TECHNICAL MANUAL

Maxwell® CSC Viral Total Nucleic Acid Purification Kit

Instructions for Use of Product
AS1780

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

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INSTRUCTIONS FOR USE OF PRODUCT
AS1780

IVD

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Promega

Maxwell® CSC Viral Total Nucleic Acid Purification 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 Viral Total Nucleic Acid Purification Kit[a] is used with the Maxwell® Instruments specified in Table 1 to provide an easy method for efficient, automated sample preparation and purification of viral total nucleic acid. Maxwell® CSC Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. The Maxwell® method for the CSC Viral Total Nucleic Acid Kit can process from one to the maximum number of Maxwell® Instrument samples in approximately 30 minutes. The low elution volume of 50µl results in concentrated purified nucleic acid for downstream applications such as quantitative PCR (qPCR) or quantitative RT-PCR (qRT-PCR). After brief initial lysis, the sample is added to the Maxwell® CSC Viral Total Nucleic Acid Purification Cartridge, and the remaining processing is fully automated.

Table 1. Supported Instruments

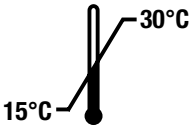
Instrument	Cat.#	Technical Manual
Maxwell® CSC	AS6000	TM457
Maxwell® CSC 48	AS8000	TM623

The Maxwell® CSC Viral Total Nucleic Acid Purification Kit purifies samples using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of nucleic acid. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind nucleic acids to the paramagnetic particle in the first well of a prefilled cartridge. The samples are processed through a series of washes before the total nucleic acid is eluted.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® CSC Viral Total Nucleic Acid Purification Kit	**48 preps**	**AS1780**

For In Vitro Diagnostic Use. Professional use only. Sufficient for 48 isolations. Cartridges are for single use only.



Includes:

- 20ml Lysis Buffer
- 2 × 1ml Proteinase K (PK) Solution
- 50 CSC/RSC Plungers
- 48 Cartridges
- 50 Elution Tubes (0.5ml)
- 25ml Nuclease-Free Water

Storage Conditions: Store components at room temperature (15–30°C).

Safety Information: The cartridges contain ethanol, isopropanol and guanidine hydrochloride. Ethanol and isopropanol should be considered flammable, harmful and irritants. Guanidine hydrochloride should be considered toxic, harmful and an irritant. Refer to the SDS for detailed safety information.

Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) 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; edges may be sharp.

Additional Information: The Maxwell® CSC Viral Total Nucleic Acid Purification 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. For additional safety information, see the Safety Data Sheet, available at: **www.promega.com**

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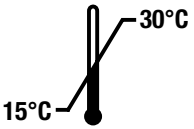
Symbols Key

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

3. Product Intended Use

The Maxwell® CSC Viral Total Nucleic Acid Purification Kit is intended for use, in combination with Maxwell® CSC Instruments and the Maxwell® CSC Viral Total Nucleic Acid purification method, as an in vitro diagnostic (IVD) medical device to perform automated isolation of total viral nucleic acid from human samples. The purified nucleic acid is suitable for use in amplification-based in vitro diagnostic assays.

The Maxwell® CSC Viral Total Nucleic Acid Purification Kit is intended for professional use only. Diagnostic results obtained using the nucleic acid purified with this system must be interpreted in conjunction with other clinical or laboratory data.

4. Product Use Limitations

The Maxwell® CSC Viral Total Nucleic Acid Purification 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.

This Maxwell® CSC Viral Total Nucleic Acid Purification Kit has been validated with serum, plasma and nasopharyngeal swabs in Universal Transport Medium for Virus (UTM). The user is responsible for validating its use to extract viral nucleic acid from other sample types. Compatibility of the system with stabilized saliva has been demonstrated during development.

Appropriate controls must be included in any downstream diagnostic applications using nucleic acid purified using the Maxwell® CSC Viral Total Nucleic Acid Purification System. The user is responsible for validating the performance characteristics necessary for downstream diagnostic applications.

Users may choose to add exogenous internal controls (IC) to the sample or lysate. Certain nucleic acid internal controls smaller than 100bp may not be efficiently purified using the system.

5. Sample Preparation

Materials to Be Supplied By the User

- tubes for plasma, serum, UTM or stabilized saliva samples

Blood-borne pathogen precautions are recommended when handling any human-derived specimens.

For plasma samples, collect blood in EDTA- or ACD-anticoagulant Vacutainer® tubes. Avoid heparin as it may inhibit downstream amplifications.

The following general recommendations are for preparing and storing samples (1–3):

1. Separate plasma from cells within 1 hour of drawing blood by centrifuging at 1,500 × *g* for 20 minutes at 25°C, and then transfer plasma layer into a clean tube.
2. Separate serum from clotted blood by centrifuging at 1,000 × *g* for 10 minutes at 25°C, and then decant into a clean tube.
3. For swabs in UTM, use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 2–3ml of viral transport medium.

Store plasma and serum samples at 2–8°C for up to 24 hours, or freeze samples that are not processed within 24 hours at –20°C for up to 5 days. Store UTM and stabilized saliva samples at 2–8°C for up to 72 hours, or freeze samples at –70°C. Avoid repeated freeze-thaw cycles, and do not store samples in a frost-free freezer. Specific collection and storage conditions may vary, depending on the virus isolated.

6. Before You Begin

Materials to Be Supplied by the User

- 1.5–2.0ml tubes for incubation of samples (e.g., ClickFit Microtube, 1.5ml [Cat.# V4741]; recommended to prevent the cap from opening during heating)
- 15ml or 50ml conical tube for preparation of Lysis Solution
- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- heating block or water bath set to 56°C

6.A. Lysis Solution Preparation

If the Lysis Buffer is cloudy or contains precipitates, heat at 37–56°C until the Lysis Buffer clears.

Prepare fresh Lysis Solution for each batch of samples as described in Table 2. Invert tube to mix.

Table 2. Preparing Lysis Solution.

For 100µl and 200µl of plasma or serum samples, or 200µl of UTM or stabilized saliva samples:

Reagent	Amount/Reactions	Reactions (Number to be run + 2	Total
Lysis Buffer[1]	200µl	n + 2	200µl × (n + 2)
Proteinase K Solution	20µl	n + 2	20µl × (n + 2)

For 300µl of plasma or serum samples:

Reagent	Amount/Reactions	Reactions (Number to be run + 2)	Total
Lysis Buffer[1]	300µl	n + 2	300µl × (n + 2)
Proteinase K Solution	30µl	n + 2	30 µl × (n + 2)

[1]If an internal control is used, it may be added to the Lysis Solution. Internal controls are not provided in this kit.

Note: Some respiratory viruses from sample types such as nasophyrangeal swabs may not require the use of Proteinase K.

6.B. Sample Preparation for Maxwell® Viral Total Nucleic Acid Purification Cartridges

Samples may be fresh or frozen. Thaw frozen specimens at room temperature or on ice, and mix by vortexing for 10 seconds before use.

1. Pipet each plasma or serum sample or 200µl of UTM or stabilized saliva into a 1.5ml or 2ml microcentrifuge tube with a cap.
2. Add Lysis Solution prepared in Section 6.A.
   a. For sample volumes of 100µl or 200µl, add 220µl of Lysis Solution.
   b. For sample volume of 300µl, add 330µl of Lysis Solution.
3. Close tubes, and vortex for 10 seconds.
4. For serum samples, incubate at room temperature (15–30°C) for 10 minutes, and then proceed to Step 5.
5. Incubate at 56°C in a heat block or water bath for 10 minutes. During this incubation, proceed to Section 6.C to prepare the cartridges.

   Note: Some viruses, such as hepatitis B virus, may require incubation at 80°C for optimal nucleic acid recovery. due to secondary structure of the viral genome.

6.C. Maxwell® CSC Viral Total Nucleic Acid Purification Cartridge Preparation

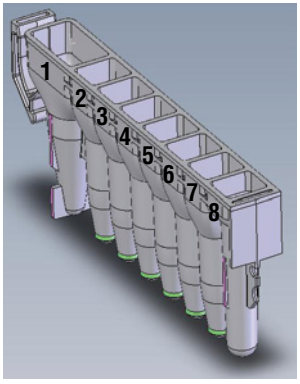
1. Change gloves before handling Cartridges, Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
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).

6.C. Maxwell® CSC Viral Total Nucleic Acid Purification Cartridge Preparation (continued)

4. Add 50µl of Nuclease-Free Water to the bottom of each elution tube.
5. Pulse samples in a microcentrifuge to collect liquid at the bottom of the tube. Transfer sample lysate to well #1 (the largest well) of the cartridge.
6. Proceed to Section 7, Maxwell® Instrument Setup and Run.

Notes:

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



User Adds to Wells
1. Sample lysates
8. CSC/RSC Plunger

Figure 1. Maxwell® Viral Total Nucleic Acid Purification Cartridge. Preprocessed sample is added to well #1, and a plunger is added to well #8.

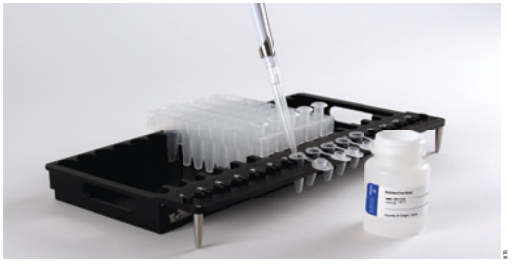


Figure 2. Setup and configuration of the deck tray(s). Nuclease-Free Water is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

7. Maxwell® Instrument Setup and Run

For detailed information, refer to the Technical Manual specific to your Maxwell® Instrument (see Table 1).

1. Turn on the Maxwell® Instrument and Tablet PC. Sign in to the Tablet PC, and start the Maxwell® IVD mode software by double-touching the icon on the desktop. The instrument will power up, proceed through a self test and home all moving parts.
2. Touch **Start** to begin the process of running a method.
3. Scan or enter the method bar code in the upper right corner of the Maxwell® CSC Viral Total Nucleic Acid Purification Kit label to automatically select the method to be run (Figure 3).

   Note: The Maxwell® CSC Viral Total Nucleic Acid Kit bar code is required for purification on the Maxwell® CSC Instruments. The kit label contains two bar codes. The method bar code is indicated in Figure 3 below. If the bar code cannot be scanned, contact Promega Technical Services.

Figure 3. Kit label indicating the method bar code to scan. The bar code to scan for starting a purification run is shown in the red box, on the upper right of the kit label.

4. On the 'Cartridge Setup' screen, touch the cartridge positions to select/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 48-position Maxwell® Instruments, press the **Front** and **Back** buttons to select/deselect cartridge positions on each deck tray.

7. Maxwell® Instrument Setup and Run (continued)

5. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with Nuclease-Free Water and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto 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 the 24-position Maxwell® deck tray(s) to determine whether they should be placed in the front or back of the instrument.

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

   Note: When using a 48-position Maxwell® Instrument, if the Vision System has been enabled, the deck tray(s) 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.

   The Maxwell® Instrument will immediately begin the purification run. The screen will display information including the user who started the run, the current method step being 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**. The samples will be lost. Do not attempt to repurify samples if an instrument run has been aborted.

7. Follow on-screen instructions at the end of the method to open the 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 Technical Manual appropriate to your Maxwell® Instrument (Table 1) to perform a **Clean Up** process to attempt to unload the plungers.

8. Remove the deck tray(s) from the instrument. Remove elution tubes containing viral total nucleic acid, and cap the tubes. If paramagnetic particles are present in the elution tubes, centrifuge at 10,000–20,000 × *g* for 30 seconds to 1 minute. After the run is complete, the extraction run report will be displayed. From the 'Report View' screen, you can print or export this report or both.

9. Remove the cartridges and plungers from the deck tray(s), and discard as hazardous waste following your institution's recommended guidelines. Do not reuse reagent cartridges, plungers or elution tubes.

   Note: Ensure samples are removed before performing any required UV light treatment to avoid damage to the nucleic acid.

8. Storing Eluted Nucleic Acid

If samples are not processed immediately, store eluted viral DNA on ice or at 4°C for up to 24 hours. For longer term storage, freeze at –20°C or –70°C. Viral RNA is less stable and preferably tested in downstream assays immediately after isolation. Alternatively, store eluted viral RNA at –70°C. Consult the instructions for downstream applications for specific sample storage and handling recommendations.

9. 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.
3. Centers for Disease Control and Prevention. (2020, March 25). *Coronavirus Disease 2019 (COVID-19): Guidelines for Clinical Specimens*. Retrieved March 30, 2020, from **www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html**

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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 viral nucleic acid recovery than expected (e.g., for customer-provided internal controls)	The starting samples were compromised. Ensure that samples were collected, shipped and stored according to recommended guidelines.
	For RNA viral samples, ensure RNase-free conditions are used for sample preparation and assay setup, including RNase-free tubes and pipette tips.
	Processing step was not optimal. • Prepare Lysis Buffer and Proteinase K immediately before use, and discard unused solutions following your institution's recommended guidelines. • Use only the Lysis Buffer provided with this kit. • Incomplete mixing may reduce lysis. Vortex sample with Lysis Solution as recommended. • Incomplete protease treatment to remove viral capsids. Check the heat block or water bath temperature, and incubate for the full time recommended. • Incubation for 10 minutes at room temperature before the 56°C incubation may improve recovery for some plasma samples. • Some viruses may need higher incubation temperatures. • Adding more sample than recommended may reduce nucleic acid recovery.
Lower viral nucleic acid recovery than expected (e.g., for customer-provided internal controls)	Check that a plunger was added to the cartridge.
	Ensure that all cartridges are snapped into the deck tray properly before processing.
	Post-purification storage issues. • Remove eluates, and store at the recommended temperature immediately after the Maxwell® Instrument run. • Do not subject eluates to multiple freeze-thaw cycles before downstream assays.
	Nucleic acid internal controls smaller than 100bp may not be efficiently purified using the system. The user is responsible for establishing performance of any internal control.

Symptoms	Causes and Comments
Poor amplification	Paramagnetic particle carryover may cause interference in amplification reactions. Remove particles in elution tube by centrifugation.
	Wrong elution buffer was added. Use only the Nuclease-Free Water supplied with the Maxwell® CSC Viral Total Nucleic Acid Purification Kit.
Cross-contamination	Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
	Avoid splashing when adding lysates to cartridges. Cartridges may be removed from the deck tray for sample addition to minimize contamination of adjacent cartridges.
Instrument unable to pick up plungers	Make sure you are using a CSC-specific chemistry kit; the plungers for the Maxwell® CSC reagent kits are specific to the supported Maxwell® Instruments for this kit.

11. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® CSC 48 Instrument	1 each	AS8000
Maxwell® RSC/CSC Plungers, 50pk	1 each	AS1331
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
ClickFit Microtube, 1.5ml	1,000/pack	V4741

Maxwell® CSC Reagent Kits

For a list of available Maxwell® CSC purification kits, visit: **www.promega.com**

12. Summary of Changes

The following changes were made to the 5/21 revision of this document:

1. Updated for compliance with EU Directive 98/79/EC.
2. Added instructions for stabilized saliva samples.
3. Replaced the cover image.
4. Updated disclaimers.

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