Maxwell® 16
INSTRUMENT

Maxwell® 16 Viral Total Nucleic Acid Purification System

INSTRUCTIONS FOR USE OF PRODUCT AS1155.

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

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CE

INSTRUCTIONS FOR USE OF PRODUCT
AS1155

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Promega

Maxwell® 16 Viral Total Nucleic Acid Purification System

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

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This product meets the essential requirements of EU Directive 98/79/EC on in vitro diagnostic medical devices. The Maxwell® 16 Viral Total Nucleic Acid Purification System is intended for use in the following countries only: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

1. Intended Use

The Maxwell® 16 System, which consists of the Maxwell® 16 Viral Total Nucleic Acid Purification System[a] and Maxwell® 16 Instrument configured with the low elution volume (LEV) hardware, is used for automated viral total nucleic acid purification from 1 to 16 human plasma or serum samples. Purified total nucleic acid is eluted in 50µl of elution buffer and is suitable for use in direct, downstream analysis by standard amplification methods. These methods include a variety of polymerase chain reaction (PCR) or reverse-transcriptase polymerase chain reaction (RT-PCR) tests for human in vitro diagnostic purposes. The Maxwell® 16 System is not intended for use as part of a specific in vitro diagnostic test.

1. Intended Use (continued)

The Maxwell® 16 Viral Total Nucleic Acid Purification System is for professional use only. Diagnostic results obtained using total nucleic acid purified with this system must be interpreted in conjunction with other clinical or laboratory data.

2. Product Use Limitations

The Maxwell® 16 Viral Total Nucleic Acid Purification System is not intended for use with clinical samples from whole blood, tissue or body fluids other than human serum and plasma. It is not intended for use with nonhuman samples or for the isolation of nucleic acid from other nonviral organisms.

Maxwell® 16 Viral Total Nucleic Acid Purification System performance was evaluated using 300µl of human serum or plasma using model phage RNA and DNA viruses and inactivated hepatitis C virus (HCV) and cytomegalovirus (CMV) samples.

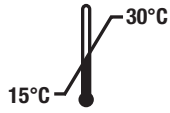
The user is responsible for validating the performance characteristics necessary for downstream diagnostic applications. Users may choose to add exogenous internal controls (IC) to the sample or lysate. Certain nucleic acid internal controls smaller than 100bp may not be efficiently purified using the system. The user is responsible for establishing performance of any IC. Appropriate controls must be included in any downstream diagnostic applications using nucleic acid purified using the Maxwell® 16 Viral Total Nucleic Acid Purification System.

Compliance with EU Directive 98/79/EC on in vitro diagnostic medical devices has been demonstrated for, and only applies to, use of the Maxwell® 16 IVD Instrument (Cat.# AS3050) in the clinical mode with the Maxwell® 16 Viral Total Nucleic Acid Purification System (Cat.# AS1155).

3. Product Components, Storage Conditions and Symbol Key

Product	Size	Cat.#
Maxwell® 16 Viral Total Nucleic Acid Purification System	48 preps	AS1155

Each system contains sufficient reagents for 48 purifications.



Includes:

- 48 Maxwell® 16 LEV Cartridges (MCC)
- 20ml Lysis Buffer
- 2 × 1ml Proteinase K (PK) Solution
- 20ml Nuclease-Free Water
- 50 LEV Plungers
- 50 Elution Tubes (0.5ml)

Storage Conditions: Store components at room temperature (15–30°C). See expiration date on the product label. Do not use product after the expiration date.

Safety Information: The Maxwell® 16 LEV Cartridges contain ethanol and isopropanol, which are flammable, and guanidine hydrochloride and urea, which are irritants and toxic. Wear gloves, and follow standard safety procedures while working with these substances.

For additional safety information, see the Material Safety Data Sheet, available at: **www.promega.com**

The Maxwell® 16 LEV Cartridges are designed for use with potentially infectious substances. Users should wear appropriate protection (e.g., gloves 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: Due to the toxicity of the chemicals used in the purification procedure and the prevalence of RNases, we recommend that gloves be worn during sample and cartridge preparation.

EC REP

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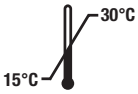
Symbol Key

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

4. Maxwell® 16 Instrument Hardware and Firmware Setup

To use the Maxwell® 16 Viral Total Nucleic Acid Purification System, the Maxwell® 16 IVD Instrument must be configured with LEV hardware. If your Maxwell® 16 Instrument contains SEV (standard elution volume) hardware, it will need to be reconfigured using the Maxwell® 16 LEV Hardware Kit (Cat.# AS1250). Reconfiguring the instrument is simple and easy. Refer to the *Maxwell® 16 IVD Instrument Technical Manual* #TM315 for directions.

5. Collection and Storage of Samples Before Purification

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

Collect blood in EDTA- or ACD-anticoagulated Vacutainer® tubes. Avoid heparin as it may inhibit downstream amplifications.

The following general recommendations are for preparing and storing plasma and serum samples (1,2). Separate plasma from cells within 1 hour of drawing blood by centrifuging at 1,500 × *g* for 20 minutes at 25°C, then decant into a clean tube. Separate serum from clotted blood by centrifuging at 1,000 × *g* for 10 minutes at 25°C, then decant into a clean tube. 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. 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. This information may be obtained from the technical information for the downstream assay system. RNA viruses are susceptible to degradation at any step during sample collection and processing. Provide an RNase-free environment during all steps of the procedure.

6. Purification of Viral Total Nucleic Acid from Plasma or Serum

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.

The isolation process includes sample lysis in the presence of Lysis Buffer and Proteinase K at 56°C in a heat block or water bath. This treatment removes the viral protein coat and inactivates RNases in the sample. Samples then are transferred to the sample well of the Maxwell® 16 LEV Cartridge, and the remaining processing is totally automated. Paramagnetic particles are mixed with the sample for optimal nucleic acid binding and subsequently washed in various buffers. Elution is performed in Nuclease-Free Water.

Materials to Be Supplied by the User

- 1.5ml or 2ml microcentrifuge tubes, nuclease-free
- tube for Lysis Solution
- heat block or water bath set to 56°C
- 0.5ml tubes, nuclease-free
- RNase-free, sterile, aerosol-resistant pipette tips

6.A. Preparation of Lysis Solution

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 1. We recommend preparing approximately 20% extra Lysis Solution to compensate for potential pipetting losses.

Table 1. Preparation of Lysis Solution for 300µl Plasma or Serum Samples.

Reagent	Volume for One Sample	Volume for 16 Samples[1]
Lysis Buffer[2]	300µl	5,700µl
Proteinase K Solution	30µl	570µl

[1]The volumes listed for Lysis Buffer and Proteinase K Solution for 16 samples include approximately 20% extra volume.

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

6.B. Preparation of Samples for Maxwell® 16 LEV Cartridges

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

Label microcentrifuge tubes used for sample lysis. The bar code labeling option may be used.

1. Pipet each plasma or serum sample into a 1.5ml or 2ml microcentrifuge tube with a cap.
2. Add Lysis Solution prepared in Section 6.A.. To 300µl samples, add 330µl of Lysis Solution.
3. Close tubes, and vortex for 10 seconds.
4. For plasma samples proceed to Step 5.

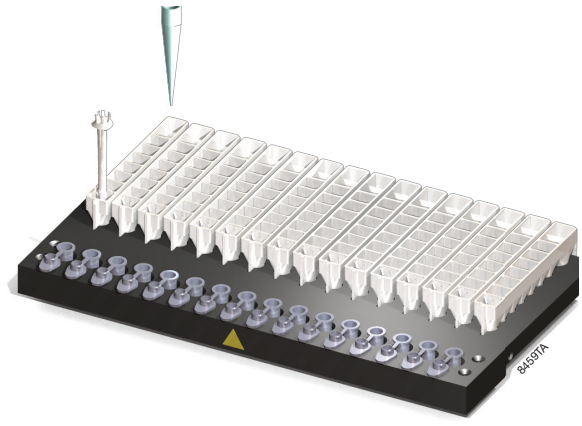
   For serum samples incubate at room temperature (15-30°C) for 10 minutes, then proceed to Step 5.
5. Incubate at 56°C in a heat block or water bath for 10 minutes. During this incubation, proceed to Step 6 to prepare the cartridges.

   Note: Samples containing virus such as hepatitis B virus require incubation at 80°C for optimal nucleic acid recovery due to secondary structure of the viral genome.
6. Change gloves before handling cartridges, LEV Plungers and Elution Tubes. Place the cartridges to be used in the Maxwell® 16 LEV Cartridge Rack (Cat.# AS1251). Place each cartridge in the rack with the label side facing away from the Elution Tube. 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 the cartridges in the instrument.

Notes:

1. If you are processing fewer than 16 samples, center the cartridges on the platform.
2. Specimen or reagent spills on any part of the Maxwell® 16 LEV Cartridge Rack should be cleaned with a detergent-water solution, followed by a bacteriocidal spray or wipe, then water. Do not use bleach on any instrument parts.

7. Place an LEV Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.



8. Place 0.5ml Elution Tubes in the front of the Maxwell® 16 LEV Cartridge Rack. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube.

   Notes:

   1. If Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
   2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may not work with the Maxwell® 16 Instrument.

9. Transfer sample lysate to well #1 of the cartridge. Well #1 is the well closest to the cartridge label and furthest from the Elution Tube.

6.C. Maxwell® 16 IVD Instrument Run

Refer to the *Maxwell® 16 IVD Instrument Technical Manual* #TM315 for detailed information about setting up and running the Maxwell® 16 IVD Instrument.

1. Turn on the Maxwell® 16 IVD Instrument. The instrument will power up, display the firmware version number, proceed through a self-check and home all moving parts.
2. Verify that the Home screen indicates "LEV" and the LEV hardware is present. Press "Run/Stop" to continue.
3. Enter user and PIN, if this option is enabled.
4. At the Protocols screen, select "Viral".
5. On the next screen, verify that the correct method and user were chosen. Select "Run/Stop" to continue.
6. Open the door when prompted on the screen, then select "Run/Stop".

Warning: Pinch point hazard.

7. Follow on-screen instructions for bar code reader input if this option is enabled.

8. Transfer the Maxwell® 16 LEV Cartridge Rack containing the prepared cartridges on the Maxwell® 16 IVD Instrument platform. Ensure that the rack is placed in the Maxwell® 16 IVD Instrument with the Elution Tubes closest to the door. The rack will only fit in the instrument in this orientation. If you have difficulty fitting the rack on the platform, check that the rack is in the correct orientation. Ensure the rack is level on the instrument platform.

   Note: Hold the Maxwell® 16 LEV Cartridge Rack by the sides to avoid dislodging cartridges from the rack.

9. Verify that samples were added to well #1 of the cartridges, cartridges in the rack are loaded on the instrument, Elution Tubes are present with 50µl of Nuclease-Free Water and LEV Plungers are in well #8.
10. Select "Run/Stop" at the LEV Set Up screen. The platform will retract. Close the door.

Warning: Pinch point hazard.

11. The Maxwell® 16 IVD Instrument will immediately begin the purification run. The screen will display the approximate time remaining in the run.

    Notes:

    1. Pressing the Run/Stop button or opening the door will pause the run.
    2. If the run is abandoned 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.

12. When the automated purification run is complete, follow instructions on the screen for data transfer. For detailed instructions, consult the *Maxwell® 16 IVD Instrument Technical Manual* #TM315 and *Maxwell® Sample Track Software Technical Manual* #TM314.

13. 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 were not removed from the magnetic plunger bar, push them down gently by hand to remove them.

14. Press "Run/Stop" to extend the platform out of the instrument.

Warning: Pinch point hazard.

15. Remove the Maxwell® 16 LEV Cartridge Rack from the instrument. Remove Elution Tubes containing viral nucleic acid, and close the tubes.

16. Centrifuge the elution tubes at 10,000 x *g* for 2 minutes. Transfer the supernatant to a clean tube (not provided). Avoid transferring paramagnetic particles and any floating debris.

17. Remove the cartridges and plungers from the Maxwell® 16 LEV Cartridge Rack, and discard as hazardous waste. Do not reuse reagent cartridges, LEV Plungers or Elution Tubes.

Ensure samples are removed from the Maxwell® 16 IVD Instrument before UV light treatment to avoid damage to the nucleic acid.

7. Storing Eluted Nucleic Acid

If samples are not processed immediately, store the 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.

8. 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.

9. 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.
	The Maxwell® 16 Instrument was set for the wrong method. Ensure that the correct Viral method is chosen.
	Processing step was not optimal. • Prepare Lysis Buffer and Proteinase K immediately before use, and discard unused solutions. • 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. • Some viruses may need higher incubation temperatures. • Adding more sample than recommended may reduce nucleic acid recovery.
	Check that an LEV Plunger was added to the cartridge.
	Ensure that all cartridges are snapped into the rack properly before processing.
	Post-purification storage issues. • Remove eluates, and store at the recommended temperature immediately after the Maxwell® 16 IVD Instrument run. • Do not subject eluates to multiple freeze-thaw cycles before downstream assays.
	Nucleic acid internal controls smaller the 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 reaction. Remove particles in Elution Tube by centrifugation.
	Wrong elution buffer was added. Use only the Nuclease-Free Water supplied with the Maxwell® 16 Viral Total Nucleic Acid Purification System.
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 rack for sample addition to minimize contamination of adjacent cartridges.
Viral method not an option on the instrument	For the Maxwell 16 MDx Instrument (Cat.# AS3050), verify that the instrument is in LEV mode.
Power failure during instrument run	To recover samples after a power failure, first ensure that the particles are in one of the wells of the cartridge and are not attached to the plunger. If the power failure occurred at a point where the magnetic particles were captured on the outside of the plungers, manually move the plungers up and down in the wells to wash the particles off, then manually remove the plungers from the instrument and restart the purification from the beginning with new plungers.

[a] U.S. Pat. Nos. 7,329,488, 7,721,947 and 7,891,549 and other patents pending.