

TECHNICAL BULLETIN

Maxwell[®] 16 Viral Total Nucleic Acid Purification Kit

Instructions for Use of Products
AS1150 and ASB1150

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



Maxwell[®] 16 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 Bulletin.
 E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The Maxwell[®] 16 Viral Total Nucleic Acid Purification Kit^(a,b) is used with the Maxwell[®] 16 Instrument (Cat.# AS2000 or AS3000) configured with low elution volume (LEV) hardware. This total nucleic acid purification procedure is an easy method for efficient, automated purification of viral total nucleic acid from 1 to 16 samples. The low elution volume of 50µl results in more concentrated purified nucleic acid for downstream applications such as quantitative PCR (qPCR) or quantitative RT-PCR (qRT-PCR). The Maxwell[®] 16 Instrument is supplied with preprogrammed purification procedures and uses prefilled reagent cartridges, maximizing simplicity and convenience. After brief initial lysis the sample is added to the Maxwell[®] 16 cartridge, and the remaining processing is fully automated. The Maxwell[®] 16 Instrument purifies high-quality viral total nucleic acid from 100µl, 200µl or 300µl of plasma or serum.



2. Product Use Limitations

Performance of this total nucleic acid purification system was evaluated using model phage RNA and DNA viruses, inactivated hepatitis C virus (HCV), inactivated hepatitis B virus (HBV) and inactivated cytomegalovirus (CMV) samples. This system is intended for use with human plasma or serum. This system is not intended for use with any specific virus. Detection limits depend on viral titer and downstream assay systems and may vary between viral types.

The user is responsible for validating the performance of purified nucleic acid in downstream 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.

3. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® 16 Viral Total Nucleic Acid Purification Kit	48 preps	ASB1150

For Laboratory Use. 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)

PRODUCT	SIZE	CAT.#
Maxwell® 16 Viral Total Nucleic Acid Multi-Pack Kit	144 preps	ASB1150

For Laboratory Use. Each system contains sufficient reagents for 48 purifications. Includes:

- 144 Maxwell® 16 LEV Cartridges (MCC)
- 3 × 20ml Lysis Buffer
- 6 × 1ml Proteinase K (PK) Solution
- 3 × 20ml Nuclease-Free Water
- 3 × 50 LEV Plungers
- 3 × 50 Elution Tubes (0.5ml)

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

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.

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 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.

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

4. Maxwell® 16 Instrument Hardware and Firmware Setup

To use the Maxwell® 16 Viral Total Nucleic Acid Purification Kit, the Maxwell® 16 Instrument must be configured with LEV hardware. If your Maxwell® 16 Instrument contains standard elution volume (SEV) 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 Instrument Technical Manual specific for your instrument 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 $1,500 \times g$ for 20 minutes at 25°C, then decant into a clean tube. Separate serum from clotted blood by centrifuging at $1,000 \times g$ 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.

6. Purification of Total Viral 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
- 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 100µl and 200µl plasma or serum samples

Reagent	Volume for One Sample	Volume for 16 Samples¹
Lysis Buffer ²	200µl	3,800µl
Proteinase K Solution	20µl	380µl

For 300µl plasma or serum samples

Reagent	Volume for One Sample	Volume for 16 Samples¹
Lysis Buffer ²	300µl	5,700µl
Proteinase K Solution	30µl	570µl

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

²If 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.

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 100µl or 200µl samples, add 220µl of Lysis Solution.
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 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.

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.



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

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.
3. The elution volume may require optimization for downstream applications. The recommended elution volume for the Maxwell® 16 Viral Total Nucleic Acid Purification Kit is 50µl of Nuclease-Free Water.



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. Instrument Run: AS2000 and AS3000 Instruments

Setup for AS2000 Maxwell® 16 Instruments

Refer to the *Maxwell® 16 Instrument Operating Manual #TM295* for more detailed information.

To run the “Viral” protocol, you must have Maxwell® 16 firmware version 4.61 or higher installed on your instrument.

1. Turn on the Maxwell® 16 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 instrument settings indicate an “LEV” hardware configuration and “Rsch” operational mode setting.
3. Select “Run” on the Menu screen, and press the Run/Stop button to start the method.
4. Select “Viral” on the Menu screen, then select “OK” at the Verification screen.
5. Open the door when prompted to do so on the screen. Press the Run/Stop button to extend the platform.



Warning: Pinch point hazard.

6. Transfer the Maxwell® 16 LEV Cartridge Rack containing the prepared cartridges on the Maxwell® 16 Instrument platform. Ensure that the rack is placed in the Maxwell® 16 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 that the cartridge 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.

7. 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.
8. Press the Run/Stop button. The platform will retract. Close the door.



Warning: Pinch point hazard.

9. The Maxwell® 16 Instrument will immediately begin the purification run. The screen will display the steps performed and 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 sample will be lost.
10. When the automated purification run is complete, the LCD screen will display a message that the method has ended.

6.C. Instrument Run: AS2000 and AS3000 Instruments (continued)

End of Run

11. 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 magnetic plunger bar, push them down gently by hand to remove them.
12. Press the Run/Stop button to extend the platform out of the instrument.
13. Remove the Maxwell® 16 LEV Cartridge Rack from the instrument. Remove Elution Tubes containing total viral nucleic acid, and close the tubes.
14. It is recommended to centrifuge the elution tubes at $10,000 \times g$ for 2 minutes. Transfer the supernatant to a clean tube (not provided). Avoid transferring paramagnetic particles and any floating debris.
15. 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.

Setup for AS3000 Maxwell® 16 MDx Instruments

Refer to the *Maxwell® 16 MDx Instrument Technical Manual #TM320* for detailed information.

1. Turn on the Maxwell® 16 MDx 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” 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 Instrument platform. Ensure that the rack is placed in the Maxwell® 16 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. Press the Run/Stop button. The platform will retract. Close the door.



Warning: Pinch point hazard.

The Maxwell® 16 MDx 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.
11. When the automated purification run is complete, follow instructions on the screen for data transfer. For detailed instructions, refer to the *Maxwell® 16 MDx Instrument Technical Manual #TM320* and *Maxwell® Sample Track Software Technical Manual #TM314*.

End of Run

12. 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 magnetic plunger bar, push them down gently by hand to remove them.
13. Press the Run/Stop button to extend the platform out of the instrument.
14. Remove the Maxwell®16 LEV Cartridge Rack from the instrument. Remove Elution Tubes containing total viral nucleic acid, and close the tubes.
15. It is recommended to centrifuge the elution tubes at $10,000 \times g$ for 2 minutes. Transfer the supernatant to a clean tube (not provided). Avoid transferring paramagnetic particles and any floating debris.
16. Remove cartridges and plungers from the cartridge rack, and discard as hazardous waste. Do not reuse reagent cartridges, LEV Plungers or Elution Tubes.

For the Maxwell® 16 MDx Instrument, ensure samples are removed before the 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

Lower viral nucleic acid recovery than expected (e.g., for customer-provided internal controls)

Causes and Comments

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.
- 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.

Symptoms

Lower viral nucleic acid recovery than expected (e.g., for customer-provided internal controls; continued)

Causes and Comments

The Maxwell® 16 Instrument was set for the wrong method. Ensure that the correct method is chosen in Research Mode.

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 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.

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 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 rack for sample addition to minimize contamination of adjacent cartridges.

Viral method not an option on the instrument

For the Maxwell® 16 SEV Instrument (Cat.# AS2000 series), verify that the instrument is in LEV Research mode. Verify that the instrument has firmware version 4.61 or higher, which includes the Viral method.

For the Maxwell 16 MDx Instrument (Cat.# AS3000 series), 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.



10. Summary of Change

The following change was made to the 5/20 revision of this document:

1. Added new size ASB1150.

^(a)U.S. Pat. Nos. 7,329,488, 7,721,947 and 7,891,549 and other patents pending.

^(b)U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Japanese Pat. No. 3253638, European Pat. No. 1 204 741 and other patents pending.

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