessing, the samples can be stored after Step 7 at –30°C to –10°C for up to 5 days. At these storage temperatures, the samples may or may not freeze completely. When you are ready to resume sample purification, thaw the tubes at room temperature for 10 minutes before continuing with the next step.
8. Incubate at room temperature for 10 minutes. During this time, prepare cartridges as described in Section 6.B.
9. Add lysate to well #1 of the Maxwell® CSC RNA Blood Cartridge (the largest well in the cartridge).

6.B. Maxwell® CSC RNA Blood Cartridge Preparation

1. Change gloves before handling Maxwell® CSC RNA Blood 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 a CSC/RSC 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 RNA Blood Kit. Other elution tubes may not be compatible with the Maxwell® CSC Instruments and may affect RNA purification performance.
4. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube. The Elution Tubes must remain open during the RNA purification.

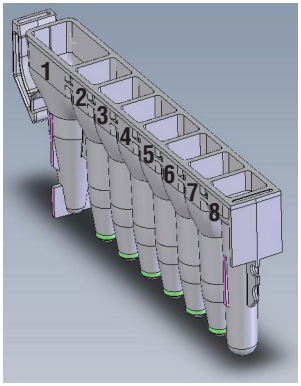
   Note: Use only the Nuclease-Free Water provided in the Maxwell® CSC RNA Blood Kit. Use of other elution buffers may impact RNA purification performance or downstream use.

6.B. Maxwell® CSC RNA Blood Cartridge Preparation (continued)

5. Add 10µl of reconstituted DNase I (blue) to well #4 (yellow) of each cartridge. The resulting green color is a visual indicator that DNase I solution has been added to well #4.

Maxwell® CSC RNA Blood 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.



Well Content User Adds:
1. Preprocessed sample lysate
4. 10µl prepared DNase I
8. CSC/RSC Plunger

Figure 1. Maxwell® CSC Cartridge. The preprocessed blood sample lysate is added to well #1, 10µl of DNase I is added to well #4 and a CSC/RSC Plunger is added to well #8.

Figure 2. Setup and configuration of the deck tray. Nuclease-Free Water (50µl) is added to the elution tubes as shown.

7. Instrument Run

The Maxwell® CSC RNA Blood Method for the Maxwell® CSC Instrument can be downloaded from the Promega web site: **www.promega.com/resources/tools/maxwellcscmethod**. The Maxwell® CSC RNA Blood Method for the Maxwell® CSC 48 Instrument can be downloaded from the Promega web site: **www.promega.com/resources/software-firmware/maxwell-maxprep/maxwell-csc-48-methods/**

If you suspect your instrument may be contaminated with RNase, clean the instrument prior to running it using a detergent solution such as Steris LpH®. Follow the instructions in the Cleaning and Maintenance section of the appropriate Maxwell® CSC Instrument Operating Manual.

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. Touch **Start** on the 'Home' screen.
3. Scan or enter the bar code in the upper right corner of the Maxwell® CSC RNA Blood Kit label and touch **OK** to automatically select the method to be run (Figure 3).

   Note: The Maxwell® CSC RNA Blood Kit method bar code is required for RNA purification on the Maxwell® CSC Instrument. 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 touch the **Proceed** button to continue.

   Note: When using the Maxwell® CSC 48 Instrument, touch the **Front** or **Back** button to select or deselect cartridge positions on each deck tray.

7. Instrument Run (continued)

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. Confirm that all of the indicated preprocessing has been performed, and touch **Start** to close the instrument door and begin processing.

   Note: When using a 48-position Maxwell® Instrument, if the Vision System has been enabled, the deck trays will be scanned as the door retracts. Any errors in deck tray setup (e.g., plungers not in well #8, elution tubes not present and open) will cause the software to return to the 'Cartridge Setup' screen and problem positions will be marked with an exclamation point in a red circle. Touch the exclamation point for a description of the error and resolve all error states. Touch the **Start** button again to repeat deck tray scanning and begin the extraction run.

   Warning: Pinch point hazard.

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 indicating that the method has ended.

End of Run

9. Follow the on-screen instructions at the end of the method to open the door. Verify that the 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 for your Maxwell® Instrument (see Table 1) to perform a **Clean Up** process to attempt to unload the plungers.

10. Cap and remove the Elution Tubes containing RNA immediately following the run to prevent evaporation of the eluates. Remove the Maxwell® deck tray(s) from the instrument.

    Note: To remove the deck tray from the instrument platform, hold the deck tray by its sides. RNA samples may be stored overnight at −30°C to −10°C, or at lower than −60°C for longer term storage.

    Ensure that the 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) and discard as hazardous waste according to your institution's procedures. Cartridges, plungers and elution tubes are intended for single use only. Do not reuse Maxwell® CSC Cartridges, CSC/RSC Plungers or Elution Tubes.

8. Post-Purification

Determine that the purified RNA sample yield and purity meet the input requirements for the downstream diagnostic assay prior to use in that assay.

9. Analytical Performance Evaluation

Analytical performance of the Maxwell® CSC RNA Blood Kit was evaluated using human whole blood specimens on the Maxwell® CSC Instrument. Equivalent performance of the Maxwell® CSC RNA Blood Kit with the Maxwell® CSC 48 Instrument was demonstrated as part of development of the instrument.

9.A. RNA Quantity and Quality

Table 2. RNA Extracted from Replicate Specimens of Shipped Whole Blood. RNA was extracted from eight replicate 2.5ml specimens of shipped whole blood and eluted in 50µl. Absorbance of purified RNA was measured at 230nm, 260nm, 280nm and 340nm. RNA concentration was determined using absorbance at 260nm after subtracting absorbance of the blank and correcting for instrument noise (absorbance at 340nm), and A_{260}/A_{280} and A_{260}/A_{230} ratios were calculated to assess RNA quality. The Maxwell® CSC RNA Blood Kit yielded RNA concentrations averaging 127.54ng/µl with an average A_{260}/A_{280} ratio of 2.12 and an average A_{260}/A_{230} ratio of 2.10.

Replicate	RNA Concentration (ng/µl)	A_{260}/A_{280} Ratio	A_{260}/A_{230} Ratio
1	118.22	2.11	2.01*
2	137.85	2.12	2.08
3	107.23	2.12	2.09
4	133.07	2.12	2.09
5	137.18	2.12	2.11
6	93.90	2.12	2.09
7	145.42	2.13	2.12
8	147.45	2.13	2.12
Average	**127.54**	**2.12**	**2.10**

*Dixon's Outlier Test allowed exclusion of one replicate in this set as an outlier at the 95% confidence threshold. This replicate was excluded from analysis.

9.B. RNA Amplifiability

Table 3. Assessing Amplifiability of RNA from Whole Blood. To assess the amplifiability of RNA extracted from whole blood using the Maxwell® CSC RNA Blood Kit and the Maxwell® CSC Instrument, each extracted RNA sample was amplified in duplicate using an RT-qPCR assay targeting a housekeeping gene, HPRT1 (hypoxanthine phosphoribosyltransferase 1). All RNA samples amplified within the linear range of the assay, with yields ranging from 117.84–182.79ng/µl and an average yield across all samples of 151.58ng/µl.

RNA Sample	RNA Concentration (ng/µl) Determined by RT-qPCR
1	144.70
2	136.90
3	117.84
4	151.06
5	173.55
6	124.25
7	182.79
8	181.55
Average	**151.58**

9.C. Reproducibility

Table 4. User-to User Variability in RNA Extraction from Whole Blood. To assess user-to-user variability, RNA was extracted from whole blood specimens by three different users using the Maxwell® CSC RNA Blood Kit and Maxwell® CSC Instrument. The extracted RNA was amplified in an RT-qPCR assay targeting the HPRT1 gene and RNA concentrations were calculated from the C_q values. Each sample set included eight replicates. The percent coefficient of variation (% CV) across the three user sample sets was 9.83.

User	Average Yield (ng/µl)	% CV
1 (n = 7)*	134.44	3.24
2 (n = 7)*	134.42	4.64
3 (n = 8)	122.95	15.13
% CV, Users 1, 2, 3		9.83

*Dixon's Outlier Test allowed exclusion of one replicate in this set as an outlier at the 95% confidence threshold. This replicate was excluded from analysis.

9.D. Inhibition (Interfering Substances)

Table 5. Testing for RNase Contamination and Inhibition of RNA Amplification due to Interfering Substances. RNA was extracted from eight replicate samples of the same whole blood specimen using the Maxwell® CSC RNA Blood Kit and Maxwell® CSC Instrument. A commercially available RNase detection assay was used to test for the presence of active RNase in the eluates. No detectable RNase activity was observed. The effect of interfering substances that copurify with RNA from whole blood was also assessed. Two sets of amplifications were assembled for each RNA eluate—one using 2µl of undiluted RNA per RT-qPCR for the HPRT1 target and a second set using 2µl of an eightfold dilution—and ΔC_q values were calculated. ΔC_q values ranged from a low of 2.256 cycles to a high of 3.116 cycles. All ΔC_q values were in the 3±1 cycles range expected for an eightfold dilution, confirming that any substances that copurified with RNA from whole blood had minimal effect on amplification.

Sample Number	C_q for Undiluted RNA (Cycles)	C_q for Eightfold RNA Dilution (Cycles)	ΔC_q (Cycles)
1	25.528	27.881	2.353
2	23.530	26.647	3.116
3	23.836	26.888	3.052
4	23.602	26.618	3.016
5	24.139	26.407	2.268
6	23.567	25.824	2.256
7	23.694	26.469	2.774
8	24.298	27.189	2.891

9.E. Cross Contamination

RNA was extracted from 8 replicates of a single whole blood specimen and 8 negative controls (water) using the Maxwell® CSC RNA Blood Kit and Maxwell® CSC Instrument. Maxwell® CSC cartridges containing whole blood samples or negative control (water) were processed in alternating deck positions in the Maxwell® CSC Instrument. The resulting RNA eluates were tested in duplicate by RT-qPCR targeting the HPRT1 to detect any RNA contamination in the negative controls from neighboring whole blood samples. No contaminating RNA was detected in the negative controls.

10. Clinical Performance Evaluation

Clinical performance of the Maxwell® CSC RNA Blood Kit was evaluated by an external clinical laboratory using human whole blood specimens and the Maxwell® CSC Instrument.

10.A.RNA Quantity, Quality and Amplifiability

Table 6. Method Comparison. RNA was extracted from 2.5ml samples from a total of 24 different whole blood specimens using the Maxwell® CSC RNA Blood Kit and Maxwell® CSC Instrument. RNA was extracted from the same specimens using the laboratory's standard nucleic acid purification method (Laboratory Reference Method) for comparison purposes. The extracted RNA was tested in RT-qPCR for BCR-ABL1 targeting the wild-type ABL1 (ABL proto-oncogene 1) transcript according to the clinical laboratory's standard procedures and yield was determined as the number of ABL1 copies detected. RNA eluates from the same whole blood specimen were tested in the same RT-qPCR assay to minimize the effects of assay variability on the results. RNA extracted using the Maxwell® CSC RNA Blood Kit yielded ≥10,000 ABL1 copies in the RT-qPCR assay. Yields obtained using RNA extracted from the same blood specimen were concordant for RNA extracted using the Maxwell® CSC RNA Blood Kit and the Laboratory Reference Method.

	RNA Yield (ABL1 copies)		Average C_q		ΔC_q (Maxwell® CSC–
Blood Sample	Maxwell® CSC System	Laboratory Reference Method	Maxwell® CSC System	Laboratory Reference Method	Laboratory Reference Method)
1	69,083.3	40,833.2	22.06	22.86	–0.80
2	223,597.5	80,162.6	20.29	21.84	–1.55
3	124,624.0	45,716.7	21.17	22.69	–1.52
4	108,277.1	46,241.3	21.39	22.71	–1.32
5	121,341.0	64,340.2	21.21	22.17	–0.96
6	83,351.0	52,905.5	21.78	22.46	–0.68
7	143,918.0	61,427.5	20.95	22.24	–1.29
8	118,185.0	50,284.7	21.25	22.54	–1.29
9	58,022.8	40,434.3	22.76	23.30	–0.54
10	101,240.1	45,860.7	21.94	23.11	–1.17
11	59,266.0	44,276.5	22.74	23.16	–0.42
12	71,620.9	50,254.9	22.45	22.98	–0.53
13	92,923.4	60,768.8	22.07	22.69	–0.62
14	62,285.3	47,983.2	22.66	23.04	–0.38
15	81,094.3	50,554.7	22.27	22.97	–0.70
16	88,203.5	56,790.2	22.14	22.79	–0.65
17	35,307.2	29,342.7	22.85	23.11	–0.26
18	30,544.4	30,890.3	23.08	23.04	0.04
19	35,016.3	37,702.2	22.86	22.74	0.12
20	26,850.8	29,618.1	23.25	23.10	0.16
21	26,792.4	34,277.7	23.25	22.88	0.37
22	35,179.1	30,662.9	22.84	23.05	–0.20
23	47,123.3	40,675.5	22.41	22.63	–0.22
24	50,146.0	30,248.6	22.31	23.07	–0.75

10.B. Reproducibility

Table 7. Reproducibility of RNA Extraction by Different Testers. To confirm consistency of results between users in the typical user environment, RNA was extracted from eight different whole blood specimens by two separate testers using the Maxwell® CSC RNA Blood Kit and Maxwell® CSC Instrument. The resulting RNA eluates were amplified using an RT-qPCR assay for BCR-ABL1 targeting the wild-type ABL1 transcript and the results obtained from each specimen were compared between the two testers. RNA extracted by both testers from all specimens were amplifiable and yielded ≥10,000 ABL1 copies, with an average C_q difference (ΔC_q) between testers of less than 1 cycle. The ΔC_q ranged from 0.03 to 1.25.

Blood Sample	RNA Yield (ABL1 copies)		Average C_q		ΔC_q Between Testers
	Tester 1	Tester 2	Tester 1	Tester 2	
1	69,083.3	68,042.8	22.06	22.09	0.03
2	223,597.5	102,244.2	20.29	21.47	1.18
3	124,624.0	68,351.6	21.17	22.08	0.91
4	108,277.1	48,271.9	21.39	22.60	1.21
5	121,341.0	133,669.5	21.21	21.53	0.32
6	83,351.0	64,549.4	21.78	22.60	0.82
7	143,918.0	84,824.5	20.95	22.20	1.25
8	118,185.0	83,599.3	21.25	22.22	0.97

10.C. Cross Contamination

Cross contamination occurring between samples during RNA extraction using the Maxwell® CSC RNA Blood Kit in the typical user environment was assessed. RNA extraction using the Maxwell® CSC RNA Blood Kit was performed with eight different whole blood specimens and eight negative controls (water) in the same instrument run. Maxwell® CSC cartridges containing whole blood specimens or negative controls were processed in alternating adjacent deck positions in the Maxwell® CSC Instrument. The resulting eluates were tested in duplicate by RT-qPCR targeting the wild-type ABL1 gene to determine if the negative control samples contained any contaminating RNA from the blood specimens. No contaminating RNA was detected in the negative controls, confirming that there was no detectable cross contamination during RNA extraction using the Maxwell® CSC system.

11. 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	Possible Causes and Comments
Lower than expected concentration of RNA in eluate (A typical sample should yield >50ng/µl of purified RNA.)	Blood sample WBC count was below or above the product's intended use range of 4×10^6 to 10×10^6 WBC/ml. The kit is optimized to purify RNA from blood samples with a WBC count of 4×10^6 to 10×10^6 WBC/ml.
	Incorrect volume of whole blood was used. Adding greater or less than 2.5ml of whole blood can result in lower yield.
	Blood sample was too old. Best yields are obtained with fresh blood samples. Samples that have been stored at 2–10°C for more than 3 days may result in reduced yields.
	Sample was stored below 2°C or above 10°C prior to purification. Improper storage temperatures can cause WBC lysis or RNA degradation.
	RNases may have been introduced during sample processing or quantification. See Section 12 for information on creating a ribonuclease-free environment.
	Inadequate supernatant removal following differential lysis. Ensure that the supernatant is removed as completely as possible.
	WBC pellet was dislodged during supernatant removal. Avoid touching the WBC pellet when removing the supernatant.
	Incorrect sample type. The kit is intended for use with whole human blood. Other sample types (e.g., bone marrow, plasma, buffy coat, etc.) have not been tested with this kit.
	Incorrect blood collection tube type. The kit is intended for use with whole human blood in EDTA collection tubes. Other tube types have not been tested with this kit and may not be compatible with the purification chemistry.

11. Troubleshooting (continued)

Symptoms	Possible Causes and Comments
Low RNA quality (Eluates should have an A_{260}/A_{280} ratio greater than 1.8 and an A_{260}/A_{230} ratio between 1.8 and 2.4.)	Blood sample WBC count was above the product's intended use range of 4×10^6 to 10×10^6 WBC/ml. The kit is optimized to purify RNA from blood samples with a WBC count of 4×10^6 to 10×10^6 WBC/ml.
	Incorrect volume of whole blood was used. Adding greater than 2.5ml of whole blood can result in poor eluate purity.
	Blood sample was too old. Best yields are obtained with fresh blood samples. Samples that have been stored at 2°C–10°C for more than 3 days may result in reduced RNA quality.
	Sample was stored below 2°C or above 10°C prior to purification. Improper storage temperatures can cause WBC lysis or RNA degradation.
	Inadequate supernatant removal following differential lysis. Ensure that the supernatant is removed as completely as possible.
	Incorrect sample type. The product is intended for use with whole human blood. Other sample types (e.g., bone marrow, plasma, buffy coat, etc.) have not been tested with this product.
High levels of DNA are present in eluates (The eluates are contaminated with DNA, which can interfere with downstream assays.)	Blood sample WBC count is above the product's intended use range of 4×10^6 to 10×10^6 WBC/ml.
	Incorrect volume of whole blood was used. Adding greater than 2.5ml of whole blood can lead to DNA contamination in eluates.
	DNase I was not added to cartridge. If possible, examine well #4 in the spent cartridges. Well #4 should appear green (not yellow) if DNase I was added to the cartridges in Section 6.B, Step 5.
Preprocessed lysate is too viscous to pipet	Blood sample WBC count was above the product's intended use range of 4×10^6 to 10×10^6 WBC/ml.
	Incorrect volume of whole blood was used. Adding greater than 2.5ml of whole blood can lead to lysates that are viscous and difficult to pipet.

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.

12. Creating a Ribonuclease-Free Environment

Ribonucleases are extremely difficult to inactivate. Take care to avoid introducing RNase activity into your RNA samples during and after isolation. This is especially important if the starting material was difficult to obtain or is irreplaceable. The following notes may help prevent accidental RNase contamination of your samples.

1. Two of the most common sources of RNase contamination are the user's hands and bacteria or molds that may be present on airborne dust particles. To prevent contamination from these sources, use sterile technique when handling the reagents supplied with this system. Wear gloves at all times. Change gloves whenever ribonucleases may have been contacted.
2. Whenever possible, sterile, disposable plasticware should be used for handling RNA. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
3. Treat non-sterile glassware, plasticware and electrophoresis chambers before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plasticware with 0.1N NaOH, 1mM EDTA, followed by RNase-free water. Commercially available RNase removal products also may be used, following the manufacturer's instructions.

   Note: Electrophoresis chambers may be contaminated with ribonucleases, particularly RNase A, from analysis of DNA samples. Whenever possible, set aside a new or decontaminated apparatus for RNA analysis only.
4. Treat solutions not supplied with the system by adding diethyl pyrocarbonate (DEPC) to 0.1%v/v in a fume hood. Incubate overnight with stirring at room temperature in the hood. Autoclave for 30 minutes to remove any trace of DEPC.

Caution: DEPC is a suspected carcinogen and should only be used in a chemical fume hood. DEPC reacts rapidly with amines and cannot be used to treat Tris buffers.

Note: For all downstream applications, it is essential that you continue to protect your RNA samples from RNases. Wear clean gloves and use RNase-free solutions and centrifuge tubes.

13. References

1. Clinical Laboratory Standards Institute (2007) Handling, transport, and storage of specimens for molecular methods. This can be viewed online at: **www.clsi.org**
2. Murray, P.R. *et al.* (2007) *Manual of Clinical Microbiology*, 9th Edition, ASM Press.

14. 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.

15. 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 9 and 10 were added and subsequent sections renumbered.
3. Document updated for compliance with Regulation (EU) 2017/746 on in vitro diagnostic medical devices.

[a]U.S. Pat. No. 7,329,488 and S. Korean Pat. No. 100483684.